Food and Agriculture Organization
of the United Nations

Organisation des Nations Unies
pour l'alimentation et l'agriculture

Organización de las Naciones Unidas
para la Alimentación y la Agricultura

59
2016

ISSN 2078-6336

ANIMAL GENETIC RESOURCES

an international journal

RESSOURCES GÉNÉTIQUES ANIMALES

un journal international

RECURSOS GENÉTICOS ANIMALES

una revista internacional

United Nations Decade on Biodiversity

Animal Genetic Resources is an international journal published under the auspices of the Animal Genetic Resources Branch of the Animal Production and Health Division, Food and Agriculture Organization of the United Nations (FAO).

Ressources génétiques animales est un journal international publié sous les auspices de la Sous-Division des ressources génétiques animales de la Division de la production et de la santé animales, Organisation des Nations Unies pour l'alimentation et l'agriculture (FAO).

Recursos genéticos animales es una revista internacional publicada bajo los auspicios de la Subdivisión de los Recursos Genéticos Animales de la División de Producción y Sanidad Animal, la Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO).

Print edition and institutional subscriptions / Édition imprimée et abonnements pour institutions / Edición de la impresión y suscripciones institucionales: Sales and Marketing Group, Office of Knowledge Exchange, Research and Extension, FAO, Viale delle Terme di Caracalla, 00153 Rome, Italy; Fax: (39) 06 5705 3360; E-mail / courrier électronique / correo: Publications-Sales@fao.org or through FAO sales agents / ou auprès des agents de vente des publications de la FAO / o a través de los agentes de venta de la FAO.

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ANIMAL GENETIC RESOURCES

RESSOURCES GÉNÉTIQUES ANIMALES

RECURSOS GENÉTICOS ANIMALES

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Animal Genetic Resources, 2016, 59, ii. © Food and Agriculture Organization of the United Nations, 2016
doi:10.1017/S2078633616000266

Editorial

Dear reader,

In 1983 the first issue of *Animal Genetic Resources,* at that time entitled *Animal Genetic Resources Information,* was published. Now, 33 years later, with its 59th issue, the journal comes to an end, mainly due to a lack of resources. Already the editorial of the 50th commemorative issue provided you with some information on the history of the journal and some statistics. I would now like share with you the final numbers.

The 50th issue was made available online mid-2012. To that time more than 400 articles had been published. Since then, other 123 articles have been accepted for publication. Whereas the proportion of articles published in English during the first 50 issues was below 80%, it increased to almost 90% in the last nine issues. Regarding the livestock species, the analyses of the papers reveal that the majority of publications still focus on ruminants. More specifically, in these last nine issues, two-thirds of the publications dealt with large or small ruminates, each with an equal share, while a fifth of the articles have been dedicated to poultry, and to a lesser extent, pigs and equines. While this result substantiates the important role of ruminants for human livelihoods, it may also be interpreted as call for more research on other livestock species playing an essential role in certain areas of the world that might be of increasing importance in the light of changing environmental conditions.

The papers published in the journal can be related to the various strategic priority areas of the *Global Plan of Action for Animal Genetic Resources (GPA)*[1]. I would like to recall that the international community adopted the GPA at the International Technical Conference on Animal Genetic Resources for Food and Agriculture in September 2007. The GPA was later endorsed by the 34th FAO Conference. It includes 23 strategic priorities for action grouped into four priority areas: characterization and monitoring; sustainable use and development; conservation; and policies, institutions and capacity-building. The *Second Report on the State of the World's Animal Genetic Resources for Food and Agriculture*[2] (published in 2015) provided the basis for a review and possible update of the GPA. It confirmed that the four strategic priority areas remain valid. A large majority of journal's articles deals with the first strategic priority area. While 63% of all articles published in the first 50 issues fell under this strategic priority area, this percentage increased to 73% in the final nine issues. Sixteen percent of the articles published after the 50th issue dealt with sustainable use and development, compared to 11 percent before. The percentage of articles dealing with conservation dropped from 17 to 8 and only a low percentage of articles have dealt with strategic priority area 4 on policies, institutions and capacity building. The unbalanced distribution over the strategic priority areas may be explained by the feasibility of certain studies supported by research projects limited in terms of time and money but also by the frequent lack of structured breeding programmes and related data.

In total, three special issues of the journal have been published. The last one, entitled "adding value" was published in 2013. The articles on this topic describe various strategies that have been used to add value to local breeds in various countries and regions, with the aim of increasing the competitiveness of these breeds and promoting their sustainable use in the long term.

Since 2009, FAO has been publishing the journal jointly with Cambridge University Press. All issues and articles will continue to be available through Cambridge Journals Online and on FAO's website at http://www.fao.org/AG/AGAInfo/programmes/en/genetics/journal.html.

I would like to thank the readership of the journal for its loyalty and interest over so many years and the authors for their valuable submissions. You made the journal to an exciting source of information for everybody interested in the diversity of animal genetic resources and their manifold products and services. We sincerely hope that past *Animal Genetic Resources* authors will find a suitable place among the broad field of journals related to livestock issues to submit their future publications to continue facilitating the spread of knowledge on animal genetic resources.

Last but not least, I would also like to thank the reviewers and members of the editorial board of the journal who did a fabulous job over so many years, taking the capacity building role of the journal seriously. This was reflected in the review process, which aimed, where necessary, to support and advise authors who might otherwise have difficulty presenting their work in an appropriate form for publication in a scientific journal.

Yours sincerely,

Roswitha Baumung

[1] http://www.fao.org/docrep/010/a1404e/a1404e00.htm
[2] http://www.fao.org/publications/SoWAnGR/en/

Animal Genetic Resources, 2016, 59, iii–iv. © Food and Agriculture Organization of the United Nations, 2016
doi:10.1017/S2078633616000278

Editorial

Estimado lector,

En 1983 se publicó el primer volumen de *Recursos Genéticos Animales,* en aquella época con el nombre de *Boletín de Información sobre Recursos Genéticos Animales*. Ahora, 33 años después, con su 59° volumen, la revista llega a su fin, debido principalmente a la falta de recursos. Ya en el editorial del volumen conmemorativo número 50 le aportábamos cierta información sobre la historia de la revista y algunos datos estadísticos. Me gustaría ahora compartir con Usted los datos finales.

El 50° volumen se publicó en línea a mediados de 2012. Hasta esa fecha se habían publicado más de 400 artículos. Desde entonces, otros 123 artículos han sido aceptados para su publicación. Mientras que a lo largo de los 50 primeros volúmenes el porcentaje de artículos publicados en inglés era inferior al 80 por ciento, este porcentaje se ha incrementado hasta cerca del 90 por ciento en los nueve últimos volúmenes. En lo que concierne a las especies ganaderas, el análisis de los artículos desvela que la mayoría de las publicaciones siguen centrándose en los rumiantes. Más concretamente, en estos nueve últimos volúmenes, dos tercios de las publicaciones trataron sobre pequeños o grandes rumiantes, a partes iguales entre ellos, mientras que los artículos dedicados a las aves sólo representaron una quinta parte y los dedicados a cerdos y equinos una proporción aún menor. Si bien este resultado corrobora el importante papel de los rumiantes para la subsistencia de los seres humanos, también puede ser interpretado como una llamada de atención para una mayor investigación en otras especies ganaderas que desempeñan un papel esencial en ciertas áreas del Mundo, las cuales podrían adquirir mayor importancia, habida cuenta de las condiciones ambientales cambiantes.

Los artículos publicados en la revista pueden relacionarse con varias áreas estratégicas prioritarias del *Plan de Acción Mundial sobre los Recursos Zoogenéticos (PAM)*[1]. Quisiera recordar que la comunidad internacional adoptó el PAM en la Conferencia Técnica Internacional sobre los Recursos Zoogenéticos para la Agricultura y la Alimentación en septiembre de 2007. El PAM fue posteriormente avalado por la 34ª Conferencia de la FAO. Incluye 23 prioridades estratégicas para la acción agrupadas en cuatro áreas prioritarias: caracterización y seguimiento; utilización sostenible y desarrollo; conservación; y políticas, instituciones y creación de capacidad. El *Segundo Informe sobre la Situación de los Recursos Zoogenéticos Mundiales para la Alimentación y la Agricultura*[2] (publicado en 2015) ha proporcionado la base para una revisión y posible actualización del PAM. Esto ha confirmado que las cuatro áreas estratégicas prioritarias siguen siendo válidas. La gran mayoría de los artículos de la revista tratan sobre la primera área estratégica prioritaria. Mientras que el 63 por ciento del total de artículos publicados en los 50 primeros volúmenes se enmarcaba bajo esta área estratégica prioritaria, este porcentaje aumentó hasta el 73 por ciento en los últimos nueve volúmenes. El 16 por ciento de los artículos publicados tras el 50° volumen trataron sobre la utilización sostenible y el desarrollo, en comparación con el 11 por ciento anterior. El porcentaje de artículos en relación con la conservación disminuyó del 17 al 8 por ciento y únicamente un escaso porcentaje de artículos han abordado la cuarta área estratégica prioritaria sobre políticas, instituciones y creación de capacidad. Esta distribución desequilibrada entre las áreas estratégicas prioritarias puede explicarse por la factibilidad de algunos estudios, financiados por proyectos de investigación de corta duración y con limitaciones presupuestarias, así como por la frecuente falta de programas estructurados de selección y de datos relacionados.

En total, se han publicado tres volúmenes especiales de la revista. El último de ellos, titulado "Añadiendo valor", fue publicado en 2013. Los artículos sobre este tema describen varias estrategias que han sido empleadas para dotar de más valor a las razas locales en varios países y regiones, con el fin de hacer más competitivas a estas razas y promover su utilización sostenible a largo plazo.

Desde 2009, la FAO ha venido publicando la revista conjuntamente con Cambridge University Press. Todos los volúmenes y artículos seguirán estando disponibles a través de Cambridge Journals Online y en la página web de la FAO http://www.fao.org/AG/AGAInfo/programmes/es/genetics/journal.html.

Me gustaría agradecer a los lectores de esta revista su lealtad e interés a lo largo de tantos años y a los autores sus valiosas contribuciones. Ustedes han hecho de esta revista una fuente apasionante de información para todas aquellas personas interesadas en la diversidad de los recursos zoogenéticos y en sus múltiples productos y servicios. Confiamos sinceramente en que los antiguos autores de *Recursos Genéticos Animales* encuentren un lugar idóneo, en el amplio campo de las revistas relacionadas con las cuestiones ganaderas, para mandar sus futuras publicaciones y, así, seguir contribuyendo a la difusión del conocimiento sobre los recursos zoogenéticos.

Por último, pero no por ello menos importante, quisiera también dar las gracias a los revisores y miembros del consejo editorial de la revista, que han realizado un magnífico trabajo a lo largo de tantos años, asumiendo

[1] http://www.fao.org/docrep/010/a1404s/a1404s00.htm
[2] http://www.fao.org/publications/sowangr/es/

con seriedad el rol de creación de capacidad de la revista. Esto se ha reflejado en el proceso de revisión, con el cual se pretendía, en caso de que fuera necesario, ayudar y aconsejar a los autores que, de otra manera, hubieran podido tener dificultades para presentar su trabajo en un formato adecuado para su publicación en una revista científica.

Atentamente,

Roswitha Baumung

Animal Genetic Resources, 2016, 59, v. © Food and Agriculture Organization of the United Nations, 2016
doi:10.1017/S207863361600028X

Editorial

Cher lecteur,

Le premier volume de *Ressources Génétiques Animales* fut publié en 1983, sous le nom à cette époque de *Bulletin d'Information sur les Ressources Génétiques Animales*. Maintenant, 33 ans plus tard, le journal arrive à son terme avec son 59ème volume, et ce principalement en raison du manque de ressources. Dans l'éditorial du volume commémoratif 50 quelques informations sur l'histoire du journal et quelques données statistiques vous étaient déjà fournies. Je voudrais maintenant partager avec vous les chiffres finals.

Le 50ème volume a été mis en ligne à la mi-2012. Jusqu'à cette date plus de 400 articles avaient été publiés. Dès lors, 123 autres articles ont été acceptés pour publication. Alors qu'au cours des 50 premiers volumes, le pourcentage d'articles publiés en anglais était inférieur à 80 pour cent, ce pourcentage a augmenté à près de 90 pour cent dans les neuf derniers volumes. En ce qui concerne les espèces d'animaux d'élevage, l'analyse des articles révèle que la plupart des publications continuent de se concentrer sur les ruminants. Plus précisément, dans ces neuf derniers volumes, les deux tiers des publications ont traité des petits ou des grands ruminants, à parts égales entre eux, tandis que les articles consacrés aux volailles n'ont représenté qu'un cinquième et ceux dédiés aux porcs et aux équidés une proportion encore plus faible. Bien que ce résultat confirme le rôle important des ruminants pour la subsistance des êtres humains, il peut également être interprété comme un appel à davantage de recherches sur d'autres espèces animales jouant un rôle essentiel dans certaines régions du monde, qui pourraient devenir plus importantes, étant donné les conditions environnementales changeantes.

Les articles publiés dans ce journal peuvent être mis en rapport avec plusieurs domaines prioritaires du *Plan d'Action Mondial pour les Ressources Zoogénétiques (PAM)*[1]. Je souhaiterais rappeler que la communauté internationale a adopté le PAM à la Conférence Technique Internationale sur les Ressources Zoogénétiques pour l'Alimentation et l'Agriculture en septembre 2007. Le PAM a ensuite été approuvé par la 34ème Conférence de la FAO. Il inclut 23 priorités stratégiques d'action regroupées en quatre domaines prioritaires: caractérisation et surveillance; utilisation durable et mise en valeur; conservation; et politiques, institutions et renforcement des capacités. Le *Deuxième Rapport sur l'État des Ressources Zoogénétiques pour l'Alimentation et l'Agriculture dans le Monde*[2] (publié en 2015) a fourni la base pour une révision et une possible mise à jour du PAM. Ceci confirme que les quatre domaines prioritaires sont toujours valables. Une grande majorité des articles du journal traitent du premier domaine prioritaire. Alors que 63 pour cent de tous les articles publiés dans les 50 premiers volumes s'inscrivaient dans ce domaine prioritaire, ce pourcentage est passé à 73 pour cent dans les neuf derniers volumes. Seize pour cent des articles publiés après le 50ème volume ont traité de l'utilisation durable et la mise en valeur, contre 11 pour cent auparavant. Le pourcentage d'articles dédiés à la conservation a diminué de 17 pour cent à 8 pour cent et seul un faible pourcentage d'articles ont traité du quatrième domaine prioritaire sur les politiques, les institutions et le renforcement des capacités. Cette répartition inégale entre les domaines prioritaires peut s'expliquer par la faisabilité de certaines études soutenues par des projets de recherche limités en termes de durée et de financement, ainsi que par l'absence fréquente de programmes de sélection structurés et des données connexes.

Au total, trois volumes spéciaux du journal ont été publiés. Le dernier, intitulé "Ajouter de la valeur", a été publié en 2013. Les articles sur ce sujet décrivent plusieurs stratégies qui ont été utilisées pour ajouter de la valeur aux races locales dans divers pays et régions, afin de rendre ces races plus compétitives et de promouvoir leur utilisation durable à long terme.

Depuis 2009, la FAO a publié le journal conjointement avec Cambridge University Press. Tous les volumes et articles continueront d'être disponibles par l'intermédiaire de Cambridge Journals Online et sur le site Internet de la FAO http://www.fao.org/AG/AGAInfo/programmes/fr/genetics/journal.html.

Je tiens à remercier les lecteurs de ce journal pour leur fidélité et leur intérêt pendant tant d'années et les auteurs pour leurs précieuses contributions. Vous avez fait de ce journal une source passionnante d'informations pour tous ceux intéressés par la diversité des ressources zoogénétiques et leurs multiples produits et services. Nous espérons sincèrement que les anciens auteurs de *Ressources Génétiques Animales* trouveront un endroit approprié dans le vaste domaine des journaux consacrés à l'élevage pour y envoyer leurs futures publications et continuer ainsi à contribuer à la diffusion des connaissances sur les ressources zoogénétiques.

Dernier point mais non le moindre, je voudrais également remercier les relecteurs et les membres du comité de rédaction du journal, qui ont fait un excellent travail pendant de nombreuses années, en prenant au sérieux le rôle de renforcement des capacités du journal. Cela s'est reflété dans le processus de révision, qui visait, le cas échéant, à soutenir et conseiller les auteurs qui autrement auraient pu avoir du mal à présenter leur travail sous une forme appropriée pour la publication dans une revue scientifique.

Cordialement,

Roswitha Baumung

[1] http://www.fao.org/docrep/010/a1404f/a1404f00.htm
[2] http://www.fao.org/publications/sowangr/fr/

Animal Genetic Resources, 2016, 59, 1–6. © Food and Agriculture Organization of the United Nations, 2016
doi:10.1017/S2078633616000072

Assessment of genetic diversity of Mithun (*Bos frontalis*) population in Bhutan using microsatellite DNA markers

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Summary
Genetic diversity of Mithun population in Bhutan was studied using 14 microsatellite markers. Two sets of two-step polymerase chain reactions were performed with multiplex and individual markers for genotyping 105 hair samples collected from Arong in Samdrupjongkhar (AS, 36) and Wangdigang in Zhemgang (WZ, 69). Fifty-three alleles were detected with average of 3.89 alleles and polymorphism information content of 0.44 ± 0.03 per locus. A low level of genetic variability within population was present with observed heterozygosity at 0.50 ± 0.06 and expected heterozygosity at 0.48 ± 0.06. Analysis of molecular variance attributed 58 percent of total variation to within the individuals. Mean F_{IS} and F_{IT} were −0.056 and 0.005 respectively, indicated low level of population differentiation and limited out-breeding. The normal L-shaped distribution of allelic frequencies without any mode-shift revealed the absence of recent genetic bottleneck in Mithun populations. Therefore to manage inbreeding in the small Mithun population of Bhutan, periodic assessment of inbreeding levels and exchange of animals between farms is recommended to reduce frequency of introduction of animals from India.

Keywords: *Genetic diversity, Mithun, analysis of molecular variance (AMOVA), microsatellite DNA marker*

Résumé
La diversité génétique de la population de gayals au Bhoutan a été étudiée en utilisant 14 marqueurs microsatellites. Deux séries de réactions en chaîne par polymérase (PCR selon ses sigles en anglais) en deux étapes ont été réalisées avec plusieurs marqueurs et avec des marqueurs individuels pour génotyper 105 échantillons de poils prélevés à Arong au Samdrup Jongkhar (AS, 36) et à Wangdigang au Zhemgang (WZ, 69). Cinquante-trois allèles ont été détectés pour une moyenne de 3.89 allèles et un contenu d'information sur le polymorphisme de 0.44 ± 0.03 par locus. Un faible niveau de variabilité génétique a été observé au sein de la population avec une hétérozygotie observée de 0.50 ± 0.06 et une hétérozygotie attendue de 0.48 ± 0.06. L'analyse de variance moléculaire (AMOVA) a attribué le 58 pour cent de la variation totale à la variabilité intra-individuelle. Les valeurs moyennes des coefficients F_{ST}, F_{IS} et F_{IT} ont été de 0.054, −0.056 et 0.005 respectivement, ce qui est le reflet d'un faible niveau de différenciation dans la population et d'un manque de croisements exogames. La distribution habituelle des fréquences alléliques en L, sans aucune distorsion, a décelé l'absence de goulots d'étranglement génétique récents dans les populations de gayals. Ainsi, afin de gérer la consanguinité dans la petite population de gayals du Bhoutan, une évaluation périodique des niveaux de consanguinité et un échange d'animaux entre les fermes sont conseillés pour réduire la fréquence des importations depuis l'Inde.

Mots-clés: *diversité génétique, gayal, analyse de variance moléculaire (AMOVA), marqueurs microsatellites d'ADN*

Resumen
Se estudió la diversidad genética de la población de gayales en Bhután, utilizando para ello 14 marcadores microsatélites. Se llevaron a cabo dos tandas de reacciones en cadena de la polimerasa (PCR, por sus siglas en inglés) de dos pasos con múltiples marcadores y con marcadores individuales para genotipificar 105 muestras de pelo tomadas de Arong en Samdrupjongkhar (AS, 36) y de Wangdigang en Zhemgang (WZ, 69). Se detectaron 53 alelos, con una media de 3.89 alelos y un contenido de información polimórfica de 0.44 ± 0.03 por locus. Se constató un bajo nivel de variabilidad genética dentro de la población, con una heterocigosis observada de 0.50 ± 0.06 y una heterocigosis esperada de 0.48 ± 0.06. El análisis de varianza molecular (AMOVA) atribuyó el 58 por ciento de la variación total a la variabilidad intraindividual. Los valores medios para los coeficientes F_{ST}, F_{IS} y F_{IT} fueron de 0.054, −0.056 y 0.005, respectivamente, lo cual refleja un bajo nivel de diferenciación en la población y una escasez de cruzamientos exogámicos. La distribución típica en forma de L de las frecuencias alélicas, sin ninguna distorsión, puso de manifiesto la ausencia de cuellos de botella genéticos recientes en las poblaciones de gayales. Por tanto, para gestionar la endogamia en la pequeña población de gayales de Bhután, se recomienda una evaluación periódica de los niveles de consanguinidad y el intercambio de animales entre las granjas con el fin de recudir la frecuencia de las importaciones desde la India.

Palabras clave: *diversidad genética, gayal, análisis de varianza molecular (AMOVA), marcadores microsatélites de ADN*

Submitted 12 February 2015; accepted 4 May 2016

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Introduction

Mithun *(Bos frontalis)* is an important semi-domesticated animal species found in hilly region of India, China, Myanmar, Bhutan and Bangladesh. Within Bhutan, they are reared in small numbers throughout the country. Unlike other countries where Mithun is kept for meat purpose, utility of the species in Bhutan is unique. They are kept for crossbreeding with traditional Nublang cattle *(Bos indicus)* to produce hybrids for milk and draft purposes. These crosses are well adapted to sloppy terrain and transhumance system of cattle management in the country. The documentation on this breeding practice exists as early as the seventeenth century and also vividly described in Report of British Mission to Bhutan (RGoB, 2002).

Mithun is believed to have originated from wild Indian gaur *(Bos gaurus)* more than 8 000 years ago (Simoons, 1984; Mondal and Pal, 1999; Dorji *et al.*, 2010; Tanaka *et al.* 2011). The literature on genetic diversity studies of Mithun is generally scarce. Mithun in India are classified into four distinct strains; Arunachalee, Mizorami, Nagami and Manipuri based on their phenotypic and genetic characterization (Bhusan *et al.*, 2009). And considering the proximity and initial source of animals, Mithun in Bhutan are likely of Arunachalee strain.

To meet the demand for Mithun bulls and sustainable management of Mithun genetic resources in the country, two conservation farms were established in mid 1970s at Wangdigang, Zhemgang (WZ) and Arong, Samdrupjongkhar (AS) (Figure 1). These farms produce and supply Mithun bulls to farmers for selective inter-breeding and for production of frozen semen for artificial insemination on Nublang cattle *(Bos indicus)*. The animals are maintained under free range and forest grazing and follow random mating. The population of Mithun in Bhutan was 1 559 (DoL, 2013) and declining (1 826 (DoL, 2006)). Therefore in view of the small and declining population size, this study attempted to assess the genetic diversity of Mithun population in Bhutan to provide insights into conservation and sustainable management of the species in the country.

Fig. 1 - Colour online

Figure 1. Mithun bull at Regional Mithun Breeding Farm, Arong, Bhutan.

Materials and methods

Samples, microsatellite DNA markers and polymerase chain reaction (PCR) amplification

A total of 105 tail hair samples, including 36 from AS and 69 from WZ, were collected. These hair samples were put into an individually labelled envelope and stored at room temperature. A total of 17 highly polymorphic microsatellite DNA markers recommended by FAO (2011) for cattle and validated in Mithun (Qu *et al.*, 2012) were used in this study (Table 1). Two-step PCR was performed (Arandjelovic *et al.*, 2009) for multiplex and individual markers separately. Two sets of multiplex markers, set A containing nine non-labelled markers (ETH225, ETH10, ETH3, BM1824, BM2113, SPS115, TGLA122, TGLA126 and TGLA227) and set B containing eight non-labelled markers (BMS2533, POTCHA, ILSTS006, HEL5, HAUT24, INRA023, BoLADRBP1 and BoLADR2B), were used to perform the first PCR. In 20 µl of master reaction mixture, 10 µl of 2× buffer, 4 µl of dNTPs, 0.4 µl of polymerase (KOD FX Enzyme and kit, Toyobo Co., Ltd., Tokyo, Japan), 1.35 µl each of 20 p forward and reverse primers and H_2O were added. The mixture was divided in 10 µl aliquots and added 6 hair roots per sample (in duplicates) to test repeatability. PCR was performed with following thermal-cycling conditions: 94 °C for 2 min, 35 cycles of (98 °C for 10 s, 58 °C for 30 s and 68 °C for 30 s) and 10 °C hold. The products of the first PCR were stored at 4 °C or −20 °C until the second PCR.

For the second PCR, 12.5 µl reaction mixture contained 6.25 µl of ×2 buffer, 2.5 µl of dNTPs, 0.25 µl of polymerase (KOD FX Enzyme and kit, Toyobo Co., Ltd., Tokyo, Japan), 0.375 µl each of 5 p forward and labelled reverse primers, 2.250 µl H_2O and 0.5–1.0 µl of the first PCR products. Thermal-cycling conditions for this reaction was set up as 94 °C for 2 min, 45 cycles of (98 °C for 10 s, 58 °C for 30 s and 68 °C for 30 s) and hold at 10 °C. The PCR products were stored at 4 °C/−20 °C.

A loading cocktail containing 960 µl of Formamide (FA) and 9.0 µl of molecular size marker (ROX 400HD) enough for 6 runs (96 samples) were prepared. Each well contained 9.8 µl aliquots of loading cocktail and 1 µl of the second PCR products in 96 well plates. They were heated at 95 °C for 3 min and immediately snap cooled in ice for few minutes and applied to Genetic Analyzer (ABI PRISM 3130xl) to read the fragment size. Microsatellite data and allele scoring were performed using GeneMapper software (Version 4.1).

Statistical analysis

Microsatellite data were analyzed to estimate the observed number of alleles (N_a), the number of effective alleles (N_e), observed (H_o) and expected (H_e) heterozygosity, F-statistics, Hardy-Weinberg equilibrium (HWE) and Nei's genetic distance using GenAlEx 6.501 software (Peakall and Smouse,

Table 1. Primer sequences, chromosomes numbers and annealing temperature for 17 microsatellite loci employed for diversity analysis of Mithun population in Bhutan.

Locus	Chromosomes	Primer sequences (5′–3′)	Annealing temp(°C)	Genebank accession no.	Allele range
ETH3	19	GAACCTGCCTCTCCTGCATTGG ACTCTGCCTGTGGCCAAGTAGG	55–65	Z22744	103–113
ETH10	5	GTTCAGGACTGGCCCTGCTAACA CCTCCAGCCCACTTTCTCTTCTC	55–65	Z22739	207–231
ETH225	9	GATCACCTTGCCACTSTTTCCT ACATGACAGCCAGCTGCTACT	55–65	Z14043	131–159
BM1824	1	GAGCAAGGTGTTTTTCCAATC CATTCTCCACTGCTTCCTTG	55–60	G18394	176–197
BM2113	2	GCTGCCTTCTACCAAATACCC CTTCCTGAGAGAAGCAACACC	55–60	M97162	122–156
TGLA122	21	CCCTCCTCCAGGTAAATCAGC AATCACATGGCAAATAAGTACATAC	55–58	–	136–184
TGLA126	20	CTAATTTAGAATGAGAGAGGTTCT TTGGTCTCTATTCTCTGAATATTCC	55–58	–	115–131
TGLA227	18	CGAATTCCAAATCTGTTAATTTGCT ACAGACAGAAACTCAATGAAAGCA	55–56	–	75–105
BMS2533	15	TGAAGTAAGTAAGCACACAAGCA TTGATCATCTTTAGGTCCATCC	56		122–156
POTCHA	15	GTAAACACAGTTCCCTGGAGAG ATGCCAACTTTTCCCATCAC	59		135–151
ILSTS006	7	TGTCTGTATTTCTGCTGTGG ACACGGAAGCGATCTAAACG	55	L23482	277–309
HAUT24	22	F: CTCTCTGCCTTTGTCCCTGT R: AATACACTTTAGGAGAAAAATA	52.1		106–132
BoLA RBPI	23	ATGGTGCAGCAGCAAGGTGAGCA GGGACTCAGTCTCTCTATCTCTTT	55		110–132
BoLA DR2B	23	AGGCAGCGCCGAGGTGAGCGA TCCAACACTCACCTGGACGTAGC	60		144–152
SPS115	15	AAAGTGACACAACAGCTTCTCCAG AACGAGTGTCCTAGTTTGGCTGTG	58		240–256
HEL5	21	GCAGGATCACTTGTTAGGGA AGACGTTAGGTACATTAAC	52–57	X65204	145–171
INRA023	3	GAGTAGAGCTACAAGATAAACTTC TAACTACAGGGTGTTAGATGAACTC	55	X67830	195–225

2006, 2012). Polymorphic information content (PIC) value of marker was calculated using Cervus version 3.0.6 (Kalinowski *et al.*, 2007). Mode-shift test for the distribution of allele frequencies classified into 10 frequency classes was performed using BOTTLENECK 1.2.02 (Piry *et al.*, 1999).

STRUCTURE version 2.3 (Pritchard *et al.*, 2000) was employed for Bayesian identification of genetic clusters and the degree of admixture that best fit the data. Ten independent runs were performed for $K = 2$ with burn-in period of 100 000 iterations followed by 200 000 iterations of the Markov chain Monte Carlo algorithm. STRUCTURE HARVESTER (Earl and vonHoldt, 2012) was used to reformat data using Evanno method (Evanno *et al.*, 2005). CLUMPP version 1.1 (Jackobsson and RosenBerg, 2007) was employed to obtain optimal matrices for 10 replications for $K = 2$ and this output file was visualized using DISTRUCT version 1.1 (Rosenberg, 2004).

Results and discussion

A total of 14 loci were successfully amplified from hair samples while three loci (SPS115, HEL5 and INRA023) failed. The genetic variability estimates (H_o, H_e and inbreeding coefficient (F)) by marker and populations are presented in Table 2. N_a per locus ranged between 2 (TGLA126) and 7 (BM2113 and BoLADRBP1) in WZ and between 2 (ETH10 and BM1824) and 6 (ETH225) in AS. Mean N_a per locus was 4.14 and 3.64 for WZ and AS, respectively. These parameter estimates of the two farms were similar, which is obvious considering the same source of the animals. The microsatellite loci in current study as per Barker (1994) may be useful to evaluate the genetic diversity of Mithun in Bhutan. The overall N_a of Bhutanese Mithun population was lower than Yunnan Mithun (7.88) (Qu *et al.*, 2012).

Observed heterozygosity (H_o) ranged between 0.121 (BM1824) and 0.813 (BMS2533) for WZ and between 0.125 (TGLA227) and 0.889 (BoLA DRBP1) for AS. Expected heterozygosity (H_e) ranged between 0.115 (BMI1824) and 0.827 (BoLA DRBP1) for WZ and between 0.122 (ETH10) and 0.716 (ETH 225, BoLA DRBP1) for AS. Mean H_o and H_e were 0.56 and 0.50 in WZ, and 0.49 and 0.48 in AS respectively. The mean H_o (0.53), H_e (0.49) and PIC (0.44) for all the loci in this study are low. Ten loci from WZ and 11 from AS were

Table 2. Number of alleles (Na, observed; N_e, effective), heterozygosity (H_o, observed; H_e, expected), polymorphic information content (PIC) and Wright's fixation index (F).

Pop	Locus	N_a	N_e	*F*	H_o	H_e	PIC	Pop	Locus	N_a	N_e	*F*	H_o	H_e	PIC
WZ	ETH3	4	2.04	−0.009	0.515	0.511	0.430	AS	ETH3	3	2.40	−0.088	0.634	0.583	0.498
	ETH10	5	1.24	0.075	0.182	0.197	0.190		ETH10	2	1.14	−0.070	0.130	0.122	0.114
	ETH225	5	2.67	−0.067	0.667	0.625	0.578		ETH225	6	3.53	0.059	0.674	0.716	0.673
	BMI1824	3	1.13	−0.052	0.121	0.115	0.111		BMI1824	2	1.43	−0.082	0.326	0.301	0.256
	BM2113	7	3.91	0.023	0.727	0.744	0.700		BM2113	5	3.27	−0.120	0.778	0.694	0.649
	TGLA122	3	1.84	−0.137	0.519	0.456	0.368		TGLA122	4	1.61	−0.171	0.444	0.380	0.323
	TGLA126	2	1.91	−0.142	0.545	0.478	0.363		TGLA126	4	1.81	0.074	0.415	0.448	0.367
	TGLA227	3	1.70	−0.060	0.438	0.413	0.339		TGLA227	3	1.38	0.547	0.125	0.276	0.244
	BMS2533	3	2.07	−0.570	0.813	0.518	0.412		BMS2533	4	2.05	0.133	0.444	0.512	0.473
	POTCHA	5	2.06	−0.333	0.688	0.516	0.469		POTCHA	4	2.00	−0.111	0.556	0.500	0.450
	ILSTS006	3	1.99	−0.071	0.533	0.498	0.445		ILSTS006	3	1.66	0.059	0.375	0.398	0.354
	HAUT24	5	2.77	−0.174	0.750	0.639	0.580		HAUT24	3	2.25	0.324	0.375	0.555	0.456
	BoLA PRBPI	7	5.76	0.049	0.786	0.827	0.804		BOLAPRBPI	5	3.52	−0.241	0.889	0.716	0.667
	BoLA DR2B	3	2.09	0.041	0.500	0.521	0.451		BOLADR2B	3	2.18	−0.321	0.714	0.541	0.453
Mean		4.14	2.37	−0.102	0.560	0.500	0.446			3.64	2.16	−0.001	0.490	0.480	0.427

moderately informative ($0.25 < PIC < 0.50$) and four loci from WZ and three loci from AS were highly informative ($PIC > 0.5$), indicating a moderate polymorphism across all loci (Bolstein *et al.*, 1980). Overall, there is low level of genetic diversity among the Mithun population in Bhutan. These estimates of genetic diversity is similar to Yunnan Mithun in China (H_o (0.53), H_e (0.63) and PIC (0.60) (Qu *et al.*, 2012). But higher than two rapidly declining zebu cattle breeds of India (Mukesh *et al.*, 2004) and lower than the cattle breeds in Europe (Schmid *et al.*, 1999; Del Bo *et al.*, 2001). A low genetic diversity in current study may be attributed to small population size and repeated introduction of animals from same source (Aurnachal Pradesh, India) over the years.

The Wright's fixation indices (*F*) to measure local inbreeding ranged between −0.570 and 0.075 with average of −0.102 for WZ and between −0.321 and 0.547 with average of −0.001 for AS (Table 3). The overall population inbreeding estimate (F_{IS}) was −0.056 ($P = 0.001$) and subdivision estimate (F_{ST}) was 0.054. This indicated that there was no inbreeding within the population, which possibly is a result of random mating in the population. Test for HWE revealed that two loci (ETH10 and BMS2533) in WZ and one locus (TGLA227) in AS deviated significantly ($P < 0.05$). Overall, these values suggest a low level of population differentiation and limited out-breeding. This is evident as period of isolation of these populations is only from mid 1970s.

Analysis of molecular variances (AMOVA) showed 5 percent of total variation was between populations, 37 percent among individuals and 58 percent within individuals. A high within-individual variation is a result of high expression of genetic drift in small population size (Excoffier

Table 3. *F*-statistics and Hardy-Weinberg equilibrium (HWE) values for 14 microsatellite loci in Mithun of Wangdigang, Zhemgang (WZ) and Arong, Samdrupjongkhar (AS).

Locus	F-statistics			HWE	
	F_{IS}	F_{IT}	F_{ST}	WZ	AS
ETH3	−0.051	−0.042	0.009	0.87	0.54
ETH10	0.019	0.023	0.004	0.000***	0.64
ETH225	0.001	0.013	0.012	0.99	0.96
BMI1824	−0.074	−0.031	0.040	0.99	0.58
BM2113	−0.046	0.012	0.055	0.91	0.69
TGLA122	−0.152	−0.141	0.010	0.80	0.92
TGLA126	−0.038	−0.029	0.009	0.41	0.80
TGLA227	0.183	0.199	0.020	0.92	0.002**
BMS2533	−0.220	−0.174	0.038	0.05*	0.68
POTCHA	−0.224	0.145	0.301	0.93	0.63
ILSTS006	−0.014	0.002	0.016	0.20	0.20
HAUT24	0.057	0.138	0.086	0.22	0.47
BOLAPRBPI	−0.086	−0.069	0.016	0.51	0.36
BOLADR2B	−0.143	0.016	0.139	0.07	0.37
Mean	−0.056	0.005	0.054		

* Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Significant at $P < 0.001$.

Fig. 2 - B/W online

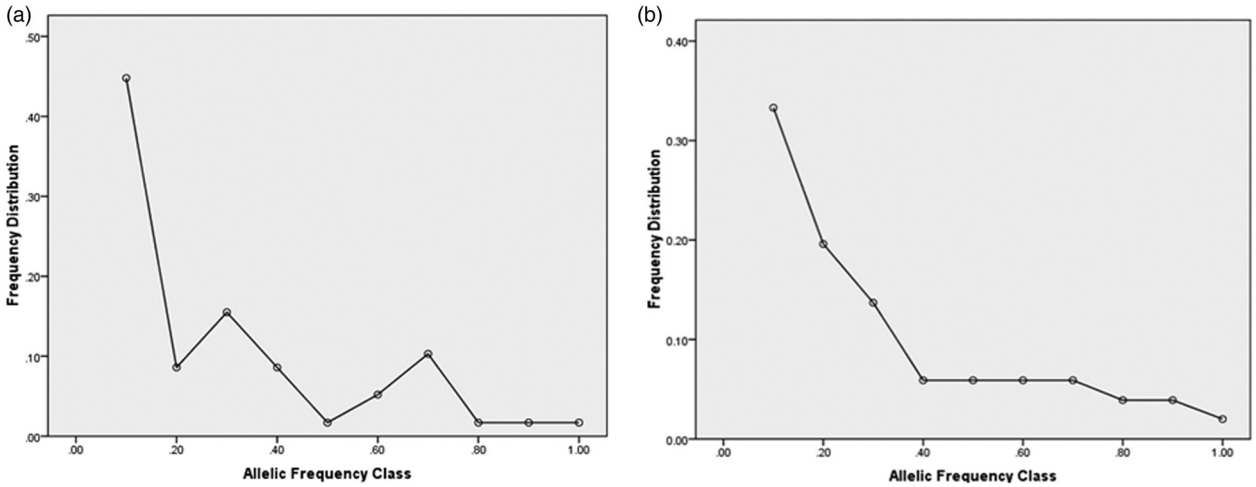

Figure 2. (a) Modeshift test for bottleneck analysis in Wangdigang Mithun population; (b) modeshift test for bottleneck analysis in Arong Mithun population.

Fig. 3 - Colour online

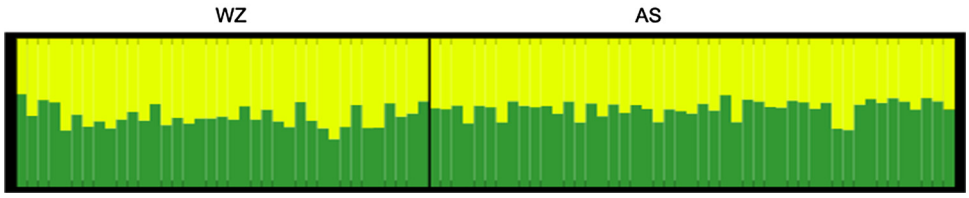

Figure 3. Distinct plot for 84 Mithun by model-based clustering in STRUCTURE for $K = 2$ with green colour representing WZ and Yello As Mithun. Individuals are represented by thin vertical lines and population separated by bold line.

et al., 1992). Further, there was a close relationship between farms (Nei's genetic distance 0.145) (Nei, 1987). Despite small population, there was no recent bottleneck as per mode-shift distortion graph (Figure 2a and 2b). The lack of recent bottleneck according to Luikart *et al.* (1998) further supports lack of inbreeding in these populations and presence of optimum population size at the source.

STRUCTURE software was employed to compute the proportion of the genome at multiple markers to observe overall population structuring and to assign individuals to one of the two populations. At $K = 2$, the populations of WZ and AS did not cluster distinctly to the inferred populations. Nevertheless, membership coefficients (Q) values indicated subtle level of genetic clusters as shown in Table 4. There is no clear differentiation of two populations further supports the absence of distinct genetic characteristic between the farms (Figure 3). Each column represents the genetic proportion in which an animal belonged to the population, with green colour representing WZ and Yellow AS Mithun.

Table 4. Population membership Q values in the two inferred clusters using STRUCTURE analysis.

Populations	Inferred cluster		Number of individuals
	1	2	
WZ	0.4569	0.5431	37
AS	0.5275	0.4725	47

WZ, Wangdigang, Zhemgang and AS, Arong, Samdrupjongkhar populations.

The animals in these two farms have not undergone genetic differentiation. They also, have low genetic diversity and very low/no inbreeding. Therefore periodic assessment of levels of inbreeding in the population, exchange of animals between the farms is recommended to guide the need for introduction of Mithun from elsewhere.

Conclusion

This study revealed that a panel of 14 polymorphic microsatellite DNA markers of cattle can be useful for assessment of genetic diversity in Mithun. There is overall a low genetic diversity in Mithun population in Bhutan. Considering the small population, periodic assessment of inbreeding levels and exchange of animals between farms is recommended to manage levels of inbreeding and guide on the need for introduction from its sources.

Acknowledgements

We would like to thank farm managers, Regional Mithun Breeding Farms, Wangdigang & Arong, Bhutan for collaboration on collection of hair samples and EU RNR SSP for

funding our travel to Primate Research Institute, Kyoto University for DNA fragment analysis and molecular genetics training.

Statement of interest

The authors do not have any conflict of interest that will influence the judgment and potentiality of being biased. Further, authors did not have any financial arrangements or connections pertinent to submitted manuscript.

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Animal Genetic Resources, 2016, 59, 7–14. © Food and Agriculture Organization of the United Nations, 2016
doi:10.1017/S2078633616000199

Assessment of the genetic variability using pedigree analysis of the Sahiwal breed in Kenya

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Summary

Pedigree analysis using genealogical information of 18 315 animals born between 1949 and 2008 was done to quantify genetic variability of the Sahiwal population in Kenya. Generation intervals for sire pathways were longer than dam pathways and increased over year periods, from about 4–16 years. The later was due to use of old bulls for breeding in the last 2 year groups and cessation of progeny testing in the year 2000. Average inbreeding level in last year period studied was 1.2 percent. Genetic variability of the population as assessed based on gene origin statistics decreased over the years. The ratio of effective number of founders to founders of 0.06 showed unequal contribution of founders to the reference population. However, since the founding population, ancestors contributed equally as shown by the ratio of f_e/f_a of 0.94, which could also be due to lack of effective selection in this population. The ratio of f_g/f_a of 0.63 indicated genetic loss of genetic variability occurred through genetic drift in the Kenyan Sahiwal population. The small number of ancestors (16) that accounted for 50 percent of the total variation in the reference population suggested overuse of a small number of some animals as parents over generations. The smaller ratio of f_g/f_e compared with f_a/f_e also confirms loss of genetic variability in the population by genetic drift than bottlenecks. Therefore the breeding strategy for the Sahiwal population in Kenya should incorporate tools that balance rate of genetic gain and the future rate of inbreeding.

Keywords: *effective population size, genealogy, genetic diversity, inbreeding, optimum contribution*

Résumé

Une analyse généalogique a été réalisée avec les données de 18 315 animaux nés entre 1949 et 2008 dans le but de quantifier la variabilité génétique de la population Sahiwal au Kenya. Les intervalles générationnels ont été plus longs sur la voie paternelle que sur la voie maternelle et se sont allongés au cours des années, d'environ 4 ans à 16 ans. Ceci a été dû à l'utilisation de vieux mâles pour la reproduction dans les deux dernières périodes d'années et à l'arrêt du contrôle de la descendance en l'an 2000. Le niveau moyen de consanguinité dans la dernière période étudiée a été de 1.2 pour cent. La variabilité génétique de la population, évaluée au moyen de statistiques sur l'origine des gènes, a diminué au fil des années. Le rapport entre le nombre effectif de fondateurs et les fondateurs a été de 0.06, ce qui met en évidence une contribution inégale des fondateurs à la population de référence. Cependant, depuis la population fondatrice, les ancêtres ont contribué équitablement, comme reflété par le rapport f_e/f_a de 0.94, qui pourrait aussi être dû à un manque de sélection efficace dans cette population. Le rapport f_g/f_a de 0.63 indique une perte de variabilité génétique causée par dérive génétique dans la population Sahiwal du Kenya. Le faible nombre d'ancêtres (16) expliquant 50 pour cent de la variation totale dans la population de référence suggère l'utilisation excessive en tant que parents d'un petit nombre d'animaux au cours de plusieurs générations. De même, le fait que le rapport f_g/f_e soit inférieur au rapport f_a/f_e confirme la perte de variabilité génétique dans la population par dérive génétique plutôt que par goulots d'étranglement génétique. Par conséquent, la stratégie de sélection pour la population Sahiwal au Kenya devrait intégrer des outils permettant d'équilibrer le taux de gain génétique et le taux futur de consanguinité.

Mots-clés: *taille effective de la population, généalogie, diversité génétique, consanguinité, contribution optimale*

Resumen

Se llevó a cabo un análisis genealógico con datos de 18 315 animales nacidos entre 1949 y 2008 con el fin de cuantificar la variabilidad genética de la población Sahiwal en Kenya. Los intervalos generacionales por la vía paterna fueron mayores que por la vía materna y aumentaron con el paso del tiempo, desde aproximadamente 4 a 16 años. Esto último se debió al uso de machos viejos para la cría en las dos últimas franjas de años y al cese del testaje de la progenie en el año 2000. El nivel medio de endogamia en el último periodo de tiempo estudiado fue del 1.2 por ciento. La variabilidad genética de la población, determinada en base a estadísticas del origen de los genes, disminuyó a lo largo de los años. El ratio entre el número efectivo de fundadores y los fundadores fue de 0.06, lo cual muestra una contribución desigual de los fundadores a la población de referencia. Sin embargo, desde la población fundadora, los ancestros contribuyeron equitativamente, tal como refleja el ratio f_e/f_a de 0.94, que también podría deberse a una falta de selección eficaz en esta población. El ratio f_g/f_a de 0.63 indicó una pérdida de variabilidad genética ocurrida por deriva genética en la población Sahiwal keniana. El pequeño número de ancestros (16) responsable del 50 por ciento de la variación total en la población de referencia hace pensar en un uso excesivo de un reducido número de animales como progenitores a lo largo de varias generaciones. También el

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hecho de que el ratio f_g/f_e sea menor que el ratio f_a/f_e confirma la pérdida de variabilidad genética en la población por deriva genética más que por cuellos de botella. En consecuencia, la estrategia reproductiva para la población Sahiwal en Kenya debería incorporar herramientas que equilibren la tasa de ganancia genética y la tasa futura de endogamia.

Palabras clave: *tamaño efectivo de población, genealogía, diversidad genética, endogamia, contribución óptima*

Submitted 19 August 2015; accepted 21 July 2016

Introduction

The Sahiwal was first introduced into Kenya in the first half of the twentieth century to improve the performance of the local Zebu cattle for milk production and growth. The original population was composed of 60 bulls and 20 cows, which were put in Livestock Improvement Centres (LICs) in different parts of the country following the recommendation of Meyn and Wilkins (1974). Since then breeding in this herd has been a closed nucleus with external germplasm in form of semen from Pakistan being introduced in 1992. In 1963, after some basic performance evaluations, superior individuals were selected and used to start a breeding herd in Naivasha, Kenya, referred to as the national Sahiwal Stud (NSS). Since then the Sahiwal has been bred under a closed nucleus.

Closed nucleus breeding programmes are popular in developing countries because they are cheaper to implement and run in terms of logistics, financing and expertise required (Kahi, Nitter and Gall, 2004). The nucleus is usually composed of high performing animals (Schierenbeck *et al.*, 2011), recording of performance traits is thorough. Best Linear Unbiased Prediction (BLUP) based genetic evaluations and reproductive technologies such as artificial insemination (AI) lead to faster genetic gains by increasing the selection intensity due to use of only a few superior individuals in an entire population. Availability and use of semen from these superior individuals can lead to reduction of genetic variability due to increasing inbreeding levels in livestock herds (Weigel and Lin, 2002). This is more likely to occur in closed nucleus breeding programmes, precipitating erosion of genetic diversity.

Inbreeding level of the Kenyan Sahiwal breed is reported to be above 2 percent and increasing in recent years (Muasya, Kariuki and Muia, 2011; Kamiti, 2014). Effective population size (Ne) was reported to be between 102 and 247 based on complete pedigree information (Muasya, Kariuki and Muia, 2011; Kamiti, 2014). Whereas the Ne is within recommended levels required for a population to maintain its evolutionary potential (FAO, 1998), the estimates are below the threshold of 500 for any breeding population to maintain its genetic variability in the long term (Franklin and Frankham, 1998). If unchecked, continued reduction in genetic variability will lead to unfavourable effects such as inbreeding depression, emergence of lethal recessive alleles when in homozygous form and increased variance of genetic progress due to chance (Falconer and Mackay, 1996).

Measures of inbreeding and effective population size are useful for long term management of genetic variability and can be used to monitor its trends in cattle breeding programmes. However, these parameters can be over- or underestimated depending on pedigree completeness (Muasya, Peters and Kahi, 2013; Faria, Madalena and Josahkian, 2009). Alternative measures of genetic variability which are less sensitive to pedigree completeness are gene origin statistics (Boichard, Maignel and Verrier, 1997). These parameters are therefore more informative than Ne in describing short-term effects on genetic variability of populations (Boichard, Maignel and Verrier, 1997). They provide a better understanding of a breed's history and inform future actions in order to achieve greater genetic gains without loss in genetic diversity Carneiro *et al.* (2006) by monitoring and controlling rates of inbreeding (Fernandez *et al.*, 2011). Loss of genetic diversity can occur due to heavy use of some AI sires (Muasya, Peters and Kahi, 2013; Hammami *et al.*, 2007), periodic reductions in population size, unequal contributions of ancestors and changes in the number of contributing males (Boichard, Maignel and Verrier, 1997). This has been shown to contribute to increase of family variance (Faria, Madalena and Josahkian, 2009), and make it difficult to interpret estimates of inbreeding and Ne (Boichard, Maignel and Verrier, 1997). The objective of this study was to assess the genetic variability Sahiwal cattle in Kenya through pedigree analysis.

Material and methods

Materials

Data on pedigree of the Sahiwal cattle in Kenya were obtained from animals born in the National Sahiwal Stud at Kenya Agricultural and Livestock Research Organisation (KALRO) Naivasha and consisted of animals born between 1949 and 2008. Information on these animals included dates of birth and sex of each animal. The genealogy of each animal was traced individually as far back as possible in the birth record book database in order to include all known ancestors and relatives of each individual leading to the creation of a database of 18 315 animals.

Methods

Generation intervals

Generation intervals, or the age of parents when their progeny are born (Falconer and Mackay, 1996) were calculated for the sire–son, sire–daughter, dam–son and dam–daughter pathways.

Pedigree completeness

Parameters estimated to quantify pedigree quality included pedigree completeness index, number of maximum traced, number of complete generations and number complete generation equivalent. The parameters were estimated as follows:

Number of maximum traced generations

Number of generations was calculated as the number of generations separating the offspring from its furthest known ancestor in each path. Ancestors with unknown parents were considered as founders and assigned to generation 0.

Number of complete generations

Number of equivalent generations is the number of generations, n separating the individual from the furthest generation where both generation ancestors of the individual are known. This was computed as:

$$\mathrm{GE} = \sum_{i=1}^{n} \left(\frac{1}{2}\right)^{n},$$

where n is the number of generations separating the individual to each known ancestor, where both generation ancestors are known.

Number of complete generations equivalents

Complete generation equivalent was computed as the farthest generation for which all ancestors are known. For an individual j, the number of complete generation equivalents, CGE, was calculated as:

$$\mathrm{CGE} = \sum_{i-1}^{n_j} \frac{1}{2^{g^{ij}}},$$

where n_j is the number of ancestors for animal j; g_{ij} is the number of generations separating individual j and its ancestor i (Sölkner, Filipcic and Hampshire, 1998).

Increase in inbreeding per generation

Increase in inbreeding per generation was also calculated based on regression (b) of individual increase in inbreeding over equivalent generations as:

$$\Delta F = \frac{F_t - F_{t-1}}{1 - F_{t-1}},$$

where F_t and F_{t-1} are the average inbreeding of the ith generation.

Individual increase in inbreeding

Individual increase in inbreeding ΔF_i was calculated as (Gutierrez, Cervantes and Goyache, 2009):

$$\Delta F_i = 1 - \sqrt[\mathrm{CGE}-1]{1 - F_i},$$

where F_i is the individual coefficient of inbreeding and CGE is the complete generation equivalent (Maignel, Boichard and Verrier, 1996).

Effective population size

Effective population size, Ne is defined as the number of breeding animals expected to cause the actual increase in inbreeding if they contributed equally to the next generation (Falconer and Mackay, 1996). Effective population size, Ne for each generation was calculated based on ΔF as:

$$N_{\mathrm{e}} = \frac{1}{2\Delta F},$$

as long as $F_t > F_{t-1}$ to allow for characterise the effect of remote and close inbreeding. For small populations and or with shallow pedigrees, Ne is usually overestimated (Goyache *et al*., 2003). Additional values of Ne were estimated as the coefficient of regressing individual inbreeding coefficient on number of (i) full generations, (ii) maximum and equivalent generations traced and (iii) complete generation equivalents. The corresponding coefficient of regression represented the increase in inbreeding between any two consecutive generations ($F_t - F_{t-1} = \mathrm{b}$), such that $N_{\mathrm{e}} = 1/2b$. Estimates of Ne calculated using full, maximum and equivalent generation are useful informing lower, upper and real limits of Ne for the population under study.

Probability of gene origin statistics

Number of founders (N_{f}) was computed as all individuals in the population with unknown parents and were non-inbred. Also any individual with either parent unknown, that parent was taken as a founder.

Effective number of founders (f_{e}), defined as the number of equally contributing founders expected to produce the same diversity as observed in the reference population (Lacy, 1989) was estimated as:

$$f_{\mathrm{e}} = \frac{1}{\sum_{k=1}^{f} q_k^2},$$

where q_k is the probability of a gene origin of the kth founder.

Effective number of ancestors (f_{a}) is defined as the minimum number of ancestors required to explain the complete genetic diversity in a population (Boichard, Maignel and Verrier, 1997). This parameter was

computed as:

$$f_a = \frac{1}{\sum_j^a q_j^2},$$

where q_j is the marginal contribution of an ancestor j, which is not explained by other ancestors chosen before it. Effective number of ancestors accounts for recent bottlenecks occurring in a population and therefore partially accounts for loss of allelic diversity in descendant population (Boichard, Maignel and Verrier, 1997).

Effective number of founder genomes (f_g) is the number of founders that would give the same level of genetic variability in the population under study with equal representation of founders and no loss of alleles (Ballou and Lacy, 1995; Lacy, 1989, 1995). This parameter was computed as twice the average coancestry of the individuals in the reference population.

Mean number of progeny per sire, number of generations, inbreeding coefficient, average relatedness (AR) coefficient, individual increase in inbreeding and average offspring size and probability of gene origin in the Kenyan Sahiwal population were computed for 5 10-year groups as follows: 1(1961–1969); 2(1970–1979); 3(1980–1989); 4(1990–1999) and 5(2000–2008). All parameters were calculated using ENDOG software (Gutiérrez and Goyache, 2005).

Results

Registered sires and the average progeny per sire and their respective standard deviations are given in Table 1. Average number of progeny per sire and the respective standard deviation decreased over the years by about 50 and 60 percent, respectively, from the first to the last year period (Table 1) partly due to a decline in population size over the years (Table 4). The coefficient of variation was well over 100 percent (119 to 136 percent). Generation intervals for sire pathways were longer than dam pathways and increased over year periods, and ranged from about 4–16 years (Table 2). The long generation interval along the sire pathway was due to use of old sires because progeny testing at the National Sahiwal Stud had ceased since the year 2000. Since breeding at the National Sahiwal Stud in Kenya solely uses AI, only old progeny tested bulls had their semen available. Also as a policy of the stud, progeny tested bulls were mated to elite usually old cows to produce young bulls for progeny testing Muhuyi, Lokwaleput and Sinkeet (1999), partly explaining why the generation intervals were increasing over the year groups.

Individual inbreeding coefficient, individual AR coefficient, individual increase in inbreeding and average offspring size are given in Table 3. Individual inbreeding level and AR among individuals in the population increased over time from 0.09 and 0.7 percent to 1.2 and 2.1 percent, respectively. Values of effective population size computed by regressing individual inbreeding coefficient over number of full generations, maximum number of generations traced and complete equivalent generations were 102.4, 265.1 and 125.2, respectively.

Table 1. Mean number of progeny per sire and standard deviation in the five year periods in the Sahiwal cattle in Kenya.

Period		Number of progeny per sire	
	Number of sires	Mean	Standard deviation
1	105	34.2	46.4
2	144	32.4	43.3
3	153	26.8	32.0
4	107	22.9	30.4
5	72	20.6	23.3

Table 2. Generation intervals of the four gametic pathways in the 5-year periods the for the Kenyan Sahiwal population.

Period	Gametic pathway			
	Males		Females	
	Sire	Dam	Sire	Dam
1	4.1	5.9	4.9	4.6
2	9.5	7.7	7.5	5.9
3	10.1	8.2	8.4	6.4
4	16.2	11.8	8.7	7.5
5	15.7	14.7	12.1	7.4

Probability of gene origin statistics, their ratios and number of ancestors explaining 50 percent of total variations in each year period are shown in Table 4. Number of founders, effective numbers of founders, ancestors and founder genomes decreased over the years but increased slightly in the last year group, indicating that loss in genetic variability occurred in the Sahiwal breed. The increase in the last year group could be due to the use of old sires due to cessation of progeny testing at the NSS because of logistical reasons. The effective numbers of founders were much smaller compared with the number of founders. The marginal genetic contribution of the most important ancestor increased, while the number of ancestors required for explaining half of the total variation observed in the Kenyan Sahiwal breed decreased over time.

Discussion

Generation intervals for the sire pathways were very long compared with other European breeds, even though zebu cattle generally have delayed age at first calving. This was partly due to use of old sires, born before the year 2000 following the cessation of progeny testing at the NSS. Apart from lengthening generation intervals in the

Table 3. Number of generations, inbreeding coefficient, average relatedness (AR) coefficient, individual increase in inbreeding and average offspring size for the Kenyan Sahiwal population.

Year period	Average inbreeding coefficient (%)	AR (%)	Individual increase in inbreeding (%)	Offspring	Effective population size	
					Ne^1	Ne^2
1	0.09	0.72	0.05	2.97	1 088.3	199.3
2	0.25	1.77	0.1	2.09	484.8	150.6
3	0.59	2.19	0.18	1.47	271.0	118.5
4	1.15	2.36	0.28	1.18	175.9	72.0
5	1.19	2.07	0.27	0.23	186	70.3
Overall	0.52	1.72	0.15	1.84	–	–

Ne^1 and Ne^2 represent effective population size computed through regression on individual increase in inbreeding and regression on complete equivalent generations, respectively.

Table 4. Characteristics based on probability of gene origin in the Kenyan Sahiwal population.

Year group	*N*	Founders	Parameter						
			Effective number of founders	Effective number of ancestors	Effective number of founder genomes	Ancestors explaining 50% of genetic diversity	$f_a/_{Nf}$	f_e/f_a	f_g/f_a
1	4 583	986	118	102	78	41	0.12	0.86	0.76
2	4 802	705	51	51	47	19	0.07	1.00	0.92
3	4 366	637	42	41	33	14	0.07	0.98	0.80
4	2 602	481	40	38	26	14	0.08	0.95	0.68
5	1 962	781	49	46	29	16	0.06	0.94	0.63
Overall	18 315	1 087	59	59	58	22	0.05	1.00	0.98

sire pathways, use of few old sires led would lead to reduction in genetic variation and limited or no genetic progress. This was confirmed by Ilatsia *et al.* (2007) who reported lack of genetic progress in milk production and fertility traits for the Kenyan Sahiwal. Other studies which reported long generation intervals for the sire pathways include Faria, Madalena and Josahkian (2009) and Filho *et al.* (2010) for Brazilian zebu breeds. However, in experimental herds of some Zebu breeds, shorter generation intervals of 3.7 years in both pathways have been reported (Razook *et al.*, 1993). Generation equivalents of the Sahiwal population in the current study were lower than those reported for Brazilian Zebu breeds (Faria, Madalena and Josahkian, 2009). Elsewhere, Italian beef breeds were reported to have generation equivalents of about 5 (Bozzi *et al.*, 2006).

Inbreeding coefficient of the Sahiwal cattle breed was about 1.2 percent in the last year period studied with a general increase over the years. This could be attributed to large-scale utilization of few superior sires and use of old bulls from before the year 2000 due to cessation of progeny testing, which also led to an increase in the generation interval and the family size variance (Falconer and Mackay, 1996) and limited effective population size. In the current study, effective population size decreased as inbreeding level increased across the year periods studied (Table 3). A similar trend was reported among the Gyr, Nerole and Guzerat Zebu breed in Brazil (Faria, Madalena and Josahkian, 2009). Average inbreeding coefficients of 0.5 and 1.2 percent for the entire population and the reference population, respectively were lower than corresponding values of 7.8 and 10.8 percent for the Lidia breed in Spain (Cortés *et al.*, 2014), 4.81 percent for Burlina breed in Italy (Battagin *et al.*, 2010) and 2.82 percent for Gyr dairy cattle in Brazil (Filho *et al.*, 2010). Even higher inbreeding coefficients have been reported in other cattle breeds (Danchin-Burge *et al.*, 2012; Bouquet *et al.*, 2011; McParland *et al.*, 2007).

Estimates of inbreeding levels and effective population size are closely related to the quality of pedigree (Gutiérrez *et al.*, 2003; Muasya, Peters and Kahi, 2013). Further evidence is provided by the values of 102.4, 125.2 and 265.1, obtained based on complete, full and complete equivalent generations. According to Goyache *et al.* (2003) these estimates are useful for providing lower, real and upper limits of Ne, respectively, for the population under study since for small populations and or with shallow pedigrees, Ne is usually overestimated. In other Zebu breeds, Ne estimates ranging from 124 in polled Nelore breeds to nine in Sindi breed have been reported (Faria *et al.*, 2002). Verneque *et al.* (2006) reported Ne ranging from 16 to 125 for the Dairy Gir, with more recent generations being more inbred, similar to the present study. Muasya, Peters and Kahi (2013) found Ne ranging from 454 to 263 in Kenyan Holstein–Friesian. Cortés *et al.* (2014) reported an Ne of 37.5 for

the Lidia breed of Spain. The decline in Ne can be attributed to increase in inbreeding coefficient in the population due intense use of few superior sires (Nomura, Honda and Mukai, 2001).

Management of genetic variation in selection programmes is important for conservation purposes and short and long-term selection responses (Hill, 2000). Effective population size is an important parameter to monitor when managing genetic diversity in breeding programmes because it reduces genetic variability and depresses performance. An effective population size of 31–250 has been recommended to prevent decline in fitness of a population due to inbreeding depression (FAO, 1998; Meuwissen and Woolliams, 1994) and to maintain mid-term genetic variability. This can be achieved by ensuring that the rate of inbreeding per generation does not exceed 1 percent beyond which a population begins to lose its fitness (Franklin and Frankham, 1998). However, since in most breeding programmes the aim is to maintain genetic variability in the long-term, a Ne of at least 500 should be targeted (Franklin and Frankham, 1998). In the present study Ne in the most recent year group was within the range of 31–250 required for a population to maintain its fitness (FAO, 1998) but below the recommended levels of 500 for maintenance of genetic variability in the long term (Franklin and Frankham, 1998). Therefore strategies should be put in place to control future rates of inbreeding while achieving genetic progress for traits of economic importance in the Kenyan Sahiwal breed. Nevertheless, controlling future rates of inbreeding by constraining the degree of relatedness of mates is only effective for a few generations (Fernandez *et al.*, 2011). Incorporation of genomic selection in breeding programmes, which is capable of minimising Mendelian sampling allows for increasing effective population size (Daetwyler *et al.*, 2007) should be considered.

Effective population size is normally overestimated after a number of generations with incomplete pedigree information, becoming worse as number of generations increase. On the other hand, gene origin statistics are less sensitive to pedigree completeness and are therefore more informative than Ne for describing short term effects on genetic variability of populations (Boichard, Maignel and Verrier, 1997). In the present study, the number of founders (animals without parent information) was high compared to the total population size studied, indicating that current Ne value could be an overestimation. This is seen in the change in Ne from 265.1 to 102.4 when estimated based on full and complete generations traced, respectively.

Gene origin parameters also indicate the loss of genetic variability in the Sahiwal breed. Effective number of founders reported in the current study of 49 in the most recent year group was higher than that of 38 for the Nelore of Brazil (Faria, Madalena and Josahkian, 2009) and 27.8 for Lidia breed in Spain (Cortés *et al.*, 2014) but smaller than 284 for the Gir and 247 for Guzerat cattle breeds (Faria, Madalena and Josahkian, 2009). The effective number of ancestors of 46 found in the current study was lower than 211 and 166 for Gir and Guzerat Zebu breeds (Faria, Madalena and Josahkian, 2009), but higher than 15.8 for the Lidia breed in Spain (Cortés *et al.*, 2014). Other studies which have reported lower estimates include Sorensen, Sorensen and Berg (2005) which found values of 20.6, 23.8 and 34.6, respectively, for Danish Holstein, Jersey and Red breed populations and Bouquet *et al.* (2011), Danchin-Burge *et al.* (2012) and Melka *et al.* (2013). Effective number of founder genomes of 29 was higher than 12.3 reported for the Lidia breed of Spain (Cortés *et al.*, 2014).

Ratios of parameters of gene origin provide information on the historical development of a population since the founder population (Boichard, Maignel and Verrier, 1997). The ratio of f_e/N_f describes whether the founders were used in a balanced manner and the extend of pedigree completeness. A low ratio means that the population has gone through bottlenecks and or indicates gaps in pedigree recording. For the most recent year group studied, the ratio of 0.06 was larger than 0.0004 for Nerole but close to 0.5 for both Gir and Guzerat breeds in Brazil (Faria, Madalena and Josahkian, 2009). This implies an unequal contribution of founders to the reference population. However, from these studies, the rations are somewhat dependent on population size, since the Nelore has a large number of founders (84 452) compared with the Gir (6 081) or Guzerat (4 980) and pedigree completeness such that lower values are reported for large populations with high levels of pedigree completeness.

The ratio of f_a/f_e is useful when describing the history of a population. The ratio of 0.94 reported in the current study indicates that the Kenyan Sahiwal population has been genetically stable. This ratio is close to 0.89 reported for the Nerole breed but higher than 0.74 and 0.67 for Gir and Guzerat breeds of Brazil (Faria, Madalena and Josahkian, 2009). Other lower values include 0.15 for Tunisian (Hammami *et al.*, 2007) and 0.57 for Spanish Lidia cattle breed (Cortés *et al.*, 2014). The ratio reported in the current study implies a balanced contribution of ancestors to the reference population of the Kenyan Sahiwal breed, which could be due to lack of effective selection in this population (Ilatsia *et al.*, 2007). A ratio of f_g/f_e of 0.59 found in the current study was within the range of 0.4–0.68 reported for Brazilian Zebu breeds (Faria, Madalena and Josahkian, 2009; Vozzi *et al.*, 2006) and Spanish Lidia breed (Cortés *et al.*, 2014), indicating genetic loss of genetic variability occurred through genetic drift in the Kenyan Sahiwal population.

The number of ancestors required to explain 50 percent of the observed variation in the reference population of 16 was lower than 56, 37 and 41, for Nelore, Gir and Guzerat, respectively, (Faria, Madalena and Josahkian, 2009). In the Kenyan Holstein–Friesian population a value of 89 was reported (Muasya, Peters and Kahi, 2013). For the Lidia cattle breed of Spain, six ancestors were required to explain 50 percent of the total variation

(Cortés *et al.*, 2014). The small number of ancestors that account for 50 percent of the total variation in the reference population suggests overuse of a small number of some animals as parents over generations, explaining to some extend the loss in diversity in the Sahiwal population in Kenya. Also, the smaller ratio of f_g/f_e compared with f_a/f_e, implies loss of genetic variability in the population was more by genetic drift than bottlenecks.

In Kenya, the Sahiwal is bred under a closed nucleus breeding program where performance recording and selection is confined to the nucleus, and the pastoral herds being the main recipients of the resultant genetic superiority (Ilatsia *et al.*, 2011). Nucleus breeding programmes have been advocated for genetic improvement of cattle in developing countries (Kahi, Nitter and Gall, 2004) due to their ease of implementation as recording is done in the nucleus. However, if the current inbreeding levels continue to increased unchecked and Ne declines the nucleus may experience a reduction of genetic variability and inbreeding depression for traits of economic importance as well as possibility of emergence of lethal recessive alleles in homozygous form and increased sampling variance of breeding programmes (Malhado *et al.*, 2012; Meuwissen and Sonesson, 1998). Difficulties in planning mating systems aimed at controlling future rates of inbreeding due to increased AR among individuals may also be experienced (Fernandez *et al.*, 2011). Apart from putting into place strategies to control future rates of inbreeding, new germplasm from other Sahiwal populations such as from India and Pakistan can be introduced in order to widen the population's genetic base.

Conclusions

The Kenyan Sahiwal population is derived from a small founding population and has moderate pedigree completeness. Ratios of gene origin statistics indicate that loss of genetic variability is more through genetic drift than due to inbreeding. Effective population size was low or moderate depending on the method of estimation. Future breeding strategy should aim to control future decline in effective population size. From the perspective of long-term management of genetic variability, rate of increase in coancestry is most crucial since it uses all currently available information to predict future trend in inbreeding. Using few progeny tested sires born before the year 2000 will have consequence of limited genetic progress in the NSS and reduction in genetic variation. Therefore progeny testing should be urgently resumed coupled with a breeding strategy that incorporates tools that balance rate of genetic gain and the future rate of inbreeding.

Acknowledgements

The authors are grateful to the Kenya Agriculture and Livestock Research organisation (KALRO) for availing data, the ILINOVA project for provision of funds and Egerton University, Kenya for provision of computing facilities.

Statement of interest

The authors declare that no conflict of interest, financial or otherwise, exist concerning this research work.

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Animal Genetic Resources, 2016, 59, 15–25. © Food and Agriculture Organization of the United Nations, 2016
doi:10.1017/S2078633616000084

Multivariate analyses of morphological traits in indigenous chicken populations of Metekel zone, Northwestern Ethiopia

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Summary
An exploratory survey to phenotypically characterize indigenous chicken populations was carried out in Metekel zone of Northwestern Ethiopia in April 2013. A total of 69 males and 244 females were sampled to record their qualitative and quantitative traits. Eight quantitative and 16 qualitative variables were measured. Sampling included three districts representing different agroecological zones. Coefficient of variation for quantitative variables ranged from 6.38 to 52.37 percent in male sample populations and 4.59–21.4 percent in females. The chi-square tests for plumage colour of the neck, ear lobe colour and skeletal variant type were highly significant ($\chi^2 <$ 0.05). The correct classification percentage from discriminant analysis was 93.73 and 98.41 percent for male and female sample populations, respectively, indicating the homogeneity of the chicken populations within districts. The stepwise discriminant analysis identified five variables for male and three variables for female sample populations, which had the highest discriminating power. Canonical analyses showed that differences in body measurements between indigenous chicken populations were highly significant ($P < 0.0001$). The results obtained from on-farm performance evaluation indicated that the average age at first lay of hens, number of chicks weaned and mean number of eggs laid per bird per year were 5.5 months, 6.5, 50.1, respectively. This information will constitute the basis for further characterization and development of conservation strategies for indigenous chicken populations of Northwestern Ethiopia.

Keywords: *indigenous chicken, morphological traits, multivariate analyses, Metekel, Northwestern Ethiopia*

Résumé
Dans la zone de Metekel, au Nord-Ouest de l'Éthiopie, une étude d'exploration a été menée en avril 2013 pour caractériser phénotypiquement les populations de poules indigènes. Huit caractères quantitatifs et seize caractères qualitatifs ont été mesurés sur un échantillon total de 69 mâles et 244 femelles. L'échantillonnage s'est fait sur trois districts représentant différentes zones agro-écologiques. Le coefficient de variation des paramètres quantitatifs a varié de 6,38 pour cent à 52,37 pour cent chez les mâles et de 4,59 pour cent à 21,4 pour cent chez les femelles. Les tests du khi-carré pour la couleur du plumage du cou, la couleur des oreillons et le type squelettique ont été très significatifs ($\chi^2 < 0{,}05$). Le pourcentage d'animaux correctement classés grâce à l'analyse discriminante a été de 93,73 pour cent chez les mâles et de 98,41 pour cent chez les femelles, ce qui reflète l'homogénéité des populations au sein des districts. L'analyse discriminante pas à pas a décelé que la plus grande capacité de discrimination revenait à cinq variables dans les échantillons de mâles et à trois variables dans les échantillons de femelles. Les analyses canoniques ont montré que les différences sur les mesures corporelles entre les populations de poules indigènes ont été très significatives ($P < 0{,}0001$). Les résultats obtenus sur le terrain pour les performances des poules ont indiqué que l'âge moyen à la première ponte, le nombre de poussins élevés et le nombre moyen d'œufs pondus par poule et par an ont été de 5,5 mois, 6,5 et 50,1, respectivement. Ces informations fournissent la base pour de futures caractérisations et pour l'élaboration de stratégies de conservation pour les populations de poules indigènes du Nord-Ouest de l'Éthiopie.

Mots-clés: *analyses multivariées, traits morphologiques, poules indigènes, Metekel, Nord-Ouest de l'Éthiopie*

Resumen
En abril de 2013 se llevó a cabo un estudio exploratorio en la zona de Metekel, en el Noroeste de Etiopía, para caracterizar fenotípicamente las poblaciones de gallinas autóctonas. Se midieron 8 caracteres cuantitativos y 16 cualitativos sobre una muestra total de 69 machos y 244 hembras. El muestreo incluyó tres distritos, representativos de diferentes zonas agroecológicas. El coeficiente de variación de los parámetros cuantitativos varió de 6,38 por ciento a 52,37 por ciento en las muestras de machos y de 4,59 por ciento a 21,4 por ciento en las muestras de hembras. Los tests chi-cuadrado para el color del plumaje del cuello, el color de las orejillas y la variante esquelética fueron altamente significativos ($\chi^2 < 0{,}05$). El porcentaje de animales clasificados correctamente gracias al análisis discriminante fue del 93,73 por ciento en los machos y del 98,41 por ciento en las hembras, lo cual refleja la homogeneidad de las poblaciones de gallinas dentro de los distritos. El análisis discriminante escalonado desveló que la mayor capacidad discriminatoria la presentaban cinco variables en las muestras de machos y tres variables en las muestras de hembras. Los análisis canónicos mostraron que las diferencias en las medidas corporales entre las poblaciones de gallinas autóctonas eran muy significativas ($P < 0{,}0001$). Los

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resultados obtenidos para los rendimientos en granja de las gallinas indicaron que la edad media a la primera puesta, el número de pollitos criados y el número medio de huevos puestos por ave y año fueron de 5,5 meses, 6,5 y 50,1, respectivamente. Esta información constituirá la base para posteriores caracterizaciones y para el desarrollo de estrategias de conservación de las poblaciones de gallinas autóctonas del Noroeste de Etiopía.

Palabras clave: *análisis multivariantes, rasgos morfológicos, gallinas autóctonas, Metekel, Noroeste de Etiopía*

Submitted 17 December 2014; accepted 4 April 2016

Introduction

Indigenous breeds, also termed native breeds, originate from and are adapted to and utilized in a particular geographical region, form a sub-set of the locally adapted breeds (FAO, 2001). In Ethiopia, there are about 50.4 million chickens out of which 96.9 percent are indigenous and 3.1 percent are exotics and their crosses (CSA, 2013). The indigenous chickens produce 85.5 million eggs annually (91.8 percent), while a much less amount (8.2 percent) is contributed to the national egg production by improved strains of chickens largely kept in intensive poultry production systems (CSA, 2013). There are no defined breeds as such and they are referred to as 'ecotypes' or 'populations' and some have been a subject of morphological and genetic characterization studies (Forsido, 1986; Dessie, 2003; Duguma, 2006; Hassen, 2007; Kibret, 2008; Dana *et al.*, 2010; Moges, Melesse and Dessie, 2010; Melesse and Negesse, 2011; Aklilu, 2013; Desta *et al.*, 2013; Nigussie, 2013; Getu, Alemayehu and Wultaw, 2014). Only ten Ethiopian chicken types, namely Chefe, Gebsima, Horro, Jarso, Kei, Naked neck, Netch, Tepi, Tikur and Tilili have been listed in the Domestic Animal Genetic Resources Information System (DAGRIS) database (DAGRIS, 2007) and a large part of the indigenous chicken genetic diversity still remains non-descript.

Quantitative and qualitative morphological traits are important in the characterization and measurement of indigenous chicken genetic diversity. Metekel zone of Benishangul Gumuz is among areas of Ethiopia where no extensive introduction of exotic chicken germplasm has taken place. Preference for particular chicken phenotypes can be strongly influenced by the culture of the people and specific needs to their unique environments. In this regard, Metekel zone is considered as one of the most ethnically and agro-ecologically diverse areas. The five largest ethnic groups reported in the Zone were the Gumuz (36.78 percent), the Shinasha (21.6 percent), the Amhara (17.39 percent), the Awi (11.33 percent) and the Oromo (11.09 percent) while all other ethnic groups made up 1.81 percent of the population (CSA, 2007).

Except for Dana *et al.* (2010) who studied indigenous chicken populations in Mandura district, populations in other districts of Metekel zone in Northwestern Ethiopia have never been investigated. The present study was therefore carried out to study variations among indigenous chicken populations in three districts of the zone in terms of morphological and productive performance as a basis for the design of future genetic intervention programmes.

Materials and methods

Description of the study area

There are nine geopolitical boundaries in the Federal Democratic Republic of Ethiopia, which are referred to us Regional States or Regions. This study was conducted in Guba, Dibate and Wombera districts of Metekel zone, in Benishangul-Gumuz Region, in Northwestern Ethiopia (Figure 1). Metekel zone, one of the three zones in the Region, consists of six districts and is situated within an altitude range of 550–2500 m above sea level. Metekel is the largest zone with an area of 26 272 km^2. The average monthly temperature ranges between 20 and 25 °C. During the hottest months (January–May), it reaches between 28 and 34 °C. The amount of annual rainfall varies from 500 to 1800 (MZARDO, 2007). Map of Benishangul Gumuz Region with the three sample districts in Metekel zone of Northwestern Ethiopia is presented in Figure 1.

The agricultural production system in Metekel zone varies with agroecology. Wombera district, which has the highest altitude not only in the zone, but also in the Region, is characterized by mixed crop–livestock production system. The agricultural production system in the mid-altitude and especially lowland districts on the other hand is based on diversified livelihood strategies that include mixed crop–livestock production, hunting and gathering, and traditional gold mining. Chickens are kept as part of a village chicken production system where they subsist on scavenging and mate uncontrolledly. While ploughing with a pair of oxen is a technology being recently introduced to some of the indigenous communities, considerable area of land in the zone is currently being used by private companies for cotton, sesame and other lowland oilseeds production.

Sample size and sampling method

The sampling frame was established following an exploratory survey to all the six districts of the zone and discussions with local livestock extension officers and researchers. Districts were then clustered purposively taking into account of agroecological variability, poultry

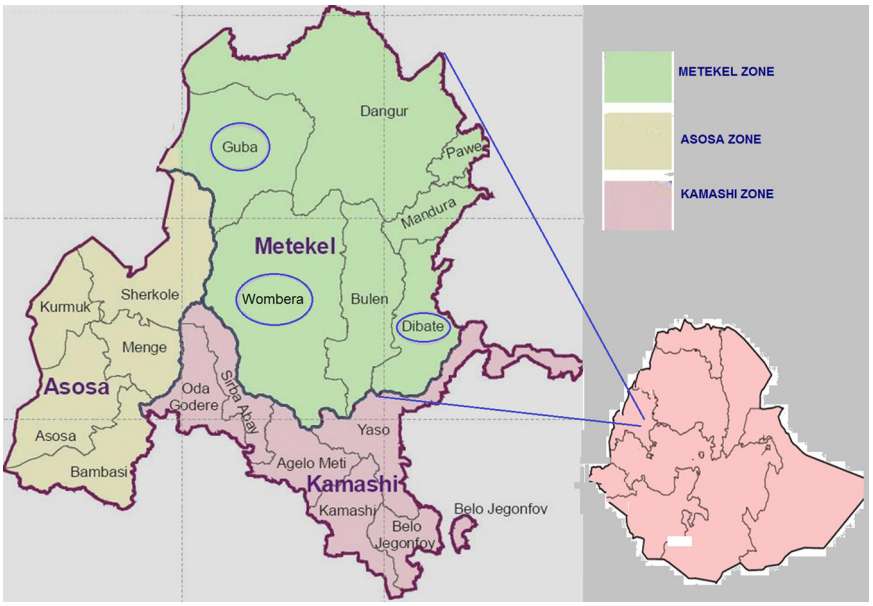

Fig. 1 - Colour online

Figure 1. Map of Metekel zone indicating the study area (adapted from GERDP, 2012).

market-sheds, and the presence of native communities who have been keeping chicken in the area for many generations. Agroecology was considered by the researchers to have implications on indigenous chicken genetic diversity through adaptive divergence, while market-shed was assumed as a major factor for the mix-up of different chicken populations in a village production setting. Selection of districts from a cluster was done randomly. The cluster of lowland districts in the zone was represented by chickens sampled from Guba district, while the mid-altitude and highland clusters were represented by Dibate and Wombera districts, respectively. Villages in each district were located within the same market-shed and were homogenous in terms of agroecology. Hence, it sufficed to sample two villages from each of the three districts on random. Households and villages were sampled through a transect walk. A maximum of three hens and two cocks were sampled from a household. The data were collected in April 2013 and the target number for sample chickens for a study district was 100–300 hens and 10–30 cocks (FAO, 2012). Measurements were taken from males aged at least 1 year and females that have already started laying based on information obtained on each chicken from the owner.

All quantitative and qualitative variables in the standard chicken descriptor list of FAO (2012) were measured in this study. A total of 69 males and 244 females were sampled to record their qualitative and quantitative traits (Table 1). Eight quantitative and 16 qualitative characters were measured. Measurements were made using textile tape in centimetre unit. A hanging spring balance was used to measure live body weight of individual chickens. Information on flock performance of indigenous chickens was collected with the help of structured questionnaires addressed to 75 farmers.

Data management and statistical analysis

All data were entered, and edited using Excel© worksheet (Microsoft Office, 2013). Qualitative data were subjected to chi-square (χ^2) tests. The Statistical Package for Social Sciences (SPSS 20.0, 2011) was used to estimate their frequency and level of association with district. Non-parametric discriminant analysis was also run on qualitative variables to confirm their importance in classifying the populations into phenotypically distinct groups. The general linear model (PROC GLM) of SAS (2003) was employed to examine the importance of quantitative variables in

Table 1. Description of the study districts and number of chickens sampled.

District[1]/district	Altitude (m asl)	Latitude	Longitude	Agroecology	Chicken[2] population	Chicken[2] density/km^2	Cocks	Hens
Dibate	1414–1531	10°46′	36°15′	Tepid to cool sub-humid, mid-altitude	28 611	18.19	21	81
Wombera	2480–2565	10°37′	35°39′	Tepid to cool moist, highland	31 605	5.81	24	81
Guba	575–879	11°16′	35°17′	Hot to warm moist, lowland	7 216	2.80	24	82
Total							69	244

[1]District is an administrative domain at the third level down a Region and immediately below a zone.
[2]Bureau of Agriculture, Benishangul-Gumuz National Regional State, 2003.

explaining phenotypic differences between sample chicken populations of the three districts. Duncan's multiple range test was performed on district means of body measurement traits. Stepwise discriminant procedure was applied using PROC STEPDISC to determine which morphological traits have more discriminant power than the others. The CANDISC procedure (SAS, 2003) was used to perform canonical analysis to derive canonical functions, linear combinations of the quantitative variables that summarize variation between districts, and compute Mahalanobis distance matrix. The percent assignment of chicken populations into respective districts was made by using DISCRIM procedure. PLOT procedure was used in conjunction with the CANDISC procedure to aid visual interpretation of group differences between districts.

Quantitative data were analysed separately for the two sexes. Taking district and sex as fixed main effects, the following model was used to analyse quantitative data:

$$Y_{ijk} = \mu + S_i + D_j + e_{ijk},$$

where $Y_{ij\ k}$ is the observed value of the linear body measurements, S_i is the fixed effect of district i ($i = 1, 2, 3$), D_j is the effect of the jth sex ($j = 1, 2$), and e_{ijk} is the residual error. Interaction effect of the ith district with the jth sex was not statistically significant and was dropped out from the final model. Discriminant analysis model used to derive classification function for the female and male sample populations is shown below:

$$Si = C_i + w_i WST + w_i WSU + w_i BL + w_i CC + w_i SL + w_i SC + w_i SPL + w_i BW,$$

where subscript 'i' denotes the respective group, C_i is a constant for the $_i$th group, w_i is the weight of the corresponding variable in the computation of the classification score for the ith group, S_i is the resultant classification score, WST is wing span at the top, WSU is wing span under, BL is body length, CC is chest circumference, SL is shank length, SC is shank circumference, SPL is spur length, and BW is body weight.

Flock performance data were also subjected to analysis-of-variance (ANOVA) procedure to identify sources of variance for reproductive and productive traits in hens and cocks (SPSS 20.0, 2011).

Results and discussion

Univariate analyses

Quantitative variation

All quantitative variables except spur length (which was mainly absent in female) were highly significantly ($P < 0.0001$) affected by sex of the animal (Table 2) and the ANOVA on quantitative variables was performed separately for the two sexes.

Table 2. Least-square means ± SE of quantitative body measurements (cm) for all districts by sex.

Dependant variable	Male ($N = 69$)	Female ($N = 244$)	Sex
Wing span top	46.46 ± 0.36	39.57 ± 0.19	$P < 0.0001$
Wing span under	48.59 ± 0.37	41.37 ± 0.20	$P < 0.0001$
Body length	42.40 ± 0.31	37.65 ± 0.17	$P < 0.0001$
Chest circumference	28.72 ± 0.30	25.64 ± 0.16	$P < 0.0001$
Shank length	10.59 ± 0.12	8.36 ± 0.06	$P < 0.0001$
Shank circumference	4.25 ± 0.06	3.35 ± 0.03	$P < 0.0001$
Spur length	1.75 ± 0.13	1.00 ± 0.41	$P < 0.0879$
Body weight (kg)	1.93 ± 0.05	1.36 ± 0.02	$P < 0.0001$

Quantitative variation in male sample populations

Phenotypic variation of all quantitative traits in males excluding spur length was highly significantly affected by district (Table 3). The highest coefficient of determination (R^2) was calculated for body weight showing that only 40 percent of the variability for this trait is explained by the model. The smallest (18 percent) coefficient of determination for shank circumference indicates that 82 percent of the variability in this trait is accounted for by other variables. Coefficient of variation ranged from 6.38 to 52.37 percent showing high heterogeneity in male sample populations.

Pairwise comparisons of the means of variables between districts (Table 3) revealed that male sample populations from Wombera had the largest measurement values for all variables followed by chicken populations from Dibate. This indicates that the chicken populations sampled from the highland and mid-altitude areas were larger in their body weights and linear measurements than those sampled from the lowlands.

The importance of shank length in estimating live body weight was explained by (Dessie, 2003) who found positive phenotypic correlations ($r_p = 0.64$–0.79) between the two traits. Shank length of males from Wombera (11.58 cm) was higher than cocks from Horro (11.32 cm) and Jarso (9.9 cm) (Aklilu *et al.*, 2014); 9.8 cm from Fogera (Kibret, 2008); 10.31 cm from Northwestern Ethiopia (Hassen, 2007). The average live body weight for cocks at Wombera (2.29 kg) was higher than the reported values for Horro (1.63 kg) and Jarso (1.45 kg) by Desta *et al.* (2013) from Western and Eastern Ethiopia; from the central highlands (1.5 kg) by Yami and Dessie (1997); from Amhara Region of Ethiopia (2.05 kg) by Hassen (2007). The body weight of sampled chickens from Dibate (1.83 kg) was also higher than reported weight of 1.62 kg by Dana *et al.* (2010) and 1.43 kg by (Melesse and Negesse, 2011).

Quantitative variation in female sample populations

Similar to the male sample populations, the ANOVA showed that district had highly significant ($P < 0.0001$) effect on the total variation of all quantitative traits except shank length ($P < 0.01$) for female sample populations (Table 4). R^2 values ranged from 18 percent for spur length

Table 3. Means of linear body measurements (cm), body weight (kg) and associated R^2 values for the male sample population by district.

Dependent variable	Mean value	Dibate	Wombera	Guba	R^2	CV
Wing span top	46.43	45.92[b]	49.08[a]	44.00[b]	0.29	7.23
Wing span under	48.57	47.63[b]	51.71[a]	46.05[b]	0.35	6.80
Body length	42.62	41.75[b]	45.42[a]	40.30[c]	0.39	6.38
Chest circumference	28.94	26.52[c]	31.00[a]	26.52[c]	0.39	7.99
Shank length	10.67	9.96[b]	11.58[a]	9.96[b]	0.31	9.92
Shank circumference	4.26	4.17[b]	4.58[a]	3.98[b]	0.18	12.82
Spur length	1.76	1.36	2.10	1.71	0.20	52.37
Body weight (kg)	1.93	1.83[b]	2.29[a]	1.57[c]	0.40	21.07

[a,b,c]Means with different superscripts within the same row are significantly ($P < 0.05$) different.

to 98 percent for body weight. Only 2 percent of the variability in body weight is accounted for by other variables and the model accounts for 98 percent of the variability for this trait. Small R^2 value for spur length indicates that the model fits the data poorly. Coefficient of variation ranged from 4.59 percent for body length to 21.4 percent for spur length showing larger variability in the latter trait.

Pairwise comparisons between districts showed significant ($P < 0.05$) differences for all quantitative traits. Like male sample populations, female sample populations from Wombera highlands had the largest measurement. Shank length for the hens from the three districts (8 cm for Dibate, 8.68 cm for Wombera and 8.09 cm for Guba) were comparable with reported values for Horro (9.22 cm) and Jarso (8.51 cm) (Aklilu *et al.*, 2014) but slightly higher than that of 7.25 cm reported by Kibret (2008). The average live weight of local adult hens in Dibate and Wombera were 1.36 and 1.55 kg, respectively, which was higher than reported values for 1.24 kg for Horro and 1.16 kg for Jarso (Desta *et al.*, 2013); 1.04 kg for central highlands of Ethiopia (Yami and Dessie, 1997); and 0.85 kg for Amhara region of Ethiopia (Hassen, 2007). Higher live body weights for hens were reported from Uganda (1.4 kg) (Ssewannyana *et al.*, 2008) and for Punjab brown chickens from India (1.57 kg) (Vij, Tantia and Vijh, 2006). Live body weights are affected by genetic and environmental factors. The higher body weights recorded for Wombera chickens could be linked with better feed resources in the area and genetic introgression from improved (exotic) chicken breeds, which were present in a relatively higher number in the highlands.

Qualitative variation

The χ^2 tests for plumage colour of the neck, ear lobe colour and skeletal variant of the feet were highly significant compared with those for feather distribution, plumage colour of body, plumage colour of tail, comb type and shank colour (Table 5). All the rest were not significant ($P > 0.05$). The least association of qualitative variable with district was observed for spur presence. The highest was for plumage colour of neck (0.48) using Phi coefficient, for ear lobe colour (0.41) using Cramer's V and for plumage colour of neck (0.43) using Contingency coefficient.

Feather distribution

Normal and crested feathers (Figure 2a–h) were most common in all the three districts. In Dibate 47 crested chickens (44.8 percent), in Wombera 46 (43.4 percent) and in Guba 59 (57.8 percent) were sampled. The proportion of crested feather in this study is larger than the one reported by Desta *et al.* (2013) in Horro (27.9 percent) and Jarso (4.1 percent) districts; Egahi *et al.* (2010), who reported 17.05 percent for Nigerian chicken; Ssewannyana *et al.*, who reported 12 percent for Ugandan chicken; Dana *et al.* (2010), who reported 34 percent for five chicken populations of Ethiopia; and Negassa, Melesse and Banerjee (2014) who reported 27.8 for hens sampled from Southeastern Oromia region of Ethiopia; but slightly

Table 4. Means of linear body measurements (cm), body weight (kg) and associated R^2 values for the female sample population.

Dependent variable	Mean value	Dibate	Wombera	Guba	R^2	CV
Wing span top	39.67	40.0[b]	40.80[a]	37.84[c]	0.25	5.18
Wing span under	41.41	41.57[b]	43.02[a]	39.49[c]	0.26	5.67
Body length	37.64	37.79[b]	38.89[a]	36.25[c]	0.26	4.59
Chest circumference	25.67	26.05[b]	26.61[a]	24.26[c]	0.28	6.26
Shank length	8.43	8.30[b]	8.68[a]	8.09[b]	0.10	7.96
Shank circumference	3.36	3.36[b]	3.57[a]	3.13[c]	0.19	11.11
Spur length	1.36	1.37[b]	1.52[a]	1.19[c]	0.18	21.40
Body weight (kg)	1.36	1.36[b]	1.55[a]	1.16[c]	0.98	5.57

[a,b,c]Means with different superscripts within the same row are significantly ($P < 0.05$) different.

Table 5. Chi-square tests and levels of association of districts with the qualitative variables.

Variable	*P*-value	Phi coefficient	Cramer's V	Contingency coefficient
Feather morphology	$P = 0.073$	0.165	0.163	0.117
Feather distribution	$P < 0.002$	0.359	0.338	0.254
Plumage pattern of neck	$P = 0.088$	0.256	0.248	0.181
Plumage pattern of body	$P = 0.231$	0.220	0.215	0.156
Plumage pattern of tail	$P = 0.392$	0.201	0.197	0.142
Plumage colour of neck	$P < 0.0001$	0.480	0.339	0.432
Plumage colour of body	$P < 0.005$	0.383	0.271	0.357
Plumage colour of tail	$P < 0.001$	0.422	0.389	0.298
Head shape	$P = 0.030$	0.150	0.148	0.150
Comb type	$P < 0.001$	0.331	0.314	0.234
Comb size	$P = 0.191$	0.144	0.143	0.102
Ear lobe colour	$P < 0.0001$	0.443	0.405	0.313
Shank colour	$P < 0.001$	0.242	0.235	0.171
Spur presence	$P < 0.570$	0.060	0.060	0.060
Eye colour	$P = 0.197$	0.225	0.220	0.159
Skeletal variant type	$P < 0.0001$	0.314	0.300	0.222

smaller than Hassen (2007) who reported 48.8 percent in Amhara region of Northwest Ethiopia.

Plumage colour of neck and body

The village chickens displayed high diversity in plumage colour of the neck and the body, which varied significantly at ($\chi^2 < 0.05$) across districts (Table 6). Most of the chickens had brown plumage colour on the neck (48.6 percent) and on the body (18.2 percent). Dana *et al.* (2010) also found out brown as the predominant (19 percent) body plumage colour for five chicken populations of Ethiopia. Black and dark brown colours were the second prevalent plumage colours on the body (each 12.1 percent).

Comb type

Seven types of comb shapes (single, pea, rose, and strawberry, buttercup, duplex and walnut) were observed in the three districts (Table 7 and Figure 3a–e). The proportion of the different types was significantly different ($\chi^2 < 0.05$). Pea (41.8 percent), single (36.1 percent) and rose (10.9 percent) combs were the most common comb shapes across the three districts. This finding is in line with Dana *et al.* (2010) and Dong Xuan *et al.* (2006) who reported 55 and 90 percent pea combs for Ethiopian and Vietnamese Dong Tao chickens, respectively. Hassen (2007) correspondingly reported pea comb as the common variant in Northwestern Ethiopian chickens, while Moges,

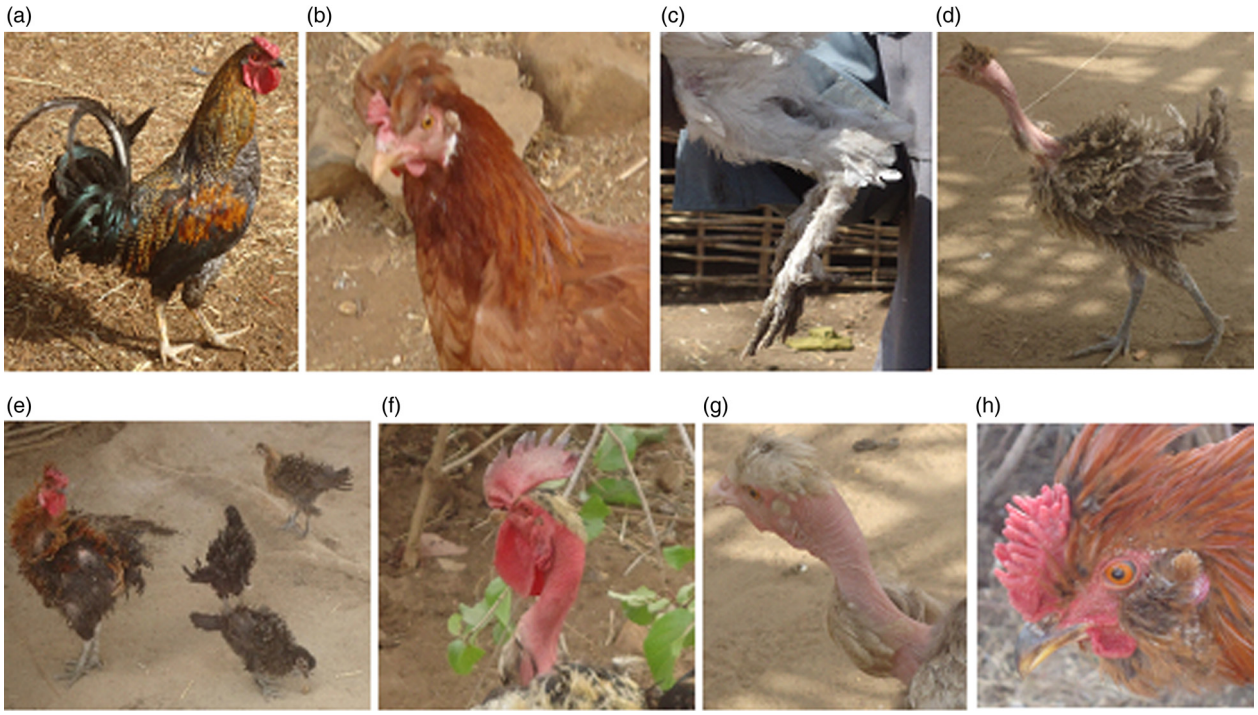

Fig. 2 - Colour online

Figure 2. Feather distribution: (a) normal, (b) crested, (c) feathered shank and feet, (d) naked neck and frizzle, (e) frizzle, (f) naked neck, (g) naked neck and crested, (h) muffs and beard.

Table 6. Variation of plumage colour of body in sample chicken populations.

Plumage colour	Dibate *N* (%)	Wombera *N* (%)	Guba *N* (%)	Overall *N* (%)
White	13[a] (12.4)	2[b] (1.9)	17[a] (16.7)	32 (10.2)
Black	10[a] (12.4)	16[a] (15.1)	9[a] (8.8)	38 (12.1)
Red	3[a] (2.9)	12[b] (11.3)	7[ab] (6.9)	22 (7.0)
Wheaten	11[a] (10.5)	8[a] (7.5)	6[a] (5.9)	25 (8.0)
Brown	16[a] (15.2)	25[a] (23.6)	16[a] (15.7)	57 (18.2)
Light brown	11[a] (10.5)	4[a] (3.8)	6[a] (5.9)	21 (6.7)
White and black	9[a] (8.6)	4[a] (3.8)	11[a] (10.8)	24 (7.7)
Grey	3[a] (2.9)	1[a] (0.9)	3[a] (2.9)	7 (2.2)
Golden	8[a] (7.6)	18[b] (17.0)	6[a] (5.9)	32 (10.2)
Silver	4[ab] (3.8)	1[b] (0.9)	7[a] (6.9)	12 (3.8)
Dark brown	13[a] (12.4)	13[a] (12..3)	12[a] (11.8)	38 (12.1)
White and red	1[a] (1.0)	2[a] (1.9)	1[a] (1.0)	4 (1.3)
White and brown	0[a] (0.0)	0[a] (0.0)	1[a] (1.0)	1 (0.3)

[a,b,ab]Indicated values among traits significantly differ at $\chi^2 < 0.05$.

Melesse and Dessie (2010) found a similar result in Bure district of Amhara region in Ethiopia.

Earlobe colour

We observed six types of earlobe colours and chickens with white and red earlobe had the highest proportion (46.3 percent) across the three districts. Red (24.9 percent) and white (20.4 percent) earlobes were also more frequent. This result disagrees with Desta *et al.* (2013) who stated (40.7 percent) and (31.7 percent) of Horro and Jarso chicken populations from Ethiopia; and Orheruata, Adegite and Okpeku (2006) who found (60 percent) of chicken populations from Edo State in Nigeria had red as their most dominant earlobe colour.

Shank colour

Three shank colour variants were observed in the study area. Slate blue/black was the commonest colour in Dibate (36.2 percent) and Guba (48 percent) districts and even from the overall population (39.3 percent) followed by white shank (31.6 percent). Slate blue was also the most prevalent phenotype among Beninese chickens (43.3 percent, Youssao *et al.*, 2010). Unlike our study populations, however, yellow was reported as the predominant shank colour by Hassen (2007, 64.4 percent), Dana *et al.* (2010, 60 percent), Melesse and Negesse (2011, 52.5 percent) and Negassa, Melesse and Banerjee (2014, 80 percent of cocks) from different parts of Ethiopia.

Table 7. Variation of comb type in sample chicken populations.

Comb type	Dibate *N* (%)	Wombera *N* (%)	Guba *N* (%)	Overall *N* (%)
Single	38[a] (38.0)	40[a] (39.2)	28[a] (30.4)	106 (36.1)
Pea	35[a] (35.0)	40[ab] (39.2)	48[b] (52.2)	123 (41.8)
Rose	17[a] (17.0)	13[a] (12.7)	2[b] (2.2)	32 (10.9)
Strawberry	0[a] (0.0)	0[a] (0.0)	2[a] (2.2)	2 (0.7)
Buttercup	5[a] (5.0)	1[ab] (1.0)	0[b] (0.0)	6 (2.0)
Duplex	5[a] (5.0)	7[a] (6.9)	12[a] (13.1)	24 (8.2)
Walnut	0[a] (0.0)	1[a] (1.0)	0[a] (0.0)	1 (0.3)

[a,b,ab]Indicated values among traits significantly differ at $\chi^2 < 0.05$.

Skeletal variants of the foot

Most of the sample populations (94.6 percent) had the wild-type foot skeleton. The frequency of chickens with extra toes was higher in Dibate (6.7 percent).

Multivariate analyses

Stepwise discriminant analysis

Eight quantitative variables for both sexes were separately subjected to the STEPDISC procedure of SAS (2003). Five variables for males and three variables for females were identified as the best discriminating variables on stepwise selection summary. Wilk's lambda test confirmed that all the selected variables had highly significant ($P < 0.0001$) contribution to discriminate the total population into separate groups. The variables with the highest discriminating power for males were chest circumference, shank length, wing span under, spur length and body length (Table 8a). Body weight, body length and wing span under were the three variables with superior discriminating power for female sample populations (Table 8b). The remaining five variables had poor discriminating power and were removed.

Discriminant analysis

The correct classification for male sample population into their district ranged from 88.89 to 100 percent (Table 9a). The overall average error count estimate was 6.27 percent for all observations and 93.73 percent of the samples were correctly classified indicating the homogeneity of chicken populations within districts for those variables included in the discriminant analysis. Even higher correct classification percentages were calculated for female sample populations than males (Table 9b). All individuals were correctly classified within district for Dibate and Guba districts while 95 percent were correctly classified for Wombera. The overall correct classification percentage for female sample populations was 98.41.

Canonical discriminant analysis

The pairwise squared Mahalanobis distances between districts for male sample populations were highly significant ($P < 0.0001$) indicating that population from each district has distinct and measurable group difference for considered quantitative variables. The shortest distance was measured between Dibate and Wombera, while the longest distance (12.94) was between Wombera and Guba districts on those quantitative variables.

The univariate statistics testing the hypothesis that class means are equal shows that each quantitative variable in male sample populations except spur length is a highly significant ($P < 0.0001$) contributor to the total variation. The multivariate statistics for differences between the districts was also significant ($P < 0.0001$) (Table 10). Wilks'

Fig. 3 - Colour online

Figure 3. Comb types: (a) single, (b) pea, (c) duplex, (d) rose, (e) walnut.

Table 8. Stepwise selection summary indicating most discriminating variables for male and female sample populations.

Step	Entered	Partial R^2	*F* value	*Pr* > *F*	Wilk's Lambda	*Pr* < lambda	Average squared canonical correction	ASCC
(a) Male sample population								
1	CC	0.60	35.77	$P < 0.0001$	0.40	$P < 0.0001$	0.30	$P < 0.0001$
2	SL	0.39	15.17	$P < 0.0001$	0.24	$P < 0.0001$	0.49	$P < 0.0001$
3	WSU	0.15	4.18	$P = 0.0215$	0.21	$P < 0.0001$	0.52	$P < 0.0001$
4	SL	0.09	2.12	$P = 0.1315$	0.19	$P < 0.0001$	0.53	$P < 0.0001$
5	BL	0.09	2.21	$P = 0.1216$	0.17	$P < 0.0001$	0.54	$P < 0.0001$
(b) Female sample population								
1	BW	0.98	3226.40	$P < 0.0001$	0.02	$P < 0.0001$	0.49	$P < 0.0001$
2	BL	0.20	15.23	$P < 0.0001$	0.01	$P < 0.0001$	0.59	$P < 0.0001$
3	WSU	0.06	4.06	$P = 0.0197$	0.01	$P < 0.0001$	0.61	$P < 0.0001$

Note: CC, chest circumference; SL, shank length; WSU, wing span under; SL, spur length; BL, body length; BW, body weight.

Table 9. Number of observations and percent classified (in bracket) in different districts using discriminant analysis.

From district	Dibati	Wombera	Guba	Total
(a) Male sample population				
Dibate	12 (92.31)	1 (7.69)	0 (0.00)	13 (100.00)
Wombera	0 (0.00)	20 (100.00)	0 (0.00)	20 (100.00)
Guba	1 (5.56)	1 (5.55)	16 (88.89)	18 (100.00)
(b) Female sample population				
Dibate	41 (100.00)	0 (0.00)	0 (0.00)	41 (100.00)
Wombera	1 (2.38)	40 (95.24)	1 (2.38)	42 (100.00)
Guba	0 (0.00)	0 (0.00)	40 (100.00)	100 (100.00)

lambda, the ratio of within-group variability to total variability on the discriminator variables, is an inverse measure of the importance of the discriminant functions. The Wilks' lambda test for the male sample population was 0.1650. This reflects that most (83.5 percent) of the variability in the discriminator variables was because of the differences between populations rather than variation within the population.

The canonical discriminant analysis extracted two canonical variates for male sample population, of which the first canonical variate (can 1) accounted for about 81.82 percent of the total variation (Table 10a). The remaining

Table 10. Multivariate statistics and *F* approximation.

Statistic				Value	*F* value	Num DF	Den DF	*Pr* > *F*
(a) Male sample population Wilks' Lambda				0.1650	7.49	16	82	$P < 0.0001$
Eigen value		Proportion	Cum.	Ratio	*F* value	Num DF	Den DF	*Pr* > *F*
1	2.75	0.8182	0.8182	0.1650	7.49	16	82	$P < 0.0001$
2	0.6124	0.1818	1.0000	0.6200	3.68	7	42	$P = 0.0035$
(b) Female sample population Wilks' Lambda				0.0123	111.02	16	226	$P < 0.0001$
1	56.6284	0.9936	0.9936	0.013	111.02	16	226	$P < 0.0001$
2	0.3621	0.0064	1.0000	0.734	5.90	7	114	$P < 0.0001$

Fig. 4 - Colour online

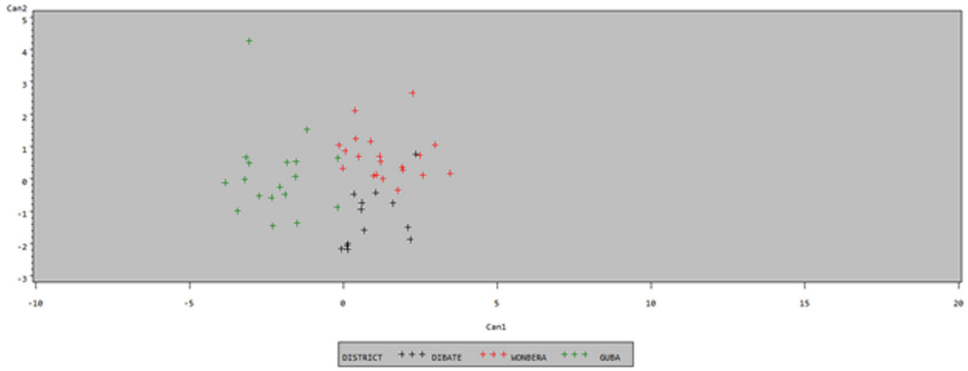

Figure 4. Plot of the two canonical variates for male sample populations.

canonical variate accounted for only 18.18 percent of the total variance. The plot for the first canonical variate for male sample populations (Figure 4) depicts that can 1 discriminated between the two groups: (1) Guba, and (2) Dibatie and Wombera. Can2 poorly separated the three populations.

The pairwise squared Mahalanobis distances for females were considerably higher than for males. The distances between districts for female sample populations were highly significant ($P < 0.0001$) between Guba and Dibate and between Guba and Wombera chicken populations. The distance between Dibate and Wombera was also significant ($P < 0.01$). The shortest distance was measured between Guba and Wombera (84.61). The longest was between Dibate and Guba (335.59) implying female sample populations from the two districts were much different in quantitative features under consideration.

Two canonical variates were also extracted from canonical discriminant analysis on the female sample populations similar to the male population. The first canonical variate (can 1) explained 99.36 percent of the total variation, with the remaining one accounting for only 0.64 percent of the total variation (Table 10b). A plot of the first two canonical variables for the female sample populations (Figure 5) shows that Can1 clearly discriminated the three chicken populations. Can2 failed to make any discrimination among the chicken populations of the three districts.

Non-parametric discriminant analysis

The overall hit ratio and the hit ratio for each sampling district obtained from non-parametric discriminant classification was 100 percent. Similar to the quantitative variables, the qualitative variables have classified the sample populations from the three districts into distinct groups.

Flock performance of chicken populations

The means and overall means for flock performance are presented in Table 11. The age at sexual maturity and marketable age varied highly significantly across districts ($P < 0.001$) and were the highest at Wombera. In addition to the cold temperature with possible negative effect on growth rate (Blahova *et al.*, 2007), the low disease prevalence and better availability of feed may have allowed the farmers in these highland areas to sell their chickens at a later age. The mean number of eggs produced by a hen per year was the lowest ($P < 0.001$) for Wombera (34.1) and the highest for Dibate (60.9). This may be attributed to the high emphasis farmers put on incubation in the highland areas resulting in significant increase of broody cycle days. Eltayeb, Wani and Yousif (2010) also observed on native Sudanese chicken that broodiness significantly affected feed intake and egg production. Analysis on hen performance history as recalled by farmers revealed that mean number of chicks hatched/set eggs (11.4), number of chicks weaned (6.5) and egg number laid/bird/year (50.1) were higher than the findings of Dessie (2003)

Fig. 5 - Colour online

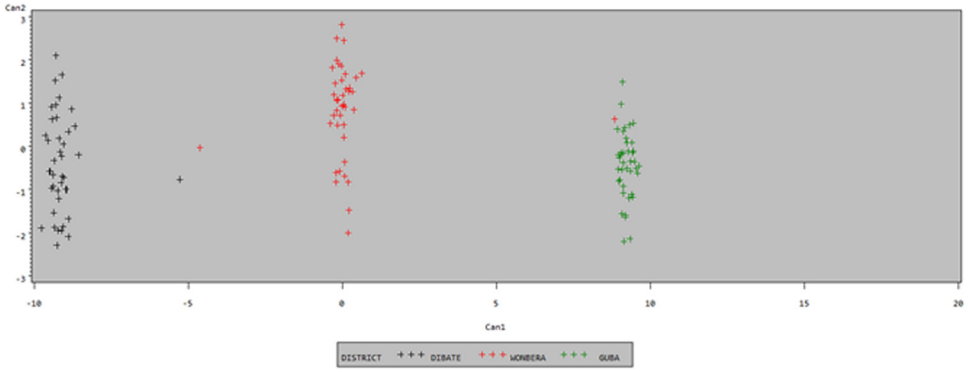

Figure 5. Plot of the two canonical variates for female sample populations.

Table 11. Flock performance of indigenous chicken populations in sample households (mean ± SE).

Reproductive parameter	District			Significance	Overall mean
	Dibate ($N = 24$)	Wombera ($N = 23$)	Guba ($N = 28$)		
Age at sexual maturity of males (month)	$3.94^{b} \pm 0.23$	$5.6^{a} \pm 0.28$	$4.5^{b} \pm 0.23$	***	4.6 ± 0.16
Age at sexual maturity of females (month)	$4.4^{b} \pm 0.25$	$5.9^{a} \pm 0.34$	$4.0^{b} \pm 0.24$	***	4.7 ± 0.18
Age at start of laying (month)	$4.8^{b} \pm 0.30$	$6.6^{a} \pm 0.31$	$5.0^{b} \pm 0.27$	***	5.5 ± 0.19
Inter-clutch interval (week)	3.0 ± 0.22	4.1 ± 0.44	3.4 ± 0.77	NS	3.5 ± 0.35
Marketable age of males (month)	$5.1^{c} \pm 0.39$	$7.6^{a} \pm 0.41$	$6.1^{b} \pm 0.34$	***	6.2 ± 0.24
Marketable age of females (month)	$5.5^{b} \pm 0.41$	$7.0^{a} \pm 0.40$	$6.1^{b} \pm 6.21$	*	6.2 ± 0.26
No. of chicks hatched/set eggs	12.1 ± 0.49	11.35 ± 0.58	10.9 ± 0.43	NS	11.4 ± 0.29
No. of chicks weaned	$7.4^{a} \pm 0.38$	$5.8^{b} \pm 0.41$	$6.2^{b} \pm 0.28$	*	6.5 ± 0.21
No. of eggs laid/clutch	$10.96^{a} \pm 0.61$	$13.19^{a} \pm 0.43$	$10.92^{b} \pm 0.45$	**	11.6 ± 0.31
Mean egg no./bird/year	$60.9^{a} \pm 1.31$	$34.1^{b} \pm 1.82$	$56.8^{a} \pm 2.78$	***	50.1 ± 1.94

[a,b,c]Means within a row followed by different superscripts show the presence of significant difference: significant level * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, NS, non-significant.

who reported 9.3, 4.8 and 46.4 for the three reproductive parameters respectively as an average performance of five Ethiopian chicken ecotypes. The average number of eggs/clutch of local hens in the study population (11.6) was comparable with the 12 eggs produced in Burkina Faso (Kondombo, 2005) but higher than that of 10 eggs/clutch reported by Mourad and Gbanamou (1997) in Guinea and 9 eggs/clutch by Kuit, Traore and Wilson (1986) in Mali. Hassen (2007) reported an average productivity of 9–19 eggs/clutch with an average total egg production ranging from 18 to 57 eggs/year per hen for local hens in Northwest Ethiopia.

Conclusion

Univariate and multivariate analyses revealed that the chicken populations in the three districts of Metekel zone are significantly different in terms of qualitative and quantitative morphological traits and flock performance. Higher between populations and lower within population variation were observed. Chicken populations sampled from the highland and mid-altitude areas had larger linear and body weight measurements compared with those from the lowlands. In the absence of comparison of animals from the different districts raised in the same environmental conditions, however, it cannot be concluded that phenotypic differences have a genetic basis. All chicken populations in the study area were extensively managed and their on-farm reproductive and productive performances generally low, implying the need for interventions in management and genetics.

Acknowledgements

This study was financed by the Food and Agriculture Organization of the United Nations (FAO) and the Ethiopian Biodiversity Institute (EBI) through the Funding Strategy for Implementation of the Global Plan of Action. Visited farmers kindly allowed us to sample their chickens. Staff at District Offices of Agriculture facilitated the fieldwork. We would also like to thank anonymous reviewers and journal editor(s) for their valuable comments.

Conflict of interest

No conflict of interest was identified in this study.

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Animal Genetic Resources, 2016, 59, 27–36. © Food and Agriculture Organization of the United Nations, 2016
doi:10.1017/S2078633616000242

Egg production and certain behavioural characteristics and mortality pattern of indigenous chicken of India

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Summary
A survey to document the behaviour characteristics and mortality pattern of indigenous chicken of Kerala and a field egg recording study to record egg production characteristics of these birds were conducted. Flight distance and height was 13.29 and 3.97 m, respectively. The territory radius of cocks was 121.15 m. The chick survivability at 4 weeks of age was 64.98 percent. The day-old and 8th week body weights were 28.83 and 347.24 g, respectively. The 20th and 40th week body weight of males were 1,428.42 and 1,936.67 g and that of females were 1,114.04 and 1,445.63 g, respectively. The mortality up to 72 weeks was 69.38 percent and major cause of mortality during chick, grower and layer stage were mongoose (44.63 percent), wolf (24.29 percent) and diseases (52.18 percent) respectively. The fertility was 71.22 percent and hatchability on total and fertile egg set were 62.26 and 87.42 percent, respectively. There were 2.13 clutches in a laying cycle with inter-clutch intervals of 1.11 days. The average clutch size and number of eggs per cycle were 7.27 and 14.32, respectively. The egg number up to 72 weeks on hen-day and hen-housed basis was 116.81 and 85.84, respectively and the eggs were laid in 7.7 cycles. The age at first egg and average age at sexual maturity were 155 and 199.26 days, respectively. The egg weight at 28, 40 and 72 weeks of age was 37.80, 40.74 and 43.31 g, respectively, and egg mass per bird was 4,659.04 g. The broodiness and incubation pause were 26.03 and 121.75 days, respectively.

Keywords: *behaviour, broodiness, egg production, mortality, native chicken*

Résumé
Une enquête pour connaître les caractéristiques du comportement et le patron de mortalité des poules indigènes du Kerala et une étude sur le terrain pour contrôler la production d'œufs de ces volailles ont été menées. La distance et la hauteur de vol ont été, respectivement, de 13,29 et 3,97 m. Le rayon du territoire des coqs a été de 121,15 m. La survie des poussins à quatre semaines de vie a été de 64,98 pour cent. Le poids corporel a été de 28,83 g à l'éclosion et de 347,24 g à la huitième semaine de vie. Aux vingtième et quarantième semaines, le poids corporel des mâles a été de 1428,42 et 1936,67 g, respectivement, et celui des femelles de 1114,04 et 1445,63 g, respectivement. Jusqu'à la soixante-douzième semaine, la mortalité a été de 69,38 pour cent, la principale cause de mortalité étant la mangouste (44,63 pour cent), le loup (24,29 pour cent) et les maladies (52,18 pour cent) aux stades de poussin, croissance et ponte, respectivement. La fertilité s'est élevée à 71,22 pour cent et le taux d'éclosion a été de 62,26 pour cent sur le nombre total d'œufs et de 87,42 pour cent sur le nombre d'œufs féconds. Il y a eu 2,13 couvées par cycle de ponte avec des intervalles entre couvées de 1,11 jours. En moyenne, la taille des couvées et le nombre d'œufs par cycle ont été de 7,27 et 14,32, respectivement. Jusqu'à la soixante-douzième semaine, le nombre d'œufs a été de 116,81 par jour de poule élevée et de 85,84 par poule logée, les œufs ayant été pondus en 7,7 cycles. L'âge au premier œuf et l'âge moyen à la maturité sexuelle ont été de 155 et 199,26 jours, respectivement. Le poids de l'œuf aux semaines 28, 40 et 72 d'âge a été de 37,80, 40,74 et 43,31 g, respectivement, et la masse d'œuf par poule s'est élevée à 4659,04 g. Le comportement de couvaison et la pause pour l'incubation ont duré 26,03 et 121,75 jours, respectivement.

Mots-clés: *comportement, couvaison, production d'œufs, mortalité, poules indigènes*

Resumen
Se llevaron a cabo una encuesta para conocer las características del comportamiento y el patrón de mortalidad de las gallinas autóctonas de Kerala y un estudio de campo para determinar la producción de huevos de estas aves. La distancia y la altura de vuelo fueron, respectivamente, de 13,29 y 3,97 m. El radio del territorio de los gallos fue de 121,15 m. La supervivencia de los pollitos a las cuatro semanas de edad fue de 64,98 por ciento. El peso corporal fue de 28,83 g el primer día de vida y de 347,24 g en la octava semana de vida. En la vigésima y cuadragésima semana, el peso corporal de los machos fue de 1428,42 g y de 1936,67 g, respectivamente, y el de las hembras de 1114,04 g y 1445,63 g, respectivamente. Hasta la semana 72, la mortalidad fue de 69,38 por ciento, siendo la principal causa de

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muerte durante las fases de pollito, crecimiento y puesta la mangosta (44,63 por ciento), el lobo (24,29 por ciento) y las enfermedades (52,18 por ciento), respectivamente. La fertilidad ascendió a 71,22 por ciento y la incubabilidad sobre el número total de huevos y sobre el número de huevos fértiles fue de 62,26 por ciento y de 87,42 por ciento, respectivamente. Se dieron 2,13 nidadas por ciclo de puesta con intervalos entre nidadas de 1,11 días. De media, el tamaño de las nidadas y el número de huevos por ciclo ascendieron a 7,27 y 14,32, respectivamente. Hasta la semana 72, el número de huevos fue de 116,81 por gallina-día y de 85,84 por gallina alojada, habiendo sido los huevos puestos en 7,7 ciclos. La edad al primer huevo y la edad media a la madurez sexual fueron de 155 y 199,26 días, respectivamente. El peso del huevo a las 28, 40 y 72 semanas de edad fue de 37,80, 40,74 y 43,31 g, respectivamente, siendo además la masa de huevo por ave de 4659,04 g. La cloquera y la pausa para la incubación duraron 26,03 y 121,75 días, respectivamente.

Palabras clave: *comportamiento, cloquera, producción de huevos, mortalidad, gallinas autóctonas*

Submitted 10 January 2016; accepted 5 September 2016

Introduction

Scavenging chicken production is a profitable enterprise that contributes to poverty reduction especially among the resource challenged rural communities in most parts of the world (Melesse, 2014). In a large numbers of developing countries in Africa and Asia, indigenous birds constitute up to 80 percent of the standing poultry population (Pym, 2010). Indigenous chicken are better adapted to scavenging systems characterized by continuous exposure to diseases, inadequate quantity and quality of feeding, poor housing and health care (Rai and Ahlawat, 1995).

The poultry species of importance in India is chicken (*Gallus gallus*), which accounts more than 95 percent of the total poultry and contributes 97.4 percent to the total eggs produced. Total egg production in India during the year 2013 was 69.2 billion eggs. Of which, 55.2 million eggs (79.8 percent) were produced from commercial farms. Out of 457 million chicken in India in the year 2006, 52 percent was of an indigenous (*desi*) type. However, Kerala, one among 29 states of India, has 70 percent *desi* chicken population (BAHS, 2015). This was possible only because chicken rearing in Kerala remained predominantly extensive backyard type unlike its neighbouring states, which switched over to intensive commercial types after 1960.

Our previous study documented the rearing practices of indigenous chicken of India (Kumar *et al.*, 2013). According to our observations, village chicken is managed mostly by womenfolk in Kerala reared under extensive farming conditions with minimum or no input. The average flock strength is 5.62. The birds must scavenge for most of their nutritional needs. Very minimal shelter or enclosure with virtually no health cover is provided. Both eggs and meat of indigenous chicken command two to threefold higher price compared with those from industrial origin (Kumar *et al.*, 2013). Despite their low growth rates and egg production, indigenous chicken are generally better in disease resistance and could maintain higher level of performance under poor nutrition and high environmental temperatures compared with commercial strains under village systems. Egg weight of the village hen reviewed by Sorensen (FAO, 2010) ranged between 42.2 and 45.8 g and the number of clutches of the village hen per year varies from 1 to 5. The data on annual egg production collected by questionnaire method ranged between 31.6 and 78 eggs (FAO, 2010). However, there is dearth of *in situ* egg recording studies in the literature revealing information on annual egg production and egg production pattern of indigenous chicken.

There were few attempts made either to record egg production of indigenous chicken of India in intensive system (Haunshi, Doley and Shakuntala, 2009) or to study the characteristics other than egg production in extensive system (Chatterjee *et al.*, 2007). Altogether, studies targeted to understand behavioural characteristics, mortality pattern, level and kind of predation, egg production and laying pattern of indigenous fowls under extensive production system in India are limited. Therefore, this study was carried out with the aim of recording the behavioural characters such as distance and height of flight, territory radius and mothering ability and mortality pattern during different stages of growth through survey and laying pattern and egg number by conducting an egg recording study in indigenous chicken of Kerala state, India.

Materials and methods

Study area

The study was undertaken in Northern Midlands agro-climatic zone, one among 13 different agro-ecological zones of Kerala comprised Kozhikode and Kannur districts (Figure 1) covering a total area of 5 311 km^2. This zone extends from latitudes 11°17′ to 12°28′N and from longitude 75°18′ to 76°14′E. The region has a humid climate with an oppressive hot season from March to the end of May followed by the Southwest Monsoon till the end of September and Northeast Monsoon from October and November. The annual average rainfall is 3 352 mm and more than 80 percent of it occurs during the period of

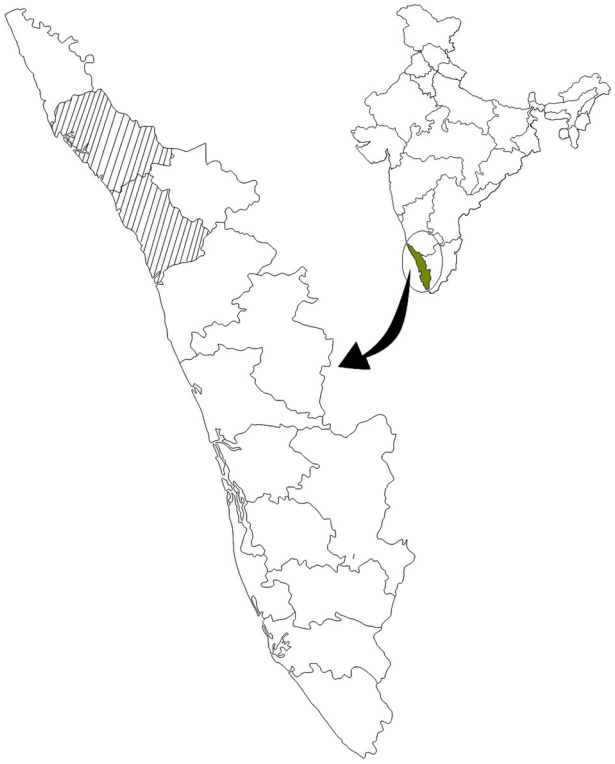

Fig. 1 - Colour online

Figure 1. Map of Kerala state of India with highlighted study area.

Southwest Monsoon. This zone is a low-altitude region of Kerala, endowed with humid tropical climate. The population density in this region is 1 677 persons km^2.

Study population and data collection

The deep pockets of Northern Midlands agro-climatic zone of Kerala is endowed with pure populations of native chicken (*desi*) by virtue of their remoteness. Only *desi* chicken with history of non-mixture of exotic germplasm in the past and also based on the phenotypic characters of the birds true to the native chicken were subjected to the study. Villages close to town and urban areas were not sampled as they tend to be influenced by urban farming. The system of management of village chicken rearing in the study area is extensive free range type with (98.4 percent) or without (1.6 percent) the provision for night shelter (Kumar *et al.,* 2013). A survey and a field egg recording were conducted in this study. A structured questionnaire is used in survey; whereas, a separate datasheet was used for field egg-recording study. The survey was conducted in 64 indigenous chicken farming families identified by two-stage cluster sampling. A structured questionnaire was designed to collect data on height and distance of flight, radius of territory, mothering ability and age at sexual maturity (ASM). The questionnaire was prepared by Centre for Advanced Studies in Poultry Science, Kerala Agricultural University. Before the commencement of the survey, the questionnaires were pretested using sample farmers and appropriate adjustments were made on specific contents. The interviews were conducted at farmers' houses with the assistance of local veterinary surgeons and ward member of local governing body.

Survey study

The survey study was conducted from 64 households of two districts of Northern Kerala, namely, Kozhikode (43) and Kannur (21). The information on flight height and distance and territory radius were retrieved from the households. A total of 52 farmers (33 from Kozhikode and 19 from Kannur districts), could provide this information. These data are of subjective type collected from farmers' knowledge. The farmers were asked to show an end point the birds might travel upon excitement. The distance was measured to flight distance. The households were also asked to show the highest places like tree branches and roof tops normally their birds climb in a single takeoff and the height was measured to record flight height. They were asked to show the farthest place their birds travel from coop and distance was measured to record territory radius.

The parameters such as length of broodiness, age at first egg (AFE) in days, clutch size, number of clutches per cycle and egg number per cycle were collected only from those farmers who can provide correct data in this respect on individual birds they reared in the recent past or during the time of survey. The earliest age of laying among all birds was expressed as AFE in the flock and the average value was expressed as average ASM. The number of observations varied from trait to trait and is given along with the mean value in Table 1.

The ability of broody hen in saving its hatchlings up to 4th week of age was considered as the mothering ability. The mortality details from day-old to 72 weeks were retrieved only from the farmers who can provide complete data about the hatches in the recent past. The data were then assembled to calculate mortality pattern at chick (up to 8 weeks), grower (9–20 weeks) and layer (21–72 weeks) stages. Egg shell colour and sex-pooled body weight at day-old and 8th week of age and sex-separate body weights at 20th and 40th weeks of age were also recorded during survey. The unhatched eggs from 21 natural settings involving 212 total hatching eggs were break-opened to determine the fertility and hatchability of indigenous chicken.

Field egg recording study

A 1-year long field egg recording study was conducted to study the production performance and laying pattern of indigenous chicken. A total of 54 birds from 17 to 20 weeks of age available in 38 households at the start were used. The exact age of the birds was determined by 'recalling method' of interviewed farmers (women farmers can easily recall the date of hatch with associating other co-incidences). Finally, 43 ready-to-lay pullets from 31

Table 1. Behavioural characters, egg production and egg production-related traits of indigenous chicken studied by the survey method.

Parameters	Kozhikode district	Kannur district	Over all
Flight distance (m) (household basis) ($n = 52$)	12.85 ± 1.26 (33)	14.05 ± 0.83 (19)	13.29 ± 0.85
Flight height (m) (household basis) ($n = 52$)	3.58* ± 0.14 (33)	4.74* ± 0.23 (19)	3.97 ± 0.14
Territory radius (m) (household basis) ($n = 52$)	139.39* ± 11.46(33)	89.47* ± 11.20(19)	121.15 ± 8.94
Chick survivability at 4th week of age (individual bird basis) ($n = 61$)	62.91 ± 4.78 (38)	68.41 ± 5.68 (23)	64.98 ± 3.66
Length of broodiness (days) (individual bird basis) ($n = 99$)	28.78 ± 2.36 (55)	27.28 ± 3.00 (44)	27.90 ± 2.00
Average age at sexual maturity (ASM) (months) (household basis) ($n = 39$)	6.39 ± 0.14 (23)	6.53 ± 0.20 (16)	6.45 ± 0.12
Average ASM (days) (individual bird basis) ($n = 40$)	175.97 ± 6.64 (21)	181.00 ± 5.58 (19)	177.60 ± 4.81
Clutch size (individual bird basis) ($n = 102$)	7.41 ± 0.55 (64)	7.84 ± 0.64 (38)	7.67 ± 0.44
Number of clutches per cycle (individual bird basis) ($n = 102$)	2.93 ± 0.38 (64)	3.48 ± 0.51 (38)	3.13 ± 0.51
Egg number per cycle (individual bird basis) ($n = 102$)	16.02 ± 0.69 (64)	15.15 ± 0.65 (38)	15.50 ± 0.48
Hatchability (%)	60.23 ± 7.97 (24)	79.05 ± 6.27 (20)	68.78 ± 5.33

*Means bearing different superscripts within a row differ significantly ($P < 0.05$).

farming households with their hatch date undoubtedly known with their owners willing to co-operate to this year-long exercise were subjected for the study. The daily egg recording was done by the farmers on individual chicken basis in a datasheet with date of hatch and identification terminology about the chicken in farmers' own description written on it. Weekly follow up was carried out to sustain the interest of farmers and to gather the data collected by them. The farmers retained the eggs at 28, 40 and 72 weeks of age to enable the author to record weight during his weekly visit. The mortality, cause of death and days of broodiness were also marked. Age at fist egg and average ASM were calculated from date of hatch and egg production record of each bird.

Data analysis

These data were assembled in a Microsoft excel spreadsheet and the parameters like AFE, average ASM, hen-housed (HH) (the number of birds at 21st week was considered as HH) and hen-day (HD) egg production, length of broodiness, clutch size, length of pause, number of clutches per cycle and number of eggs per cycle were arrived at. The mean values between the two districts of the study were compared using *t*-test. The livability percent was calculated for the period from 21 to 72 weeks of age. Linear and curvilinear regression analyses were performed on HH percent egg production to evaluate the fitness. Statistical analyses were run with SPSS software package (Version 12.0 for Windows; SPSS Inc., Chicago, IL).

Results

Behavioural characteristics

Flight distance and height

The results of survey on flight height, flight distance, territory radius and mothering ability are presented in Table 1. The overall flight distance reported was 13.29 m with vast majority of the farmers (80.77 percent) recounted values ranging between 10 and 15 m. The overall flight height reported by the farmers is 3.97 m with many farmers (46.15 percent) opining that the village chicken can fly a vertical height of 3–4 m in a single takeoff (Figure 2).

Territory radius

The overall territory radius covered by the cocks from their coops was 121.15 m with many farmers (40.39 percent) giving values ranging from 51 to 150 m.

Mothering ability

The survival rate of chicks up to 4 weeks of age collected from 61 natural broodings revealed that 40.98 percent of the mother hens could save 76–100 percent of their hatchlings to 4 weeks. Overall chick survivability at 4 weeks of age was 64.98 percent.

Broodiness

The data from 105 hens showed that majority of the indigenous hens are habitual brooders (98.1 percent) and very few were occasional brooders (1.9 percent). The survey data on 99 birds on length of broodiness revealed a wide variation among birds ranging from 7 to 65 days with a mean value of 27.90d. The length of broodiness estimated directly from egg recording study was 22.38d (Table 2).

Figure 2. Perching behaviour of indigenous chicken.

Fig. 2 - Colour online

Table 2. Egg production and related parameters of indigenous chicken under daily field egg recording study from 21 to 72 weeks of age.

Parameter	*n*	Mean + SE
Age at first egg in the flock (days)	54	155
Average age at sexual maturity (days)	50	199.26 ± 4.99
Length of broodiness (days)	34	22.38 ± 3.29
Clutch size (days)	34	7.27 ± 0.63
Inter-clutch interval (days)	34	1.11 ± 0.05
Number of clutches per cycle	34	2.13 ± 0.17
Egg number per cycle	34	14.32 ± 0.53
Cycles per year	28	7.70 ± 0.35
Broodiness pause (days)	34	26.03 ± 3.08
Incubation pause (days)	27	121.75 ± 5.62
Livability (%)	54	51.85
Hen-day (HD) egg number (21–40 weeks)	54	34.59
HD percent (21–40 weeks)	54	24.71
Hen-housed (HH) egg number (21–40 weeks)	54	33.06 ± 3.53
HH percent (21–40 weeks)	54	23.61
HD egg number (21–72 weeks)	54	116.81
HD percent (21–72 weeks)	54	32.09
HH egg number (21–72 weeks)	54	85.84 ± 10.43
HH percent (21–72 weeks)	54	23.58
Egg weight at 28 weeks	49	37.80 ± 0.97
Egg weight at 40 weeks	41	40.74 ± 0.83
Egg weight at 72 weeks	20	43.31 ± 1.19
Survivor egg number	28	114.87 ± 6.56
Survivor egg percent	28	31.56
Survivor egg mass (21–72 weeks)	28	4 659.04 ± 200.66

Mortality pattern

Overall mortality

The details on mortality in indigenous chicken collected from 41 farmers who could provide correct information on a total of 454 birds. The overall mortality in indigenous chicken from day-old to 72 weeks was 69.38 percent. Predation due to mongoose (29.52 percent) and death due to diseases (26.67 percent) are the major causes of mortality.

Chick stage

The graphical representation of the results for different stages is given in Figure 3. Out of 454 chicks at start, 278 from Kozhikode and 176 from Kannur districts, 130 (28.63 percent) died during chick stage. Among this, only 10 (7.69 percent) were due to disease, while the rest 120 (92.31 percent) was by predation. Predation due to mongoose was the most prevalent (44.63 percent), followed by shikra (16.15 percent), crow (9.23 percent), eagle and dog (6.93 percent each), cat (6.15 percent) and snake (2.31 percent).

Grower stage

During growing stage, the total mortality was 21.6 percent of 324 birds (188 in Kozhikode and 136 in Kannur districts) entered into grower stage. The mortality due to diseases was 20 percent, while the rest 80 percent was due to

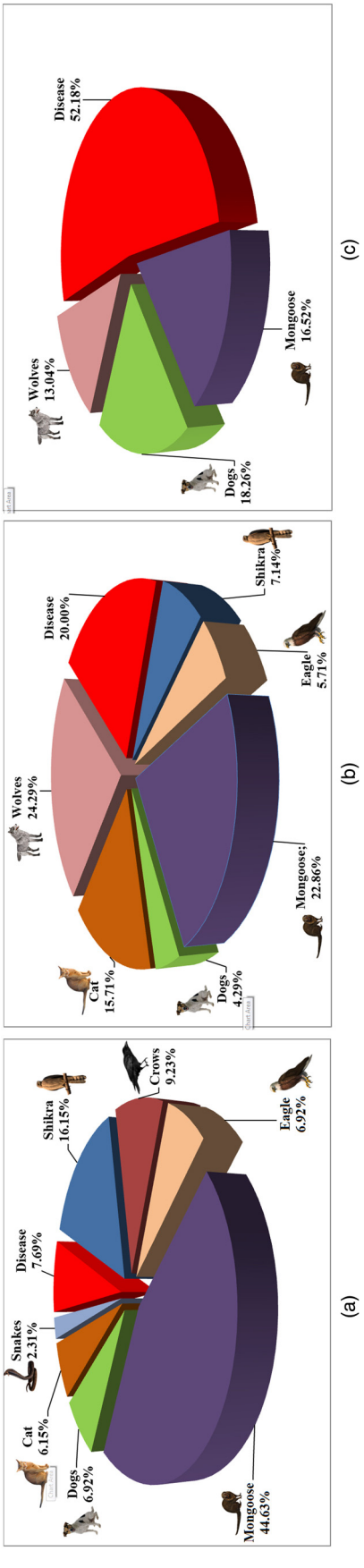

Figure 3. Mortality pattern in indigenous chicken: (a) chick stage, (b) grower stage and (c) layer stage.

Fig. 3 - Colour online

predation. The dominant predator of this stage was wolf (24.29 percent), followed by mongoose (22.86 percent), cat (15.71 percent), shikra (7.14 percent), eagle (5.71 percent) and dog (4.29 percent).

Adult stage

From 254 birds (133 in Kozhikode and 121 in Kannur districts) available at the start (21 weeks of age), 115 birds (45.28 percent) died up to 72 weeks of age. The mortality due to disease, out of total mortality was 52.18 percent and the rest was due to predation (47.82 percent) by dog (18.26 percent), mongoose (16.52 percent) and wolf (13.04 percent). The mortality during adult stage (21–72 weeks of age) recorded directly from field egg recording study was 48.15 percent.

Diseases

The important diseases affecting indigenous chicken as perceived by the farmers are Respiratory Tract Infections (RTI) (37.21 percent), Ranikhet disease (34.89 percent), fowl pox (18.60 percent) and ectoparasites (9.3 percent). Most of the farmers felt that the diseases are common in summer season (76.47 percent) compared with winter and rainy seasons (25.53 percent).

Body weight

The early (day-old) and late (8th week of age) juvenile body weights of unsexed indigenous chicken from the study area were 28.83 ± 0.31 ($n = 69$) and 347.24 ± 12.17 g ($n = 48$), respectively. The pubertal body weight (20th week of age) of males and females were $1{,}428.42 \pm 85.91$ ($n = 18$) and $1{,}114.04 \pm 45.01$ g ($n = 34$) and the adult body weight (above 40 weeks of age) for males and females were $1{,}936.67 \pm 96.83$ ($n = 22$) and $1{,}445.63 \pm 51.47$ ($n = 29$), respectively.

Egg weight

The mean egg weight recorded from the birds under egg recording study at 28, 40 and 72 weeks of age was 37.80, 40.74 and 43.31 g, respectively (Table 2). The overall egg weight and average egg mass per bird recorded in field egg recording study from 21 to 72 weeks of age among the survivors was 42.24 and 4,659.04 g, respectively.

Shell colour

The eggs of indigenous chicken ($n = 196$) were dark brown (2.04 percent) or medium brown (12.24 percent) or light brown (73.48 percent) or creamy white (12.24 percent) in colour.

Fertility and hatchability

The break-open study of unhatched eggs revealed fertility of 71.22 percent and hatchability of 62.26 percent on total eggs set and 87.42 percent on fertile egg set.

Egg production and related characteristics

The mean values of egg production traits up to 72 weeks studied by indirect (survey) method from 64 women households who could provide complete production information on 164 hens they reared in the recent past are presented in Table 1 and that of direct egg recording study are given in Table 2.

Age at first egg

A vast majority (61.54 percent) of respondents opined that the AFE in indigenous chicken is between 6 and 7 months of age with the average ASM of 6.45 months. The AFE of 40 birds retrieved from 29 farmers by recalling method was 144d with the individual values ranging from 144 to 230d and ASM of 177.60d. Thirdly, the field egg recording study revealed AFE of 155d and ASM of 199.26d with individual variations ranging from 155 to 244d.

Laying cycle

The survey on clutch pattern retrieved by recalling memory on 102 hens revealed that number of clutches in a cycle ranged from one to 16 with most of the birds (38.24 percent) having three clutches; the mean clutch number per cycle was 3.13. The clutch size ranged from one to 25 eggs with majority of the birds (53.92 percent) having clutch size of five to eight eggs with the mean clutch size of 7.67 eggs. The egg production in a laying cycle ranged from four to 30 with a mean value of 15.50 eggs.

The field egg recording study revealed that there were 2.13 clutches in a cycle with inter-clutch intervals of 1.11d. The average clutch size was 7.27 giving rise to 14.32 eggs per cycle. There was an average of 7.7 cycles in 1 year production period (21–72 weeks of age). The graphical representation of laying pattern of indigenous chicken is depicted in Figure 4.

Pause

The broodiness pause documented from egg-recording experiment was 26.03d and the combined length of natural incubation and brooding (incubation pause) studied from 27 natural settings was 121.75d.

Egg number

The graphical representation of period-wise HD egg production is presented in Table 3 and Figure 5, and the mean values are provided in Table 2. The mean egg number up to 40 weeks of age on HD and HH basis in indigenous chicken was 34.59 (24.71 percent) and 33.06 (23.61 percent) and the respective values up to 72 weeks of age were 116.81 (32.09 percent) and 85.84 (23.58 percent), respectively. The survivor egg number at 72 weeks of age was 114.87 (31.56 percent). The period-wise egg number was highest at fifth period (37–40 weeks of age) both in terms of HD (12.13 eggs or 43.33 percent) and HH (10.11 eggs or 36.11 percent) egg production. Although

Fig. 4 - Colour online

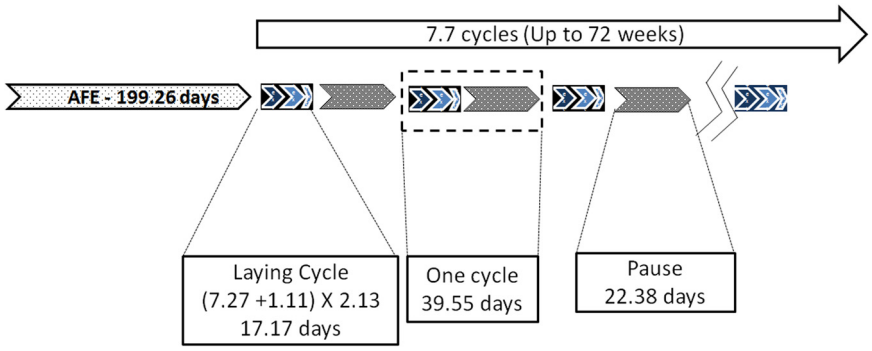

Figure 4. Laying pattern of indigenous chicken.

Table 3. Period-wise egg production of indigenous chicken collected from field egg recording study.

Period	Duration (weeks)	Number of birds	Hen-day		Hen-housed	
			HDEP	HDEP percent	HHEP	HHEP percent
1	21–24	54	1.17	4.17	1.17 ± 0.80	4.17
2	25–28	54	3.33	11.90	3.33 ± 1.11	11.90
3	29–32	54	9.56	34.13	9.56 ± 1.66	34.13
4	33–36	51	9.41	33.61	8.89 ± 1.40	31.75
5	37–40	45	12.13	43.33	10.11 ± 1.66	36.11
6	41–44	39	10.31	36.81	7.44 ± 1.55	26.59
7	45–48	39	11.00	39.29	7.94 ± 1.64	28.37
8	49–52	39	11.15	39.84	8.06 ± 1.51	28.77
9	53–56	33	11.36	40.58	6.94 ± 1.75	24.80
10	57–60	33	11.27	40.26	6.78 ± 1.60	24.21
11	61–64	31	11.32	40.43	6.50 ± 1.72	23.21
12	65–68	30	11.13	39.75	6.18 ± 1.63	22.08
13	69–72	27	10.89	38.89	5.45 ± 1.55	19.45

HDEP, hen-day egg production; HHEP, hen-housed egg production.

Fig. 5 - B/W online

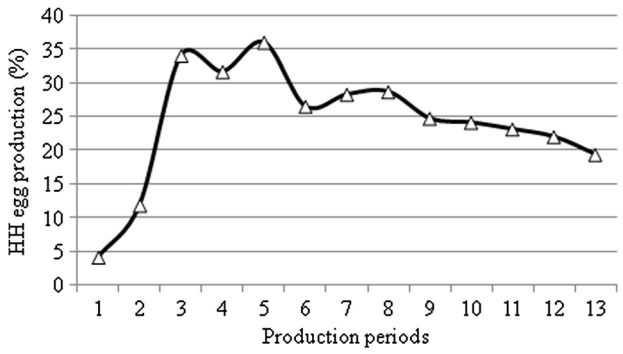

Figure 5. Egg production curve in indigenous chicken.

the period-wise HH percent egg production does not observe linear regression ($R^2 = 0.02$), a high degree of curvilinear fitness ($R^2 = 0.82$) was observed in indigenous chicken.

Discussion

The ability of chicken to fly a horizontal and vertical distance of around 13 and 4 m respectively is an adaptability trait of indigenous chicken to evade predation. The horizontal flight is the effective way of evading aerial predators; whereas, vertical takeoff helps in escaping from terrestrial predators. It is therefore the farmers feel that exotic breeds or hybrid crosses are not able to move swiftly as effective as indigenous chicken when situation demands therefore not suited to this hilly agro-climatic terrain. The light body with strong wings of indigenous chicken gives them a greater chance of evading predators by fast running and flying to a safer place. Flying also assists the birds to perch on the tree branches to evade predators while resting. Similar explanation has earlier been offered by Kumar and Kumar (2007) on local hill fowls of Uttarakhand.

The cocks in the study area cover an average radius of 121.15 m from their coop. The average land holding per household in the area is only 29.45 cents or nearly 1,200 m^2. It can be deduced from these data that the village chicken flocks are open flocks with cock of one household having overlapping territory on the neighbouring households. This possibly provides justification of our previous findings (Kumar *et al.*, 2013) of 58 percent of households not maintaining cock in their flock also incubate eggs taking chance of their hens being mated by cocks from neighbourhood.

There was 35.02 percent depletion in chicks during first month of their life. More than 92 percent mortality during chick stage ascribed to predation as observed in this study explains the challenging task before the mother hens. A very poor chick survival rate of 13.43 and 9.73 percent at the end of 8 weeks has been reported from two districts of Kenya (Olwande *et al*., 2010). According to Sørensen (FAO, 2010) indigenous chickens are ideal mothers, good setters, hatch their own eggs and excellent foragers.

The indigenous chicken exhibits strong broodiness character lasting for about 3–4 weeks as assessed by both direct and indirect methods in this study. Most of the farmers (92.7 percent) agreed that broodiness that is lacking in exotic breeds and hybrid crosses is indispensable from village chicken farming. In a similar study, Iqbal and Pampori (2008) reported 82 percent hens with 12–15d and remaining 18 percent with up to 30d broodiness in indigenous chicken of Kashmir. Most of the indigenous chicken breeds of Indian subcontinent are reported to be broody (Vijh *et al*., 2005) except Nicobari breed of Nicobar Island, India (Vijh *et al.*, 2006).

During chick stage, the death due to diseases was only 7.69 percent while the rest was due to predators (92.31 percent). Terrestrial predators (61.01 percent) such as mongoose, dog, cat and snake dominate aerial predators (32.3 percent) such as shikra (*Accipiter badius and A. virgatus*) (local name – *Prappidiyan*), crows and eagle in flock destruction. In the growing stage, the role of terrestrial predators remained high (67.15 percent), whereas that of diseases had a positive shift (20 percent) with diminishing influence of aerial predators (12.85 percent). In adult stage, the highest damage was caused by diseases (52.18 percent) followed by terrestrial predators (47.82 percent). This result was in sharp contrast with the findings of Vij *et al.* (2007), who reported almost zero mortality from Tellichery chicken of this area. However, in Danki birds of India, Vijh *et al.* (2005) documented a mortality rate of around 20–30 percent during first 2 months of age, similar to the result of present study. In grower stage, a very closer value of 21 percent mortality was documented in Baladi chicken in Saudi Arabia (Yousef and Al-Yousef, 2007). The study revealed that altogether there is 69.38 percent depletion from day-old to 72 weeks of age in village chicken much higher than that of intensively reared commercial layers (Farooq *et al*., 2012). Biswas *et al*. (2008) earlier reported that crow, eagle and mongoose are the main predators of day; while foxes, jackals and wild cats are the main predators of night. The village flocks are devastated by predators, especially during chick stage. This underlines the need for establishing Government chick nurseries to supply grownup *desi* birds as emphasized by the farmers of this region earlier (Kumar *et al*., 2013). The survey revealed that RTI and Newcastle disease (Local name: *Kozhi vasantha*) are the major disease conditions compared with fowl pox (local name: *Aakkurippu*) and ectoparasites (local name: *Kozhipaén*). Similar to the results of the present study, preponderance of Newcastle disease (Olwande *et al*., 2010; Melesse, 2014) and high disease occurrence in dry summer seasons (Sonaiya and Swan, 2004) in village chicken have already been established. The dry and windy nature of summer season that facilitates the dispersion of infectious agents in the environment in the form of dust, stress due to low availability of scavengeable feed and hot climate could be ascribed for high disease occurrence in this season.

The body weights at different age were in general, slightly higher than that of earlier reports (Yakubu, Ogah and Barde, 2008; Haunshi, Doley and Shakuntala, 2009) but similar only to that of indigenous chickens of Kenya (Olwande *et al*., 2010). The higher body weight observed in the study could partially be ascribed to the monsoon season during which the study was undertaken when abundant scavengeable feed resources (SFR) are available.

The range of AFE recorded by both direct and indirect method falls in line with the earlier report of 153–230d from Ethiopian indigenous chicken (Reta, 2009). Vij *et al*. (2007) reported that the Tellichery breed of chicken from the area of present study had ASM ranging from 150 to 240 days with a mean value of 180 days which closely compare with the results of present study. However, a higher value of 201.6d reported in Indian native chicken breeds (Vijh *et al*., 2006) might be ascribed to genetic and environmental variations between these populations.

The egg weight of indigenous chicken in literature showed high degree of variation with the present values falling above that of Ethiopia (Mogesse, 2007), Sudan (Mohammed *et al*., 2005) and Nigeria (Yakubu, Ogah and Barde, 2008) but below that of other parts of India (Iqbal and Pampori, 2008) and other countries (Olwande *et al*., 2010). The involvement of both genetics and environment could be attributed to this wide variation. Although not much information is available in the literature, the egg mass determined in this study is closer to the value of indigenous chicken of Ethiopia with Naked Neck morphology but higher than that of four other morphologies reported by Reta (2009) under on-farm conditions. With regard to shell colour, a closer observation of 77.1 percent brown and 22.9 percent white egg colours were reported from indigenous chicken of Kashmir (Iqbal and Pampori, 2008). Mogesse (2007) also reported shell colours of light brown, cream and white from scavenging indigenous chicken of West Ethiopia.

The hatchability rate observed in this study falls within the range of 70–80 percent observed earlier by Vij *et al.* (2007) in Tellicherry chicken of same area and closer to other indigenous breeds of India (Vij *et al*., 2005; Vijh *et al*., 2005) and Turkey (Sekeroglu and Aksimsek, 2009) but higher than the indigenous chicken eggs collected from Ethiopian markets (Reta, 2009). Our earlier findings of more than 58 percent households not maintaining cock in their flocks (Kumar *et al*., 2013) could explain the poor fertility obtained. The hatchability of fertile eggs

was comparable with commercial hatching using incubators indicating the ability of the indigenous mother hens in providing optimum incubation conditions to the hatching eggs as that of modern equipment. Many farmers believe that thunder and lightning can reduce the hatchability and placing pieces of iron and charcoal under the hens of natural incubation can subvert this.

The study revealed that the annual egg production of indigenous chicken is spread in 7.70 cycles separated by periods pause spanning 26.03 (direct assessment) to 27.90d (indirect assessment). The average egg production in a cycle was found to be 14.32 (direct assessment) to 15.50 (indirect assessment). The eggs in a cycle is laid in 2.13 (direct assessment) to 3.13 (indirect assessment) clutches and the clutches are separated by an inter-clutch interval of 1.11d (direct assessment). If the broody hen is allowed to incubate the eggs the length of pause extends to 121.75d. This period covers incubation (3 weeks) brooding (2–3 months) and recuperation periods after brooding (around 2 weeks). The number of eggs per cycle in the present study was higher than the previous reports (FAO, 2009) but comparable with that of Iqbal and Pampori (2008). The population under study recorded higher number of cycles per year compared with the indigenous chickens in other parts of India (Iqbal and Pampori, 2008) and other countries (FAO, 2009; Reta, 2009). The period of broodiness was also found to be shorter than that of indigenous chicken of Ethiopia (Reta, 2009). The interventions adopted by the farmers to break broodiness and expedite the resumption of next cycle are dipping in water, rattling the nest, introducing new cocks, tethering the birds in unfamiliar surroundings and by inserting a quill feather through and through the nostrils. The egg number at 72 weeks of age was higher in the studied population compared with many other indigenous chicken populations in India (Vij *et al.,* 2005; Vijh *et al*., 2005) and abroad (FAO, 2009). The better genetic potential could be partially ascribed to the practice of selective breeding largely adopted by the housewives of the region. Clearly, more studies are required to evaluate the local germplasm for drawing definite conclusions. The analysis of egg production in indigenous chicken revealed a shift in the pattern with delayed peaking compared with breeds like White Leghorn (Savegnago *et al*., 2012).

Conclusions

The present study revealed good egg-laying potential of indigenous chicken of this agro-climatic zone possibly due to their genetic merit or environment or both. The variability (CV) for egg production is very high in the studied population disclosing the possibility for genetic improvement by selective breeding. Multi-stage selection programme with selection for conformation traits like shank length during growing stage to resist predation and egg production and broodiness at laying stage can be adopted. Longer production cycle with short broodiness could be the selection criteria for improvement in egg number with retention of broodiness character. The studied zone should possibly possess a high degree of SFR as is endowed with diverse fauna and flora with very high rain fall. However, quantification of SFR needs to be taken up to ascertain the involvement of nutritional factors, if any, on high egg production of indigenous chicken of this region and also to recommend flock strength to this area. Phenotypic and molecular characterization to assess genetic diversity for evolving promising indigenous chicken varieties need to be taken up. Although, improved hybrids are not the choice of local farmers, they could hardly resist hybrid chicks when available at free of cost through Government schemes. The grownup birds of genetically improved indigenous chicken produced in state-run nurseries rather than hybrids can be disseminated to the farmers. This can revitalize the sector and protect from exotic and improved hybrids making inroads and associated problems on traditional poultry farming. As emphasized earlier by Magothe *et al*. (2012), a holistic strategy that increases productivity without increasing production costs or leading to loss of biodiversity must be developed. Such a strategy must take into account the various uses of the indigenous chicken in a rural household. A policy decision is the need of the hour to discriminate the zones which need the supply of hybrids from those in need of reintroduction of genetically improved indigenous chicken. Developing zone-specific indigenous germplasm could be the lasting solution in this direction to prevent genetic erosion.

Acknowledgements

This study was a part of the MVSc Thesis of P. Girish Kumar. The authors acknowledge the finance and infrastructure provided by the Kerala Agricultural University, Thrissur, Kerala, India for the successful conduct of the study.

Statement of interest

None.

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Animal Genetic Resources, 2016, 59, 37–45. © Food and Agriculture Organization of the United Nations, 2016
doi:10.1017/S2078633616000254

Morphometric, productive and reproductive traits of indigenous goose of Bangladesh

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Summary
The study was aimed to identify, characterize and describe the phenotypic variation of indigenous goose populations in Bangladesh. The research was conducted at Sylhet Sadar Upazilla in Sylhet district and encompassed about 141 geese (74 brown type and 67 white type). Least Square Mean ± SE of body length, wing span, shank length, beak length and head length of mature indigenous goose were 73.47 ± 0.95, 134.53 ± 1.38, 9.27 ± 0.09, 8.88 ± 0.10 and 6.42 ± 0.02 cm, respectively. Males were significantly ($p < 0.01$) higher than their female counterparts for all morphometric traits but no significant differences ($p > 0.05$) were found between two types. The body weight of indigenous goose at day old, 2-week, 1-month, 2-month and 10-month of age were 95.45 ± 0.88, 148.59 ± 1.55, 407.34 ± 7.27 gm, 1.19 ± 0.03 kg and 3.65 ± 0.06 kg, respectively. Males were significantly ($p < 0.01$) heavier than females in all age groups except day old gosling but no significant difference ($p > 0.05$) were observed for body weights between two types of goose. Egg weight, egg length, egg width, incubation period, clutch size, number of eggs in a breeding season and age at first egg were 131.85 ± 1.70 gm, 7.40 ± 0.02 cm, 5.22 ± 0.02 cm, 30.30 ± 0.07 days, 7.42 ± 0.08, 20.52 ± 0.38 and 313.22 ± 3.03 days, respectively. The number of eggs in a breeding season of brown type were significantly ($p < 0.05$) higher than that of white type goose. This study provides a bench mark for the morphometric traits and performance of goose in Bangladesh.

Keywords: *brown and white types, goose, indigenous, phenotypic characterization*

Résumé
Le but de l'étude a été d'identifier, de caractériser et de décrire la variabilité phénotypique des populations d'oies indigènes du Bangladesh. La recherche a été menée dans l'upazila de Sylhet Sadar au sein du district de Sylhet et a compris environ 141 oies (74 du type marron et 67 du type blanc). La moyenne des moindres carrés ± l'erreur-type de la longueur du corps, l'envergure des ailes, la longueur des tarses, la longueur du bec et la longueur de la tête des oies indigènes mûres ont été de 73,47 ± 0,95, 134,53 ± 1,38, 9,27 ± 0,09, 8,88 ± 0,10 et 6,42 ± 0,02 cm, respectivement. Pour tous les caractères morphométriques, les mesures des mâles ont été significativement ($p < 0,01$) supérieures à celles des femelles, alors qu'aucune différence significative ($p > 0,05$) n'a été décelée entre les deux types. Le poids corporel des oies indigènes à un jour, deux semaines, un mois, deux mois et dix mois d'âge a été de 95,45 ± 0,88 g, 148,59 ± 1,55 g, 407,34 ± 7,27 g, 1,19 ± 0,03 kg et 3,65 ± 0,06 kg, respectivement. Les mâles ont été significativement ($p < 0,01$) plus lourds que les femelles à tous les âges, hormis le cas des oisons d'un jour d'âge. Pourtant, aucune différence significative ($p > 0,05$) n'a été observée pour le poids corporel entre les deux types d'oie. Le poids de l'œuf, la longueur de l'œuf, la largeur de l'œuf, la durée de l'incubation, la taille de la couvée, le nombre d'œufs par saison reproductive et l'âge au premier œuf ont été de 131,85 ± 1,70 g, 7,40 ± 0,02 cm, 5,22 ± 0,02 cm, 30,30 ± 0,07 jours, 7,42 ± 0,08, 20,52 ± 0,38 et 313,22 ± 3,03 jours, respectivement. Le nombre d'œufs par saison reproductive a été significativement ($p < 0,05$) plus élevé chez le type marron que chez le type blanc d'oie. Cette étude sert de référence pour les traits morphométriques et la productivité des oies du Bangladesh.

Mots-clés: *indigène, oie, caractérisation phénotypique, types marron et blanc*

Resumen
El estudio pretendió identificar, caracterizar y describir la variabilidad fenotípica de las poblaciones de gansos autóctonos de Bangladesh. La investigación fue llevada a cabo en la upazila de Sylhet Sadar en el distrito de Sylhet y abarcó alrededor de 141 gansos (74 del tipo marrón y 67 del tipo blanco). La media por mínimos cuadrados ± el error estándar de la longitud del cuerpo, la envergadura de las alas, la longitud de los tarsos, la longitud del pico y la longitud de la cabeza de los gansos autóctonos maduros fueron 73,47 ± 0,95, 134,53 ± 1,38, 9,27 ± 0,09, 8,88 ± 0,10 y 6,42 ± 0,02 cm, respectivamente. Para todos los parámetros morfométricos, las medidas de los machos fueron significativamente mayores ($p < 0,01$) que las de las hembras pero no se hallaron diferencias significativas ($p > 0,05$) entre los dos tipos. El peso corporal de los gansos autóctonos a un día, dos semanas, un mes, dos meses y diez meses de edad fue, respectivamente, de 95,45 ± 0,88, 148,59 ± 1,55, 407,34 ± 7,27 g, 1,19 ± 0,03 kg y 3,65 ± 0,06 kg. Los machos fueron significativamente ($p < 0,01$) más pesados que las hembras a todas las edades, excepto en el caso de los ansarones de un día de edad, y no se detectaron diferencias significativas ($p > 0,05$), para el peso corporal, entre los dos tipos de gansos. El peso del huevo, la longitud del huevo, la anchura del huevo, el periodo de incubación, el tamaño de la nidada, el número de huevos en cada estación reproductiva y la

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edad al primer huevo fueron, respectivamente, de 131,85 ± 1,70 g, 7,40 ± 0,02 cm, 5,22 ± 0,02 cm, 30,30 ± 0,07 días, 7,42 ± 0,08, 20,52 ± 0,38 y 313,22 ± 3,03 días. El número de huevos en la estación reproductiva fue significativamente mayor ($p < 0,05$) en el tipo marrón que en el tipo blanco de ganso. Este estudio sirve de referencia en materia de rasgos morfométricos y productividad de los gansos de Bangladesh.

Palabras clave: *autóctono, ganso, caracterización fenotípica, tipos marrón y blanco*

Submitted 1 December 2015; accepted 19 October 2016

Introduction

Geese belong to the Family *Anatidae* and the Genus *Anser, they* were one of the first animals to be domesticated (Buckland and Guy, 2002). The Egyptian goose (Alopochen aegyptiacus) was tamed and possibly domesticated in Egypt in the 3rd millennium BC, but no domestic specimens of this species seem to have survived to modern days (Boessneck, 1960; MacDonald & Bench, 2000). Kear (1990) suggests that the disappearance of the Egyptian goose as a farm bird coincides with the Persian conquest of Egypt in the sixth century BC. The common domestic goose, nowadays found across the world, derives from the greylag goose (*Anser anser*), and, due to its pink beak, more likely from its eastern (*Anser anser rubirostris*) than western (*Anser anser anser*) subspecies (Harper, 1972; Crawford, 1984; Kear, 1990). In general, domestic breeds are much larger than their wild ancestors, although they have in many cases retained their ability to fly. There are two main types of domestic geese. The first are thought to have their origins in Europe, descendants of the wild Greylag goose (*A. anser*) and the second are thought to have their origins in Asia, descendants of the wild Swan goose (*Anser cygnoides*). Crosses between the domestic breeds, which have originated from these two species of wild geese are fertile and finally, these crosses have resulted in a number of recognized breeds (Buckland and Guy, 2002).

Geese can be raised effectively in integrated production systems especially in the humid tropics. The climate of Bangladesh is of tropical monsoon-type with a hot and rainy summer and a dry winter. In Bengali, the geese are known as "Raj Hash" (Sarker, 2015) due to their large size in comparison with other breeds of duck. Geese are usually found in the rural areas of tropical and sub-tropical countries where they are reared generally by the rural poor. Indigenous geese of Bangladesh are naturally very enduring, adaptive to rural surroundings. They can survive on little or no inputs, and adjust to variations in feed accessibility. Geese are among the fastest-growing avian species commonly raised for meat, large edible eggs and valuable products such as feather and down (National Research Council, 1991). Globally the ducks contribute 11 percent and geese 9 percent of the commercial poultry sector (Besbes, 2009). Though the contribution of the indigenous duck and geese is significant in the socio-economic development of the people especially of the coastal region of Bangladesh, research and pragmatic efforts for the preservation and amelioration of this species is at the elementary stage.

Characterization and inventory of poultry genetic resources are needed in countries where clearly defined poultry breeds are yet to be identified; hence properly designed scientific studies on indigenous duck and geese breeds need to be prioritized (Tixier-Boichard, Ayalew and Jianlin, 2008). Bangladesh are becoming seriously vulnerable due to the increasing rate of genetic loss resulting from numerous diseases such asaspergillosis, chlamydiosis, coccidiosis. Besides, lots of indigenous breeds are, however, on the margin of extinction because of their gradual dilution, as driven by market demands and incompatible apply of new breeding technology (Köhler-Rollefson, Rathore and Mathias, 2009). Inadequate awareness of the adaptive characteristics of indigenous livestock breeds due to insufficient scientific documentation is one of the key reasons for such dilution (Hassen *et al.,* 2007; Köhler-Rollefson, Rathore and Mathias, 2009). There are many indigenous livestock breeds that still need to be scientifically documented and characterized to make possible their conservation (Bhatia & Arora, 2005). To conserve these local breeds and ensure sustainable use of their genetic diversity, it is important to evaluate their phenotypic characteristics and performance in their home tracts and under traditional management conditions (Zarate, 1996). Phenotypic characterization of goose is very important in the way forward of preventing this valuable germplasm erosion. .In Bangladesh, most of these goose populations are non-descriptive types. Limited work has been done by some investigators on limited geese in specific region (Chittagong). But regrettably in Bangladesh, no remarkable effort has yet been taken to characterize goose. Considering the above facts and circumstances, the present study was designed to identify, characterize and describe the phenotypic variation of indigenous goose populations in Bangladesh.

Materials and methods

The experiment was conducted from February 2013 to December 2013 at Sylhet Sadar Upazilla in Sylhet district under the Department of Genetics and Animal Breeding,

Sylhet Agricultural University, Sylhet. The study area was selected on the basis of the availability of goose, favourable environment, easy communication facility and research interest of the university. In study area, there were about 100 households who reared goose. Majority of the respondents reported mating between that two type geese. Thus, the data for this research were collected directly from 40 households reared only pure brown and white type geese. The number of reared geese in selected areas varied from household to household. The geese were reared in free range system at farmer's home. There were no differences in feeding and management system of goose. In the absence of genuine practical and technical knowledge, geese are generally fed with rice grain according to traditional practice. The geese with goslings are provided with extra foods, which are usually kitchen leavings, i.e. rice leftover and rice bran. The feed supplements are generally served in earthen pottery, which are rarely cleaned and left without care. The geese are kept in backyards around the farmers' homes, which are often in marshy and damp areas with little protection. Geese are hardly provided water, as they obtain this from different natural sources such as ponds and water bodies. The houses used as night shelter are commonly made of locally available materials such as mud and wood/bamboo/corrugated sheet, while very few of the respondents had the night accommodation for their geese built with mortar and bricks.

In the study area, farmers do not use artificial incubation for the hatching of eggs, but depend on the natural way of hatching by the broody goose. After hatching, the goslings are allowed to the nearby ponds or any other water bodies to forage and roam freely with their mothers. A goose farmer may lose about twenty-five percent of his young stock during the brooding and the budding periods due to improper management system.

The morphological characteristics were measured from 42 geese (22 brown and 20 white types). The body weight of goose was measured with digital balance at day old, 2-week, 1-month, 2-month and 10-month of age. There were total 59-day old geese (32 brown and 27 white types) in 20 households. These geese were repeated at different ages for body weight trait. External marks were used to identify the goose. However, there were missed about 18 geese up to 10-month of age due to diseases and predators. The geese at different age group are shown in Figure 1, 2, 3, 4 and 5. The reproductive traits were collected from 40 geese (20 brown and 20 white types). Body length, wing span, beak length, head length, shank length and egg size (length and width) were measured with measuring scale and slide calipers. Egg weight was measured with digital balance. Incubation period was calculated from the duration of laying of last eggs of a clutch to the egg hatched. The colour of the body, feather at different regions, beak, skin, shank, eye, eyelid, eggs and shape of the body, beak and eggs were recorded carefully. For each goose under study, different record sheets with full details of each parameter were maintained. The data generated from this experiment were entered in Microsoft Excel worksheet, organized and processed for further analysis. Mean, standard errors (SE) and correlations were estimated with the help of Statistical Analysis System (SAS, 1998).

Results

Least-squares means with standard errors (LSMean ± SE) of body length, wing span, shank length, beak length and head length of mature indigenous goose are presented in Table 1. Males were significantly higher ($p < 0.01$) compared with their female counterparts in all body measurement parameters. No significant difference was found in white type and brown type goose.

LSMean ± SE of body weight of day old, 2-week, 1-month, 2-month and 10-month (mature weight) old goose are shown in Table 2. Males were significantly heavier ($p < 0.01$) than females in all age groups except day old gosling. No significant difference was found in all age groups in brown and white type goose.

LSMean ± SE of egg weight, egg length, egg width, incubation period, clutch size, number of eggs in a breeding season and age at first egg of indigenous goose are given in Table 3.

The phenotypic correlation among body length, wing span, shank length, beak length, head length and body weight of mature indigenous goose are provided in Table 4. The

Fig. 1 - Colour online

Figure 1. Day old gosling (left = brown type and right = white type).

Fig. 2 - Colour online

Figure 2. Gosling at 2 week of age (left = brown type and right = white type).

Fig. 3 - Colour online

Figure 3. Gosling at 1 month of age (left = brown type and right = white type).

highest correlation was found between body length and shank length. The lowest correlation was found between wing span and head length. All of the correlations were positive and low to high in magnitude.

The phenotypic correlation among body weights at different ages of indigenous goose are set out in Table 5. Phenotypic correlations among body weights at different ages ranged were positive and moderate to high in magnitude. The larger relationships were found between chronologically adjacent weights. The phenotypic correlation of body weight of day old gosling with the body weights at subsequent ages ranged from medium to high positive. The magnitude of this correlation declined

Fig. 4 - Colour online

Figure 4. Gosling at 2 month of age (left = brown type and right = white type).

Fig. 5 - Colour online

Figure 5. Goose at 10 month of age (left = brown type and right = white type).

Table 1. LSMean ± SE of morphometric characteristics of mature indigenous goose according to sex and colour type.

Factors	BDL (cm)	WS (cm)	SL (cm)	BL (cm)	HL (cm)
Sex	**	**	**	**	**
Male	79.08 ± 0.79 (19)	140.51 ± 1.66 (19)	9.86 ± 0.07 (19)	9.44 ± 0.10 (19)	6.53 ± 0.02 (19)
Female	68.88 ± 0.72 (23)	129.51 ± 1.52 (23)	8.79 ± 0.07 (23)	8.42 ± 0.09 (23)	6.33 ± 0.02 (23)
Type	NS	NS	NS	NS	NS
Brown	73.56 ± 0.74 (22)	134.15 ± 1.62 (22)	9.28 ± 0.07 (22)	8.84 ± 0.09 (22)	6.42 ± 0.02 (22)
White	74.40 ± 0.77 (20)	135.88 ± 1.56 (20)	9.37 ± 0.07 (20)	9.02 ± 0.10 (20)	6.44 ± 0.02 (20)
Overall	73.47 ± 0.95 (42)	134.53 ± 1.38 (42)	9.27 ± 0.09 (42)	8.88 ± 0.10 (42)	6.42 ± 0.02 (42)

NS, not significant; Figures in the parentheses indicate number of observations; BDL, body length; WS, wing span; SL, shank length; BL, beak length; HL, head length.
**, $p < 0.01$.

Table 2. LSMean ± SE of body weights of indigenous goose according to sex and colour type.

Factors	Body weights at				
	Day old (gm)	2-week (gm)	1-month (gm)	2-month (kg)	10-month (kg)
Sex	NS	**	**	**	**
Male	97.63 ± 1.36 (24)	153.54 ± 2.32 (22)	453.31 ± 8.31 (22)	1.39 ± 0.03 (19)	4.11 ± 0.05 (17)
Female	94.04 ± 1.12 (35)	145.52 ± 1.83 (35)	377.63 ± 6.93 (34)	1.05 ± 0.02 (28)	3.35 ± 0.04 (24)
Type	NS	NS	NS	NS	NS
Brown	95.25 ± 1.17 (32)	149.06 ± 2.00 (30)	414.61 ± 7.62 (29)	1.23 ± 0.03 (24)	3.77 ± 0.05 (22)
White	96.42 ± 1.29 (27)	150.00 ± 2.12 (27)	416.34 ± 7.84 (27)	1.21 ± 0.03 (23)	3.70 ± 0.05 (19)
Overall	95.45 ± 0.88 (59)	148.59 ± 1.55 (57)	407.34 ± 7.27 (56)	1.19 ± 0.03 (47)	3.65 ± 0.06 (41)

NS, not significant; Figures in the parentheses indicate number of observations.
**, $p < 0.01$.

Table 3. LSMean ± SE of reproductive characteristics of indigenous goose according to colour type.

Parameters	Brown type	White type	Overall	Level of significance
EWT (gm)	128.84 ± 2.33 (20)	134.87 ± 2.33 (20)	131.85 ± 1.70 (40)	NS
EL (cm)	7.37 ± 0.03 (20)	7.43 ± 0.03 (20)	7.40 ± 0.02 (40)	NS
EW (cm)	5.20 ± 0.02 (20)	5.25 ± 0.02 (20)	5.22 ± 0.02 (40)	NS
IP (days)	30.35 ± 0.10 (20)	30.29 ± 0.09 (20)	30.30 ± 0.07 (40)	NS
CS (no.)	7.50 ± 0.16 (20)	7.35 ± 0.14 (20)	7.42 ± 0.08 (40)	NS
NEBS	21.65 ± 0.48 (20)	19.40 ± 0.48 (20)	20.52 ± 0.38 (40)	*
AFE (days)	312.33 ± 4.29 (18)	314.17 ± 4.42 (17)	313.22 ± 3.03 (35)	NS

NS, not significant; Figures in the parentheses indicate number of observations; EWT, egg weight; EL, egg length; EW, egg width; IP, incubation period; CS, clutch size; NEBS, no. of eggs in a breeding season; AFE, age at first egg.
*, $p < 0.05$.

Table 4. Phenotypic correlation among body length, wing span, shank length, beak length, head length and body weight of mature indigenous goose.

Parameters	BDL	WS	SL	BL	HL	BW
BDL						
WS	0.65					
SL	0.86	0.61				
BL	0.58	0.37	0.63			
HL	0.55	0.26	0.49	0.52		
BW	0.64	0.43	0.70	0.69	0.67	

BDL, body length; WS, wing span; SL, shank length; BL, beak length; HL, head length.

Table 5. Phenotypic correlation among body weights at different ages of indigenous goose.

Body weights at	Day old	2-week	1-month	2-month	10-month
Day old					
2-week	0.76				
1-month	0.66	0.83			
2-month	0.62	0.70	0.86		
10-month	0.42	0.46	0.49	0.55	

Table 6. Phenotypic correlation among egg weight, egg length, egg width and incubation period of indigenous goose.

Parameters	EWT	EL	EW	IP
EWT				
EL	0.73			
EW	0.77	0.81		
IP	0.05	0.07	0.16	

EWT, egg weight; EL, egg length; EW, egg width; IP, incubation period.

Table 7. Colour and shape of different body parts of indigenous goose.

Parameters	Brown type	White type
Beak colour	Black	Orange
Eye colour	Dark brown	Blue
Eyelid colour	Black	Yellow
Skin colour	White	White
Head feather colour	Brown	White
Neck feather colour	Brown	White
Back feather colour	Brown with grey	White
Wing feather colour	Brown	White
Flight feather colour	Brown with grey	White
Tail feather colour	Brown with grey	White
Down feather colour	White	White
Shank colour	Yellow	Bright yellow or orange
Egg colour	White	White
Body shape	Long, broad, thick and the carriage was slightly upright	Long, broad, thick and the carriage was slightly upright
Beak shape	Long, broad and flattened	Long, broad and flattened
Egg shape	Oval	Oval

with age. The phenotypic correlation of body weight at 2-week of age with the body weights at subsequent ages ranged from medium to high positive.

The phenotypic correlation among egg weight, egg length, egg width and incubation period of indigenous goose are summarized in Table 6. The phenotypic correlation among egg weight, egg length, egg width and incubation period ranged from slightly positive to high positive. Lowest negligible correlation was found between egg weight and incubation period. Very high phenotypic correlations were observed among: egg length and egg width, egg weight and egg width, and egg weight and egg length. The colour and shape of different body parts of the goose are presented in Table 7.

Discussion

The body length of goose obtained in this study was lower than the findings of Madge and Burn (1987) and Dunning (1992). This may be due to the differences in breed and agro ecological area of goose rearing. The wing span of goose obtained in this study was lower than that reported by Ogilvie and Young (2004) for Graylag and Swan goose. There are a number of biological factors influencing wing span which will also influence the feather length, e.g. sex, age, population, abrasion of the feathers, moult and differences between years (Pienkowski and Minton, 1973; Visser, 1976). The shank and beak length of goose obtained in this study was in accordance with the findings of Madge and Burn (1987) and Carboneras (1992). However, the beak length of goose obtained in this study was higher than the value reported by Ogilvie and Young (2004). The present findings for body weight of day old gosling of lower than that reported by Wu (2014). Banerjee (2013) reported that the body weight of brown feathered goose and white feathered goose ranged from 2.97 to 4.4 kg, which is more or less similar to the present study. But the mature body weight of goose in this study was lower than the results of Sheikh (2013) who reported that the weight of mature Embden gander fell around 8–9 kg and the weight usually fell around 8 kg in Embden goose. The body weight of 3, 6, 9, 12 and 14-week old white Chinese geese were 1.68, 4.20, 5.74, 6.71 and 7.10 kg and those of Embden geese were 1.59, 3.80, 4.8, 5.80 and 5.95 kg, respectively (Leeson and Summers, 1991). Buckland and Guy (2002) found that the Chinese goose was relatively small in body size with mature males averaging 4.5 kg and females 4.0 kg. Diets with low nutrition supplied to geese in study area are most likely the cause for lower body weight found in present findings. In poultry, the weight of the newly hatched depends primarily on the weight of the egg from which it is hatched, a trait greatly determined by the genotype of the female; females that lay larger eggs may possess superior genetic profiles for size, growth or aggressiveness in competing for feed. Thus their offspring would receive a similar superior genetic endowment for these traits (Skogland and Seagar, 1952).

The egg weight of goose in this study was in agreement with the value reported by Mazanowski and Bernacki (2003) and Hugo (1995) but lower than that reported by Rosinski (2000); Willin (1995); Sidadolog (1999) and Leskanich and Noble (1997). Egg weight is influenced by the total egg production per year, sequence of egg in the clutch, level of protein in ration, feed and drinking water, ambient temperature, stable type and disease (Darwati *et al.,* 2010). Egg weight is also influenced by breed, body weight and sex (Ensminger, 1992). Individual egg weight is closely associated with the intake of nutrient such as methionine (Petersen *et al.*, 1983), protein (Summers and Leeson, 1985) and linoleic acid (Scragg, Logan and Geddes, 1987). The egg length and egg width are higher than the values reported by Upadhyaya and Saikia (2012). They also found a strong positive and significant relationship existed between egg length and width. Egg size varies with female age (Desrochers and Mcgrath, 1993), year (Perrins, 1970), seasonal variations (Coulson, 1963) and laying order (Murphy, 1994). Petersen, Chima and Horst (1976) and Kohne and Jones (1975) reported that high temperature and humidity lowers the egg weight and also egg production in geese.

The incubation period of goose obtained in this study was in agreement with the observations of Ralph (1969); Yuwanta (1999); Buckland and Guy (2002); and Melvin (2008). However, the present finding for the incubation of the goose is higher than the value that reported by Madge and Burn (1987). Several factors influence the length of the incubation period, e.g. breed, gender, age of eggs, the size of eggs and shell quality (Bell and Weaver, 2002).Clutch size varies widely both among and within bird species (Klomp, 1970). Different ecological factors can potentially determine inter-specific differences in clutch size in waterfowl (Anseriformes) and other birds (Rohwer, 1992 and Monaghan and Nager, 1997). The overall clutch size of goose in this study was similar to the findings of Lamon and Slocum (1922) for Canadian and Egyptian geese, but lower than the results of Upadhyaya and Saikia (2012) for Cotton Pygmy-goose. The number of eggs in a breeding season was in agreement with that of Embden goose reported by Wright (2010) while it was lower than those reported by Mazanowski, Dziadek and Adamski (2002). Banerjee (2013) reported that the less egg production in goose might be attributed to the breed, nutrition and most of all the high-temperature and humidity prevailing in the region. High temperature and humidity lowers the egg weight and also egg production in geese (Petersen, Chima and Horst (1976) and Kohne and Jones, 1975). The age at first egg of geese assessed in this study was similar to the values reported by Banerjee (2013). Lamon and Slocum (1922) found that Canadian and Egyptian geese did not lie until they were 3 years old. Buckland and Guy (2002) reported that Huoyan geese began to lay at approximately 240 days of age.

The positive and significant correlation between body weight with body length, wing span, shank length and head length suggests that selection for any of these body parameters will cause direct improvement in body weight (Bhowmik, Mia and Rahman, 2014). Many of the phenotypic correlations between body measurements were positive and high, which was also reported by Mancha (2004). If the positive phenotypic correlations translate into positive genetic correlations thus, selection for one will improve the other as a correlated response (Muhiuddin, 1993). Egg weight was positively correlated with egg length, egg width. Size of the hatching egg of broilers (and probably other chickens) influences body weight of chicks up to slaughter (Proudfoot and Hulan, 1981).

The present finding for beak colour of brown type goose was in agreement with that of brown Chinese goose

reported by Buckland and Guy (2002) and Swan goose found by Madge and Burn (1987). The beak colour of white type goose was orange, which supports the results of Buckland and Guy (2002) for white Chinese goose and Banerjee (2013) for white feathered goose. Hill (2010) reported that more than a dozen types of carotenoids are responsible for the colouration of orange and yellow beaks and phaeomelanin produced various shades of brown. Beak colour depends on a combination of the bird's hormonal state and diet. Colours are typically bright as the breeding season approaches and palest after breeding (Howell and Dunn, 2007). The colour of a bird's beak results from concentrations of pigments primarily melanins and carotenoids in the epidermal layers, including the rhamphotheca (Ralph, 1969). The eye colour of white type goose observed in this study was in agreement with that of a white Asian goose reported by Yuwanta (1999). The colour of shank, skin, and head, neck, back, wing, flight, tail and down feather of brown type and white type was in accordance with the finding of Banerjee (2013). The feather colour of white type goose supports the findings of Wright (2010). Gadow (1891) stated that shank colour became orange or yellow due to the presence of orange-yellow lipochrome pigment which, in concentrated form, gave an orange-yellow colour and in a more diluted form a light-yellow, even to an almost whitish colour. Upadhyaya and Saikia (2012) found that the Cotton Pygmy-goose eggs were light creamy white or light ivory white in colour, which partially supports the present findings. Lamon and Slocum (1922) reported that goose eggs were whitish in colour but may shade to a grey or buff tinge.

Conclusion

To conclude, this study provides a bench mark for the morphometric characteristics and performance of goose in Bangladesh. Consequently, as a genetic resource, it is imperative for the researchers, entrepreneurs and farmers should come forward for the conservation of this poultry species. Detailed information of different genres of geese needs to be amassed and evaluated to avert their possible degeneration and to uphold their prospect. Intensive and elaborate country wide studies should put emphasis on the genetic characterization of geese.

Acknowledgements

We are grateful to the Chairman, Department of Genetics and Animal Breeding, Sylhet Agricultural University, Bangladesh for providing all necessary instruments to carry out the study.

Statement of interest

Authors have no such conflict of interests.

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Animal Genetic Resources, 2016, 59, 47–54. © Food and Agriculture Organization of the United Nations, 2016
doi:10.1017/S2078633616000102

Mitochondrial DNA hypervariable region 1 diversity in Nigerian goats

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Summary
Goats make up the largest group of ruminant livestock in Nigeria and are strategic in bridging animal protein supply gap and improving the economy of rural households. The hypervariable region 1 (HVR1) of the caprine mitochondrial genome was investigated to better understand genetic diversity important for improving selection for animal breeding and conservation programs. We sequenced and analysed the mitochondrial DNA (mtDNA) HVR1 in 291 unrelated indigenous Nigerian goats (West African Dwarf (WAD), Red Sokoto (RSO) and Sahel (SAH)), randomly sampled from around the country, and compared them with the HVR1 sequences of 336 Indian goats and 12 other sequences in five different species in the genus *Capra* (*C. falconeri, C. ibex nubiana, C. aegagrus, C. cylindricornis* and *C. sibirica*). A total of 139 polymorphic sites from 291 individuals were captured in 204 haplotypes. Within and among population variations were 77.25 and 22.74 percent, respectively. Nigerian goats showed high genetic diversity (0.87) and high FST values, and separate from Indian goats and other wild species. Haplogroups in WAD separates it from RSO and SAH concomitant with a different demographic history. Clear genetic structure was found among Nigerian goat breeds with appreciable variation in mtDNA HVR1 region. This study grouped Nigerian goat breeds into two major groups suggesting two different demographic origins for Northern and Southern breeds. High genetic admixing denotes different maternal origins and in contrast to evidence from goats from Levant and Central Asia, where goats were originally domesticated.

Keywords: *genetic diversity, goats, hypervariable region, mitochondrial DNA, Nigeria*

Résumé
Les caprins constituent le plus grand groupe de ruminants domestiques au Nigéria et jouent un rôle stratégique dans l'approvisionnement en protéines animales et dans l'amélioration de l'économie des ménages ruraux. Une recherche a été menée à propos de la région hypervariable 1 (HVR1) du génome mitochondrial caprin dans le but de mieux comprendre l'importance de la diversité génétique pour améliorer la sélection dans les programmes d'amélioration génétique et de conservation des animaux. La région hypervariable 1 de l'ADN mitochondrial (HVR1) a été séquencée et analysée chez 291 chèvres indigènes du Nigéria, sans rapport entre elles (Naine d'Afrique Occidentale (NAO), Rouge de Sokoto (RS) et Sahel (S)), échantillonnées de manière aléatoire à travers le pays et comparées avec les séquences HVR1 de 336 chèvres indiennes et avec 12 autres séquences de 5 espèces différentes du genre *Capra* (*C. falconeri, C. ibex nubiana, C. aegagrus, C. cylindricornis* et *C. sibirica*). Un total de 139 sites polymorphes de 291 individus a été rassemblé en 204 haplotypes. La variation intra- et inter-populationnelle a été de 77,25 pour cent et de 22,74 pour cent, respectivement. Les caprins nigérians ont montré une grande diversité génétique (0,87) et des valeurs de FST élevées et différentes de celles des chèvres indiennes et de celles des autres espèces sauvages. D'après les haplogroupes, la chèvre NAO serait à séparer des populations concomitantes de RS et S avec une histoire démographique différente. Une structure génétique claire a été décelée entre les races caprines du Nigéria, avec une variation substantielle dans la région HVR1 de l'ADN mitochondrial. Cette étude a regroupé les races caprines nigérianes en deux groupes principaux, ce qui suggère deux origines démographiques différentes pour les races du Nord et du Sud. Le fort degré de mélange génétique dénote des origines maternelles différentes, contrairement à ce qui a été observé chez les chèvres du Levant et d'Asie Centrale, où les caprins furent d'abord domestiqués.

Mots-clés: *diversité génétique, caprins, région hypervariable, ADN mitochondrial, Nigéria*

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Resumen
Las cabras constituyen el mayor grupo de ganado rumiante en Nigeria y desempeñan un papel estratégico en el aporte de proteína animal y en la mejora de la economía de los hogares rurales. Se investigó acerca de la región hipervariable 1 (HVR1) del genoma mitocondrial caprino con el fin de comprender mejor la importancia de la diversidad genética para mejorar la selección en los programas de mejora y conservación animal. Se secuenció y se analizó la región hipervariable 1 del ADN mitocondrial (HVR1) en 291 cabras autóctonas de Nigeria no relacionadas (Enana de África Occidental (EAO), Roja de Sokoto (RS) y Sahel (S)), seleccionadas aleatoriamente a lo largo del país y comparadas con las secuencias HVR1 de 336 cabras indias y con otras 12 secuencias de 5 especies diferentes del género *Capra* (*C. falconeri, C. ibex nubiana, C. aegagrus, C. cylindricornis* y *C. sibirica*). Un total de 139 sitios polimórficos de 291 individuos se concentraron en 204 haplotipos. La variación intra- e interpoblacional fue de 77,25 por ciento y de 22,74 por ciento, respectivamente. Las cabras nigerianas mostraron una elevada diversidad genética (0,87) y unos valores de FST elevados, distintos de los de las cabras indias y de los de las otras especies salvajes. De acuerdo con los haplogrupos, la cabra EAO se desliga de poblaciones concomitantes de RS y S con una historia demográfica diferente. Se identificó una estructura genética clara entre las razas caprinas de Nigeria, con una variación apreciable en la región HVR1 del ADN mitocondrial. Este estudio agrupó las razas caprinas nigerianas en dos grupos principales, sugiriendo así dos orígenes demográficos distintos para las razas septentrionales y meridionales. El alto grado de mezcla genética denota orígenes maternos distintos, a diferencia de lo observado en cabras del Levante y Asia Central, donde se domesticaron originalmente las cabras.

Palabras clave: *diversidad genética, cabras, región hipervariable, ADN mitocondrial, Nigeria*

Submitted 25 October 2015; accepted 17 May 2016

Introduction

The domestic goat, *Capra hircus* L., also known as "poor man's cow" is an important livestock species that contributes to the economy of many developing countries through the production of meat, milk, fiber, and skin (MacHugh and Bradley, 2001; Abdul-Aziz, 2010; Rout *et al.*, 2012). In Nigeria, goats play diverse roles in the economy, culture and religion of the people, with wide distribution across all agro-ecological zones of the country from the coastal South to the Arid North (Okpeku *et al.*, 2011a, 2011b). Goats are the largest group of ruminant livestock in Nigeria totalling about 55.8 million (FAOSTAT, 2013), with various surveys showing that up to 85 percent of rural households, poor farmers, small business owners of both sexes and all age groups raise them (Bayer, 1986). Nigerian goats are able to tolerate harsh climates, thrive on poor-quality diets provided by scarce grazing on marginal lands (Okpeku *et al.*, 2011a, 2011b), and the presence of trypanotolerance in some breeds (Abdul-Aziz, 2010) are advantages for their production and management. Moreover, their small size, low maintenance requirements and short generation interval (Okpeku *et al*., 2011b) underscores their importance for increasing production efficiency in rural agricultural systems verses other livestock species (Amills *et al.*, 2009; Rout *et al.*, 2012).

Recent archaeological and molecular biological studies suggested that goats originated in West Asia. However, very little is known of the processes leading to their domestication such as the timing of population expansion and the dynamics of their selection pressures (Nomura *et al.*, 2013).

The hypervariable region I (HVR1) of mitochondrial DNA (mtDNA) contains highly informative polymorphic sites due to its: (1) simple maternal inheritance without recombination, (2) relatively rapid evolution rate; endpoints useful for diversity studies in many organisms and species (Brown *et al.*, 1986; Bradley *et al.*, 1996; Luikart *et al.*, 2001; Amills *et al.*, 2009; Zhong *et al.*, 2013; Akis *et al.*, 2014; Zhao *et al.*, 2014). The mtDNA polymorphisms have been largely applied not only to better understand phylogenetic relationships in many animal species, including cattle (Bradley *et al.*, 1996; Mannen, Nagata and Tsuji, 2001; Troy *et al.*, 2001; Kim *et al.*, 2010; Taberlet *et al.*, 2011), chickens (Niu *et al.*, 2002; Liu *et al.*, 2006), horses (Vilà *et al.*, 2001; Lira *et al.*, 2010) and goats (Mannen, Nagata and Tsuji, 2001; Sultana, Mannen and Tsuji, 2003; Chen *et al.*, 2005; Pereira *et al.*, 2005; Naderi *et al.*, 2007; Okpeku *et al.*, 2011a, 2011b; Zhong *et al.*, 2013; Akis *et al.*, 2014; Zhao *et al.*, 2014), but also to understand maternal origin and migration of livestock. Based on the phylogenetic analyses of caprine mtDNA polymorphisms, at least four mtDNA lineages (A–D) have been discovered, with the lineage A group being the most diverse and widely distributed in comparison with other lineages (Luikart *et al.*, 2001; Sultana, Mannen and Tsuji, 2003; Chen *et al.*, 2005; Akis *et al.*, 2014). Luikart *et al.* (2001) assessed the phylogenetic history and matrilineal population structure of 406 domestic goats representing 88 breeds across the world and identified three highly divergent lineages A–C. More recently, Naderi *et al.* (2007) analysed 2 430 domestic goat samples from all over the old world and identified three additional lineages (A–G).

Although a few studies examining the nuclear microsatellite DNA of Nigerian goats have been reported (Adebambo, 2003; Muema *et al.*, 2009; Okpeku *et al.*, 2011a, 2011b), there is no published report on mtDNA diversity in Nigerian goats. Therefore, we have investigated

Fig. 1 - B/W online

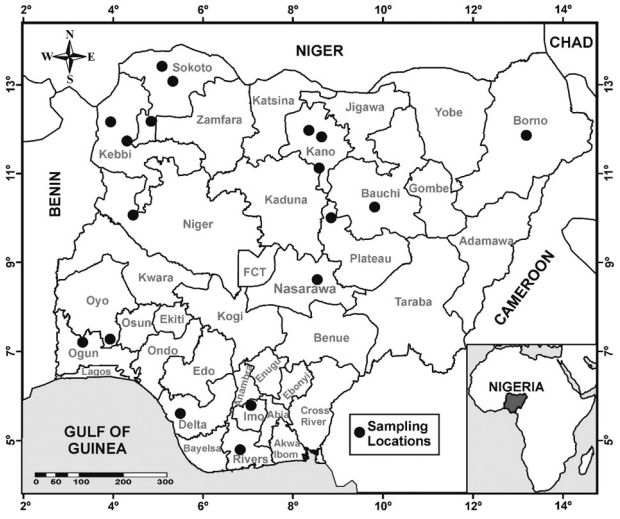

Figure 1. Map of Nigeria showing sampled locations.

the HVR1 of the caprine mitochondrial genome in three major Nigerian goat breeds sequences along with archived sequences from Africa and Asia available on NCBI to gain a better understanding of matrilineal origin of Nigerian goats, gain insight into demographic distribution and understanding goat domestication and adaptation. This will be important in facilitating the development of new approaches to improve selection and mating systems for animal breeding and conservation programmes.

Materials and methods

Sample collection and DNA isolation

Whole blood was collected from 291 unrelated goats belonging to three major goat breeds [140 Red Sokoto (RSO); 53 Sahel (SAH) and 97 West African Dwarf (WAD)]. Samples were collected from 19 different locations covering the five agro-ecological zones in the country (Figure 1). Efforts were made to collect samples from unrelated goats, based on the pedigree information provided by owners. Blood samples were collected by jugular venipuncture and DNA was isolated using ZymoBead™ Genomic DNA kit (Zymo Research Corporation, Irvine, CA, USA) following the manufacturer's instructions. Quantification of DNA yield and assessment of quality were done using a Nanodrop; ND-100 UV/Vis Spectrophotometer (Nanodrop Technologies, Inc., Wilmington, DE, USA).

PCR amplification and DNA sequencing

To amplify the HVRI of goat mtDNA, a pair of primers was designed using the known goat mtDNA sequence (AB044304, FAOSTAT, 2013) in the GENETOOL package and synthesized in an ABI 392 Oligosynthesizer (Perkin Elmer, Foster City, CA). Primer sequences were as follows: forward 5′-CATCCATATAACGCGGACAT-3′ and reverse 5′-GTGTGAGCATGGGCTGATTA-3′. PCR amplification was carried out in a 10 µl reaction volume containing 5 ng of DNA, 10 pM of each primer, 200 pM of dNTPs, 1× PCR buffer containing 2 mM $MgCl_2$, and 2 U of AmpliTaq Gold (Perkin Elmer, Foster City, CA). Amplification was performed in a Veriti thermal cycler (Applied Biosystems, Carlsbad, CA) employing the following conditions: 94 °C for 5 min; 35 cycles at 94 °C for 30 s, 54 °C for 45 s and 72 °C for 2 min; and final extension at 72 °C for 5 min. The amplicons were sequenced using BigDye™ (Applied Biosystems, Foster City) Terminator cycle sequencing kit in ABI Prism 3730 DNA Analyser (Applied Biosystems, Foster City) following manufacturer's protocol.

Data analysis

Five hundred and thirty three (503) base pairs from the mtDNA HVRI region of 291 goats of three Nigerian breeds, 366 sequences from 11 breeds of Indian goats, one breed each from Kenya, China and Korea and a sequences from Sheep (*Ovis aries*) was used as an outgroup (downloaded from NCBI; accession numbers AY155674 to AY156039, KJ420485, KJ420476, KJ420443, KJ420458, KJ420481, KJ420480.1 KJ420474, EU126792, AY853293, KP164694, KP164705, DQ188864, GQ141240, DQ188851, DQ121618, KP120664, KP120665, KP120646, KP120646, KP120622, EF490471 and AJ317864 to AJ31787 respectively), were aligned using ClustalW software package (Thompson, Higgins and Gibson, 1994). The MEGA 6.0 (Tamura *et al*., 2013) and DNASP software package version 5.10.01 (Librado and Rozas, 2009) were used for formatting the sequences to make them compatible with the desired software. Median joining networks (Bandelt, Forster and Rohl, 1999) were drawn using the Network 3.1.1.1 software (http://www.fluxus-engineering.com). Haplotype diversity, SE, Fu's Fs statistics (Fu, 1997), mismatch analysis (Schneider *et al.*, 1999), analysis of molecular variance (AMOVA), mean number of pairwise differences and population pairwise F_{ST} values were computed using ARLEQUIN version 2.001 (http://anthropologie.unige/arlequin) (Excoffier, Smouse and Quattiro, 1992). F_{ST} values were calculated using 1 000 bootstraps. These F_{ST} values were used to reconstruct an NJ/UPGMA tree in comparison with Indian and wild goat breeds using the MEGA package version 6.0 (Tamura *et al*., 2013). DnaSP program, version 5.10.01 (Librado and Rozas, 2009) was used for estimating haplotype and nucleotide diversity.

Results

mtDNA HVR1 variation in Nigerian goats

The sequences from the study have been deposited in GenBank and published with accession numbers KM582169–KM582416. Analysis of mitochondrial HVR1 sequences in Nigerian goats revealed 204 different

Table 1. Genetic diversity parameters for HVRI sequences of the Nigerian goat breeds.

	Diversity parameters										
Breed	**NS**	***H***	H_d	***K***	π	π_s	π_a	***S***	**SP**	**PIP**	**d*N*/d*S***
Wd	98	58	0.982	6.203	0.0259	0.0237	0.2708	76	42	34	1.0112
So	140	24	0.465	0.764	0.0070	0.0059	0.0078	23	9	14	1.3220
Sh	53	40	0.985	7.143	0.0204	0.0386	0.0154	56	27	29	0.3989
All sequences	291	7	0.245	0.270	0.0079	0.0158	0.0073	6	0	6	2.6524

NS, number of sequences; *H*, number of haplotypes; H_d, haplotype diversity; *K*, average number of nucleotide differences; π, nucleotide diversity π_s, synonymous nucleotide diversity; π_a, non-synonymous nucleotide diversity; *S*, number of polymorphic sites; SP, singleton variable sites; PIP, parsimony informative sites; d*N*/d*S*, Tajima ratio of non-synonymous to synonymous nucleotide diversity.

haplotypes. In addition to base substitutions and deletions, a 75 base insertion was observed in one individual belonging to WAD breed. The number of haplotypes found in each breed ranged from 24 in RS to 58 in WAD. Haplotype diversity values for the breeds were 0.465, 0.985 and 0.982 for RSO, SAH and WAD, respectively (Table 1). Transition–transversion ratios were found to be 1.6:1, 2:1 and 3.5:1 for RSO, SAH and WAD, respectively, while the total calculated for the study was 7.1:1. Median joining networks (Figure 2) were constructed based on the data obtained from transition and transversion ratios using the Network for phylogeny software. The network indicated considerable diversity, with the central node having the highest haplotype frequency of 105 made up of 3.81 percent SAH goats, 5.71 percent RS and 90.48 percent WAD. Of interest, other nodes in the network also contained more than one haplotype group.

Genetic diversity and population expansion

For comparison, a neighbour-joining (NJ) tree (Figure 3) was constructed using MEGA 6.0 (Tamura *et al.*, 2013), with Nigerian goat sequences, 363 Indian goat sequences and ten sequences from wild goats. All Nigerian goats clustered together at the top of the NJ tree and were followed by a cluster of Indian domestic goats. The wild goat types used as an out-group diverged from Indian

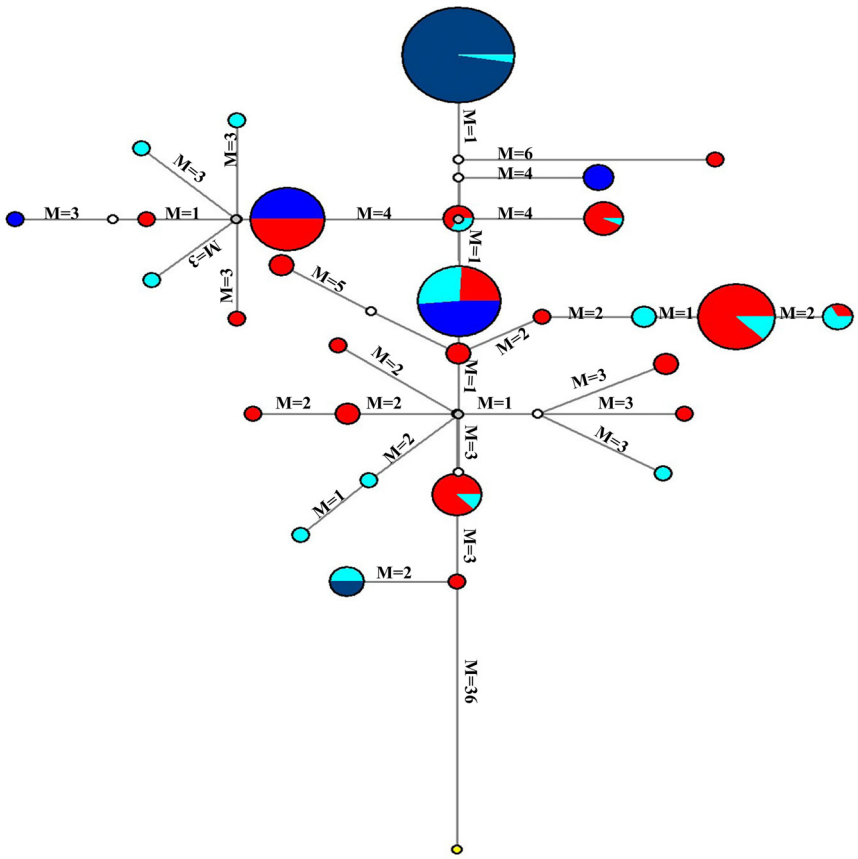

Fig. 2 - Colour online

Figure 2. Median joining network of goat mtDNA distribution among Nigerian goat breeds with sheep mtDNA as an out group. Circles represent haplotypes and have a size proportional to frequency. Mutational differences are shown on lines. Colour Legend: Red Sokoto = Red, West African Dwarf (WAD) = Deep Blue, Sahel = Sky Blue, sheep = Yellow.

Fig. 3 - B/W online

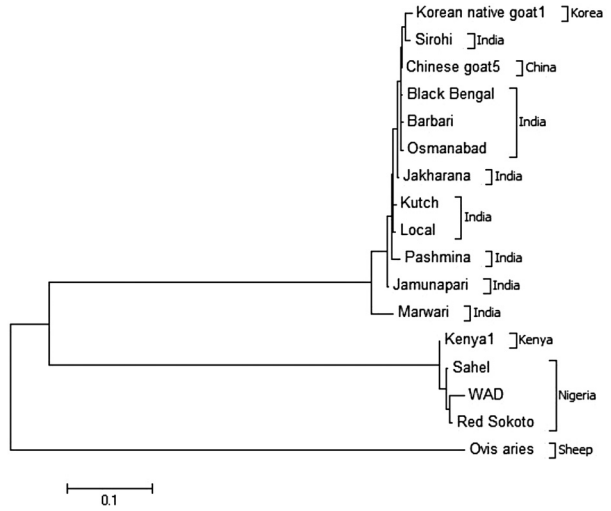

Figure 3. NJ tree of Nigerian, Indian and wild goat breeds compared with wild goat mtDNA sequences.

Table 2. Result of AMOVA among Nigerian goat breeds base on mtDNA HVR1 sequence.

Source of variation	Variance components	Percentage variation
Among breeds	1.346	22.749
Within breeds	4.573	77.251
Total	5.920	

goats as expected. However, clear differences were observed within the domestic goats of India and the Nigerian goat populations. In the Nigerian goat populations, WAD clearly separated from RSO and SAH goats. Further, genetic analysis based on AMOVA (Table 2) demonstrated that the largest genetic variation (77.25 percent) in all of these goat breeds is accounted for by within-group variation, while among-group variation accounted for approximately a quarter (22.75 percent) of the total genetic variation. Genetic distances between breeds were calculated taking into account the molecular distances between haplotypes, and the resulting F_{ST} values were positively correlated (Figure 3). Mismatch distributions (Figure 4) showed smooth and predominantly unimodal curves but not for WAD (Figure 4, No. 3). This breed appeared to have had a different demographic history from the other two studied breeds.

The results of DnaSP analysis indicated that the selected region (1–620) of the 291 sequences from the three Nigerian goat breeds have sites excluding sequences with gaps (35). The number of polymorphic sites, singleton variable sites and parsimony informative sites for individual breed sequences are higher than those for the entire sequences. SAH goat sequences had the highest value of haplotype diversity and average number of nucleotide difference (0.982 and 6.203), respectively followed by WAD goats. WAD had highest haplotype number (58) and nucleotide diversity (0.259) followed by SAH (40 and 0.204), respectively. Synonymous nucleotide diversity is less than non-synonymous nucleotide diversity in WAD and RSO goats (Table 1). The ratio of d*N*/d*S* was greater than 1.0 in WAD and RSO goats while less than 1.0 in SAH goats. The pictures of the three breeds of Nigerian goats used in this study are presented in Figure 5.

Discussion

The present work is the first substantial diversity study of Nigerian goats based on mtDNA HVR1 sequences. Nigerian goat mtDNA sequences showed some degree of diversity in the HVR1 region. The total number of haplotypes generated in this study for Nigerian goats was similar to the results reported in the literature, which supports a high degree of diversity in goat populations (Joshi *et al.*, 2004; Çinarkul and Ertugrul, 2011; Zhong *et al.*, 2013; Akis *et al.*, 2014; Zhao *et al.*, 2014). In Indian goats, Joshi *et al.* (2004) reported 200 haplotypes in 363 sequences from ten populations. Çinarkul and Ertugrul

Fig. 4 - B/W online

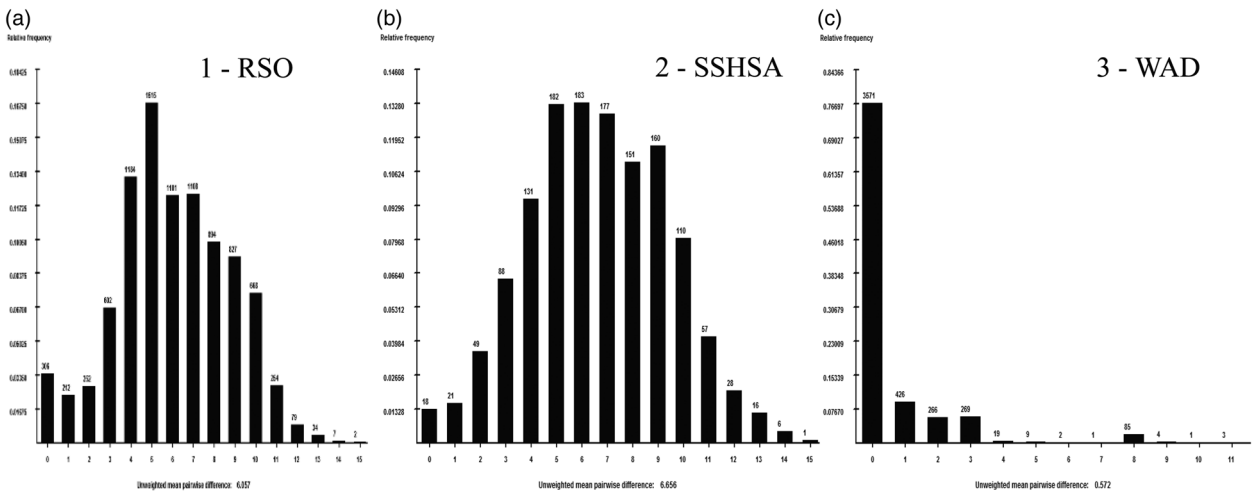

Figure 4. Mismatch distributions for mtDNA types of Nigerian goat breeds. (a) Red Sokoto, (b) Sahel, (c) WAD.

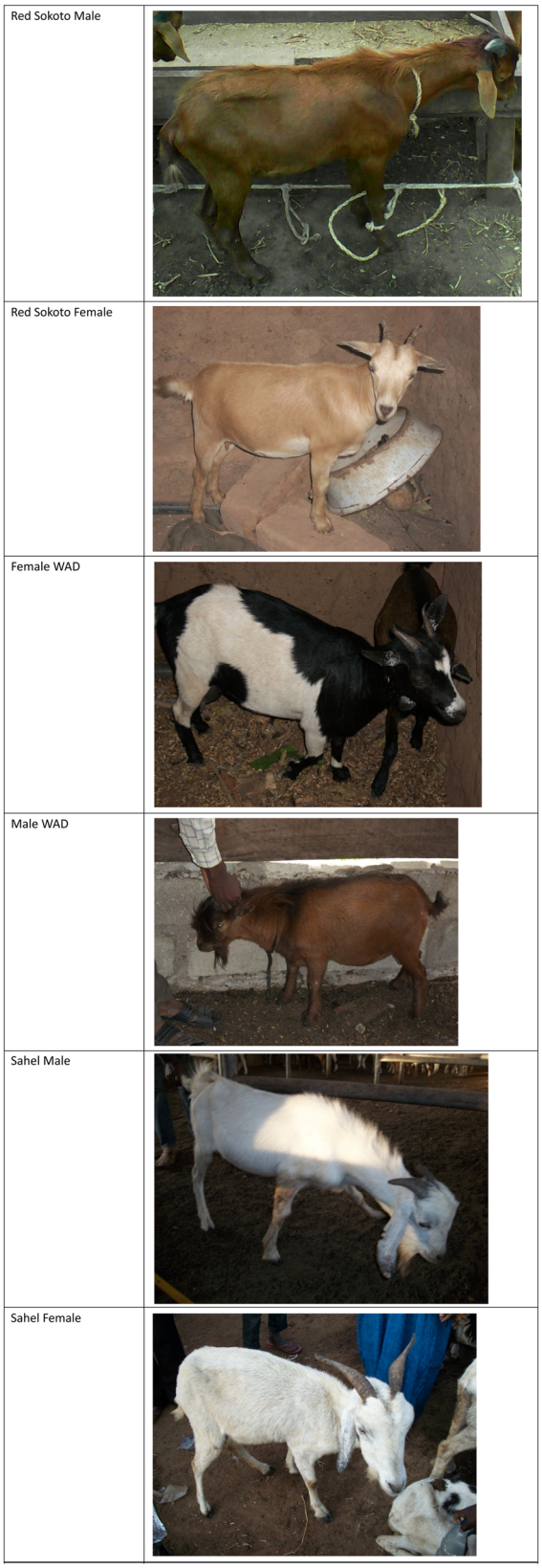

Fig. 5 - Colour online

Figure 5. Pictures of the three breeds of Nigerian goats.

(2011) reported 208 haplotypes in Turkish goat populations. These results also compare well with a recent study in Chinese goats, which observed a total of 192 haplotypes with 141 variable sites with haplotype and nucleotide diversities based on 481-bp segment of the mtDNA D-loop region, indicating a relatively high genetic diversity in Chinese black goats (Zhong *et al.*, 2013). Median joining network constructed from transition and transversion data revealed considerable diversity and interrelatedness among the different Nigerian goat's breeds in the present study. Okpeku *et al.* (2011a, 2011b) working with microsatellites reported interrelatedness in Nigerian goat breeds, which could be attributed to uncontrolled breeding among different breeds and a subsequent reduction in heterozygosity. This heterozygote deficiency have been reported to arise due to population sub-structure from pooling together different populations resulting in genetic admixture (Cerda-Flores *et al.*, 2002; Muema *et al.*, 2009; Rout *et al.*, 2012), overlapping generations, mixing of populations from different geographical locations and natural selection favouring heterozygotes or subdivision accompanied by genetic drift (Agha *et al.*, 2008). Similar results of high genetic diversity resulting from average expected heterozygosity (0.671 across loci, 10.7 alleles per locus) in Iranian goats accounting for the within-breed component (G_{ST} = 5.9 percent) have recently been reported using microsatellite DNA markers (Vahidi *et al.*, 2014). In addition, positive and highly significant F_{IS} values in the Naini, Turki-Ghashghaei, Abadeh and Markhoz breeds indicate some level of inbreeding in these populations (Vahidi *et al.*, 2014). Some studies indicate poor genetic diversity and low differentiation in Chinese domestic goat breeds (Li *et al.*, 1997; Jia *et al.*, 1999; Li and Valentini, 2004) in contrast to Nigerian goats.

Our results contrast somewhat with a recent study of HVR1 in Anatolian goats using 295 individuals that observed high genetic diversity values and a weak phylogeographical structure (Akis *et al.*, 2014), although it is consistent with overall findings of (Luikart *et al.*, 2001; Naderi *et al.*, 2007). Recent re-analysis of a consensus fragment of 481 bp in the D-loop region from 339 individuals of indigenous Chinese goat mtDNA showed high diversity but poor geographic specificity (Zhao *et al.*, 2014), although the network and NJ tree revealed three divergent maternal haplogroups (A, B1 and B2) in 17 local breeds captured in 198 different haplotypes. The discrepancies between published reports can be partially explained to differences in methodologies the investigators used in those aforementioned reports (Liu *et al.*, 2006). The NJ tree placed Nigerian goats into two distinct breeds or groups; the Northern (SAH and RSO) and Southern (WAD). Generally, similar classifications based on microsatellite markers obtained in earlier studies (Okpeku *et al.*, 2011a, 2011b) support our findings.

Within group variation was far higher and accounted for 77.25 percent of the genetic diversity observed in this study. High genetic diversity observed within breeds from mtDNA analysis can be attributed to maternal effect of multiple ancestors (Naderi *et al.*, 2007; Rout *et al.*, 2012; Akis *et al.*, 2014). In an analysis involving 2 430 individuals from all over the world, Naderi *et al.* (2007)

also reported that 77 percent of mtDNA variation is distributed within breeds. Similarly, in Indian goats, 83 percent of the total molecular variance was observed in the within-breed component (Joshi *et al.*, 2004), and more than 75 percent of the variations in Anatolian goats have been found between haplotypes. The higher within-breed variation observed has favoured genetic exchange, as goats are portable food resources that often accompany humans through migratory movements and trade routes (Azor *et al.*, 2005; Amills *et al.*, 2009; Liu *et al.*, 2009).

Mismatch distributions (pairwise comparisons) of mtDNA have also been widely used to explore demography (Rogers and Harpending, 1992). In the present study, mismatch analysis was done to evaluate population expansion among the different breeds, vital for predicting breeds with more population growth potentials. Populations with a constant size usually demonstrate ragged multimodal distribution, whereas a smooth, unimodal distribution is observed in expanding populations. The match of a real data set to these models can be assessed by the "raggedness" (Joshi *et al.*, 2004). In this study, the WAD goat breed showed a higher raggedness in this regard while the Northern breeds showed more uniform curves. These differences suggest a greater population expansion in the Northern population (SAH and RSO) than in the South (WAD). This suggests that the Northern goat breeds may have demographic origins different from WAD. However, distinctive demarcation of the breeds could also be attributed to the high genetic diversity of mtDNA HVR1 sequences. High polymorphic sites, singleton variable sites and nucleotide diversity were found in WAD, indicating that WAD exhibited the highest genetic diversity potentially more useful for selection. The higher genetic diversity of HVR1 in WAD may be related to its extensive adaptability and survival across the Southern Nigeria. In terms of nucleotide and haplotype diversities, SAH and WAD showed high values compared with other goat breeds worldwide (Luikart *et al.*, 2001; Pereira *et al.*, 2005; Naderi *et al.*, 2007). Higher non-synonymous nucleotide diversity relative to synonymous nucleotide diversity in WAD and RSO may be due to direct selection and a ratio of d*N*/d*S* greater than 1 suggests that variation at the mtDNA HVR1 is under positive selection (Kryazhimskiy and Plotkin, 2008).

In conclusion, a clear genetic structure was found among Nigerian goat breeds with appreciable variation in mtDNA HVR1 region. This study grouped Nigerian goat breeds into two major groups with higher within-breed variation than variation among breeds. Results suggest two different demographic origins for northern and southern breeds within goat populations in Nigerian. The present study suggests a high degree of genetic admixing among Nigerian goats arising from possibly different maternal origins, which is in contrast to evidence from goats in the Levant and Central Asia, where goats where originally domesticated.

Acknowledgements

We thank the International Foundation for Science (IFS), Stockholm, Sweden and the Indian Government through the Indian National Science Academy Junior Research Development Tata fellowship for financial support to Moses Okpeku. Additional support from the College of Agriculture and Life Sciences, Cornell University, Ithaca, NY is gratefully acknowledged.

Statement of interest

The authors declare that we do not have any conflicting interest during and after this research.

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Animal Genetic Resources, 2016, 59, 55–62. © Food and Agriculture Organization of the United Nations, 2016
doi:10.1017/S2078633616000114

Multivariate analysis for morphological traits of the Hamra goat population in two regions of Morocco

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Summary

The goal of this study was to characterize the Hamra goat population and to determine if Hamra goats of Beni Arouss and Rommani regions belong to the same population. Eleven morphometric traits of 157 Hamra animals (94 from Beni Arouss and 63 from Rommani) were used for this study. Overall, heart girth, body length, height at withers (HaW), height at rump (HS), chest depth (ChD), pelvis width (PW), chest width (CW), cannon circumference, head length (HeL), head width (HeW) and horn length (HL) of Hamra goats averaged 81.3, 61.5, 64.8, 65.3, 40.9, 19.3, 20.2, 9.67, 28.0, 26.3 and 23.4 cm, respectively. The effect of region was significant only on HaW, PW, HeL, HeW and HL, indicating certain homogeneity among goats of the two regions. Moreover, the inter region variance component ranged from 0 percent (absence of variability) for HS, CW, ChD and ChD to 18.5 percent for HeL, suggesting that the variability of body measurements between Beni Arouss and Rommani regions is very low. The factor analysis revealed four factors, which accounted for 73.5 percent of the total variance. The most discriminant variables between the two populations were HeL, HeW, PW and CW. The Mahalanobis distance between the two populations was 1.197, suggesting that there was genetic exchange between the two populations. The discriminant analysis showed that 80.9 percent of Rommani and 50.0 percent of Beni Arouss individuals were classified into their respective population. Results obtained will help in developing improvement and preservation strategies for the Hamra goat population.

Keywords: *Body measurement, goats, Hamra population, Morocco, multivariate analysis*

Résumé

Le but de cette étude est de caractériser la population caprine Hamra et de déterminer si les caprins Hamra des régions Beni Arouss et Rommani appartiennent à la même population. Onze mensurations corporelles de 157 caprins Hamara (94 de Beni Arouss et 63 de Rommani) ont été utilisées pour cette étude. Globalement, les moyennes du tour de poitrine, la longueur du corps, la hauteur au garrot, la hauteur au sacrum, la profondeur de la poitrine, la largeur du bassin, la largeur de poitrine, le tour de canon, la longueur de la tête, la largeur de la tête et la longueur des cornes des caprins Hamra sont respectivement de 81,3, 61,5, 64,8, 65,3, 40,9, 19,3, 20,2, 9,67, 28,0, 26,3 et 23,4 cm. L'analyse de la variance a révélé un effet significatif de la région sur la hauteur au garrot, la largeur du bassin, la longueur et la largeur de la tête, et la longueur des cornes, indiquant certaine homogénéité entre les caprins des deux régions. La composante de la variabilité entre régions a varié de 0% (absence de variabilité) pour la hauteur au sacrum, la largeur et la profondeur de poitrine et le tour de poitrine à 18,5% pour la longueur de la tête. Par conséquent, il y a une faible variabilité entre les caprins des régions Beni Arouss et Rommani. L'analyse factorielle a révélé quatre facteurs représentant 73,5% de la variance totale. Les caractères les plus discriminants sont la longueur de la tête, la largeur de poitrine, la largeur de la tête et la largeur de bassin. La distance de Mahalanobis entre les deux populations de caprins est égale à 1,197. L'analyse discriminante a montré que 80,9% et 50% des individus respectivement des populations Beni Arouss et Rommani sont correctement classés dans leurs populations d'origine. Les résultats obtenus vont aider à élaborer des stratégies d'amélioration et de préservation de la population Hamra.

Mots-clés: *Mensurations corporelles, Caprins, Population Hamra, Maroc, Analyse multivariée*

Resumen

El objetivo de este estudio es caracterizar la población de cabra Hamra y para determinar si Hamra cabras de las regiones Beni Arouss y Rommani pertenecen a la misma población. Once medidas corporales en 157 animales (94 Beni Arouss y 63 Romaníes) se utilizaron para este estudio. En general, la ronda del pecho, la longitud corporal, la altura de garrotte, la altura de sacro, la profundidad del pecho, la anchura del pelvis, la anchura del pecho, la circunferencia de cañón, la longitud de la cabeza, la anchura de la cabeza, y la longitud de los cuernos de cabras Hamra promediado 81.3, 61.5, 64.8, 65.3, 40.9, 19.3, 20.2, 9.67, 28.0, 26.3 y 23.4 cm, respectivamente. El análisis de la varianza reveló un efecto significativo de la región solamente en los caracteres: la altura del garrote, la anchura de la pelvis, la longitud y la anchura de la cabeza, y la longitud de los cuernos, lo que indica una cierta homogeneidad entre las cabras en las dos regiones. El componente de la variabilidad entre región varía desde 0% para la altura de sacro, la anchura y la profundidad del pecho, y la ronda del pecho (falta de variabilidad entre las regiones) hasta 18,5% para la longitud de la cabeza. Por lo tanto, hay poca variabilidad entre las dos regiones (Beni Arouss y Rommani). El análisis factorial reveló cuatro factores, que representa el 73,5% de la varianza total de los caracteres. Las características más discriminantes para separar las dos poblaciones son: La longitud de la cabeza, la anchura del pecho, la anchura de la cabeza, y la anchura de la pelvis. La distancia de Mahalanobis entre las dos

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poblaciones de cabras es igual a 1,197. El análisis discriminante ha mostrado que 80,9% y 50% de los individuos están correctamente clasificados, respectivamente, en la población Beni Arouss y Rommani. Estas informaciónes ayudarán a elaborar estrategias para la mejora y la preservación de la población Hamra.

Palabras clave: *Menstruaciónes corporales, Cabras, Población Hamra, Marruecos, Análisis multivariante*

Submitted 4 January 2016; accepted 20 May 2016

Introduction

The number of goats in Morocco was estimated to 6.5 million heads (FAO, 2014). Goat farming has a major economic role and contributes to the income of more than 70 percent of rural communities in the country (Chentouf *et al*., 2011). Goats are also important because they exploit marginal and harsh areas. Despite of their importance, information about Moroccan goat resources is scarce and mainly focused on the assessment of production and reproduction performance (Boujenane, 2008; Boujenane, Lichir and El Hazzab, 2010; Ibnelbachyr, Boujenane and Chikhi, 2015b). In Morocco, the Hamra goat is located in two different regions; Beni Arouss and Rommani that are distant from each other (about 400 km). The Hamra goats in both regions have a red coat colour. The head is strong often carrying twisted horns in both sexes, ears are often dressed in males and dropped in females. The neck is long, very often carrying wattles, and the tail insertion is low (Hilal *et al*., 2013) (Figure 1). Although animals of both regions have almost the same external appearance, the Ministry of Agriculture still hesitates to consider them as belonging to the same population.

The phenotypic characterization of local genetic resources depends on the variation of morphological traits, and the selection on them may constitute an effective tool to breed preservation and improvement (Nsoso *et al*., 2004; Sowande, Oyewale and Iyasere, 2010). Many authors highlighted the importance of using the multivariate analysis of morphological traits to assess phenotypic variation within and between goat populations (Herrera *et al*., 1996; Dekhili, Bounechada and Mannalah, 2013).

Therefore, the objective of this study was to characterize the Hamra goat population and to identify the relationship between Beni Arouss and Rommani populations through a multivariate analysis of morphological characteristics.

Materials and methods

Data collection

This study was carried out on Hamra goats raised in two regions of Morocco; Beni Arouss, located in the north (between latitudes 35°30′N and 35°33′N and longitudes 5°52′E and 5°63′E), and Rommani, located in the centre (between latitudes 33°35′N and 33°66′N and longitudes 6°31′E and 6°89′E) (Figure 2). Moreover, the Beni Arouss region is characterized by its mountains and high annual rainfall, whereas the Rommani region is characterized by its hills and low annual rainfall. From May to December 2012, 157 adult goats (2–5 years old); 94 in Beni Arouss (85 females and nine males) and 63 in Rommani (58 females and five males) regions were measured. These goats originated from 19 herds in Beni Arouss and nine herds in Rommani regions that were managed under an extensive system. In each herd, 4–12 adult goats were randomly selected and measured.

Figure 1. Photograph of Hamra goats.

Fig. 1 - Colour online

Studied variables

The studied variables were heart girth (HG), body length (BL), height at withers (HaW), height at rump (HS), chest depth (ChD), pelvis width (PW), chest width (CW), cannon circumference (CC), head width (HeW), head length (HeL) and horn length (HL). Measurements were taken to the nearest centimetre on animals placed on a flat ground using a tape measure and a calliper. Measurements were taken in the morning before the animals were released for grazing. All measurements were carried out by the same person in order to avoid between-individual variations.

Statistical analyses

Statistical analyses were carried out using the SAS/STAT package (SAS, 1999). Descriptive statistics for body measurements were obtained using the PROC MEANS.

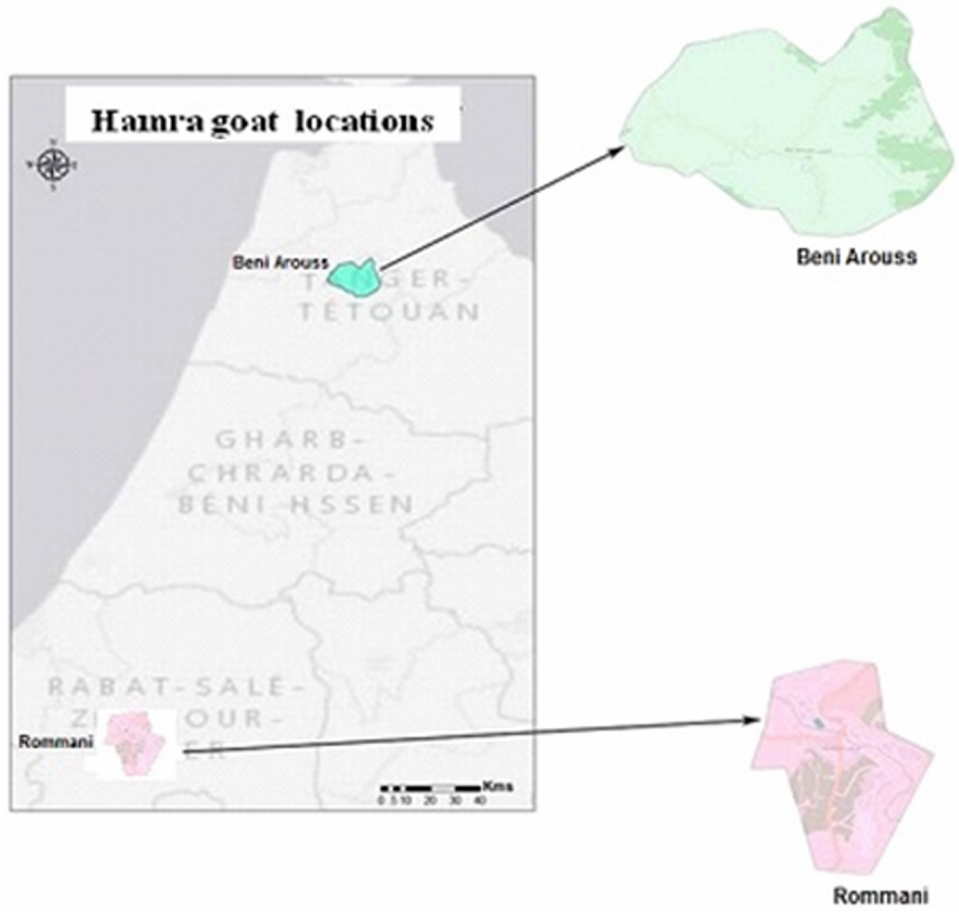

Fig. 2 - Colour online

Figure 2. Map of Beni Arouss and Rommani locations.

Environmental effects on body measurements were assessed using the PROC GLM. The mixed model used included the fixed effect of sex (female and male) and region (Beni Arouss and Rommani) and the random effects of herd nested within region to account for the non-independency of sampling in each region. To assess the between and within region variance components of body measurements, the PROC VARCOMP was used by fitting a model that included region as random effect. PROC CORR was also used to compute Pearson correlations among body measurements. Moreover, to extract a minimum number of uncorrelated components that account for most of the variance in the 11 traits, the factor analysis was realized using the PROC FACTOR. Moreover, the stepwise discriminant analysis was applied using PROC STEPDISC to determine the most discriminant traits. The significance level used for adding or excluding a variable was 0.15. Variables in the final model were then submitted to a canonical discriminant analysis, using PROC CANDISC, in order to derive canonical functions that are linear combinations of the morphological variables and compute the between regions Mahalanobis distance. The ability of the canonical functions to assign each individual to its populations was calculated as the percentage of correct assignment to each population using PROC DISCRIM.

Results

Arithmetic means and region effect

Arithmetic and least-squares means for body measurements are given in Table 1. Overall, HG, BL, HaW, HS, ChD, PW, CW, CC, HeL, HeW and HL of Hamra goats averaged 81.3, 61.5, 64.8, 65.3, 40.9, 19.3, 20.2, 9.67, 28.0, 26.3 and 23.4 cm, respectively. The coefficients of variation for different measurements ranged from 8.71 percent for ChD to 30.7 percent for HL.

The region did not have a significant effect ($P > 0.05$) on HS, CW, ChD, HG, BL and CC, but had a significant effect ($P < 0.05$) on HeL, HeW, HL, HaW and PW showing a certain homogeneity among the Hamra goats of the two regions. Rommani goat population had the highest values for HeL, HeW, HL and HaW. Differences were 1.5 cm for HeL, 1.2 cm for HeW, 2.6 cm for HL and 2.3 cm for HaW.

Table 2 shows the proportion of between region variability of Hamra population. It ranged from 0 percent (absence of variability between regions) for HS, CW, ChD and CC to 18.5 percent for HeL. Therefore, there was a low variability between goat populations of Beni Arouss and Rommani regions indicating a close relationship between them.

Table 1. Arithmetic means, coefficients of variation (CV), least-squares means ± SE for the 11 body measurements of Hamra goats according to region and sex[1].

Trait	Arithmetic mean (SD)[2]	CV (%)	Region		Sex	
			Beni Arouss	Rommani	Female	Male
Heart girth (cm)	81.3 (0.56)	8.71	80.5 ± 0.79	82.5 ± 0.75	80.5 ± 0.51[b]	87.6 ± 1.64[a]
Body length (cm)	61.5 (0.78)	16.0	61.0 ± 1.10	62.3 ± 1.08	61.1 ± 0.63[b]	65.6 ± 2.04[a]
Height at withers (cm)	64.8 (0.51)	9.80	63.9 ± 0.44[b]	66.2 ± 1.06[a]	64.7 ± 0.34[b]	68.4 ± 1.08[a]
Rump height (cm)	65.3 (0.58)	11.1	65.0 ± 0.42	65.7 ± 1.30	65.0 ± 0.47	69.6 ± 1.54
Chest width (cm)	20.2 (0.30)	20.8	20.5 ± 0.38	19.7 ± 0.48	20.0 ± 0.29	21.6 ± 0.95
Chest depth (cm)	40.9 (0.31)	9.55	40.8 ± 0.46	41.1 ± 0.37	40.7 ± 0.30	42.3 ± 0.97
Pelvis width (cm)	19.3 (0.31)	19.9	19.8 ± 0.46[a]	18.4 ± 0.31[b]	18.8 ± 0.32	22.2 ± 1.04
Cannon circumference (cm)	9.67 (0.09)	11.8	9.74 ± 0.11	9.55 ± 0.15	9.39 ± 0.08[b]	11.3 ± 0.28[a]
Head length (cm)	28.0 (0.20)	10.3	27.4 ± 0.27[b]	28.9 ± 0.26[a]	28.0 ± 0.18[b]	29.9 ± 0.60[a]
Head width (cm)	26.3 (0.21)	10.2	25.8 ± 0.29[b]	27.0 ± 0.29[a]	26.0 ± 0.20[b]	28.8 ± 0.63[a]
Horn length (cm)	23.4 (0.59)	30.7	22.4 ± 0.60[b]	25.0 ± 1.19[a]	22.2 ± 0.63[b]	33.7 ± 1.88[a]

[1]Least-squares means within a line for each factor that do not have a common superscript (a,b) are significantly different ($P < 0.05$).
[2]SD, standard deviation.

Table 2. Between region variance component, total variance and percentage of the between region variability.

Traits	Between region component	Total variance	Proportion of between region variability (%)
Heart girth	0.64	52.2	1.22
Body length	2.12	99.8	2.12
Height at withers	4.22	39.1	10.8
Rump height	0	48.1	0
Chest width	0	14.0	0
Chest depth	0	15.9	0
Pelvis width	0.03	14.2	0.19
Cannon circumference	0	1.04	0
Head length	1.41	7.62	18.5
Head width	0.91	6.67	13.7
Horn length	3.73	45.5	8.19

Correlations among measurements

Correlation coefficients among morphological traits of Beni Arouss and Rommani goats are shown in Table 3. High and significant correlations were found between HS and HeL in Beni Arouss (0.812) and between HS and HaW in Rommani populations (0.830). HeW in Beni Arouss and HG in Rommani regions were highly correlated with the other morphological traits. Also, the correlation coefficient between the ChD and HG was higher for Rommani goats (0.703) than for Beni Arouss goats (0.351). The lowest and significant correlation coefficient was recorded between HeW and BL in Beni Arouss region (−0.231) and between PW and CW in Rommani region (0.281).

Multivariate analyses

Results of the factor pattern and communality of the body measurements of Hamra goat population are reported in Table 4. The Kaiser–Meyer Measure of Sampling Adequacy, which determines the proportion of the variance in difference measurements caused by the underlying factors, was equal to 0.75. Four factors with eigenvalues >1 were extracted (Figure 3). They accounted for 73.5 percent of the total variance. The first factor explained 37.6 percent of variability, and loadings were highest for BL, PW, HeL and ChD. The second factor described 14.7 percent of the total variability. It was represented by significant positive high loading for HS, HaW and HeW. Factor three (HL, CW and HG) explained 12.2 percent of the total variance. CC was more associated with the fourth factor accounted for 9.0 percent of the variation. Likewise, variables' communalities, which represent the proportion of variance of each of the 11 variables shared by all remaining body measurements, were medium to high. They varied from 0.57 to 0.91 (Table 4).

The stepwise discriminant analysis showed that among the 11 measurements, HeL, CW, HeW and PW had the most discriminant power as indicated by their partial R^2, suggesting that only few measurements are needed to separate between the two populations.

The canonical discriminant analysis generated only one discriminant function (CAN1) that may be used to differentiate between the populations. The canonical function, which is the linear combination of the four most discriminant traits, is as follows:

$$\text{CAN1} = 0.475 + 0.490\ \text{HeW} + 0.635\ \text{HeL} - 0.375\ \text{PW} - 0.214\ \text{CW}.$$

Figure 4 shows an association among the individuals of Beni Arouss and Rommani populations. The first axis, which separated the two populations, indicated homogeneity among individuals of the two populations of Hamra breed. Moreover, the Mahalanobis pairwise distance between the two goat populations was equal to 1.197.

The percentage of classified individuals into their source population obtained from discriminant analysis was

Table 3. Pearson's correlation coefficients among measurements of Beni Arouss (above the diagonal) and Rommani (below the diagonal) goat populations[1].

Traits	HeL	HeW	HL	HaW	HS	CW	ChD	HG	BL	PW	CC
HeL		−0.019 ns	0.284**	0.192 ns	0.182 ns	0.132 ns	0.040 ns	0.418 ***	−0.231*	−0.134 ns	−0.111 ns
HeW	0.058 ns		0.566***	0.417***	0.442***	0.387***	0.544***	0.425***	0.644***	0.585***	0.657***
HL	0.248 ns	0.357**		0.440***	0.462**	0.311**	0.557***	0.421***	0.372***	0.306**	0.500***
HaW	0.533***	0.421***	0.445***		0.812***	0.205 ns	0.517***	0.470***	0.482***	0.380***	0.384***
HS	0.487***	0.374**	−0.022 ns	0.830***		0.181 ns	0.528***	0.415***	0.564***	0.365***	0.401***
CW	0.105 ns	0.087 ns	0.705***	0.314*	−0.121 ns		0.104 ns	0.316**	0.318**	0.384***	0.242*
ChD	0.406***	0.557***	0.502***	0.528***	0.455**	0.196 ns		0.351***	0.466***	0.400***	0.339***
HG	0.459***	0.444***	0.647***	0.532***	0.342**	0.444***	0.703***		0.282**	0.249*	0.298**
BL	−0.137 ns	0.534***	0.1798 ns	0.112 ns	0.153 ns	0.073 ns	0.451***	0.198 ns		0.691***	0.541***
PW	0.440***	0.285*	0.357**	0.454***	0.355**	0.281*	0.361**	0.237 ns	0.220 ns		0.472***
CC	0.329**	0.124 ns	0.450***	0.323**	0.138 ns	0.400**	0.227 ns	0.479***	−0.123 ns	0.313*	

[1]BL, body length; HaW, height at withers; HL, hom length; ChD, chest depth; CW, chest width; HG, heart girth; HeL, head length; HeW, head width; HS, rump height; PW, pelvis width; CC, cannon circumference.
ns, Not significant: $P > 0.05$.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

80.9 percent in Rommani and 50.0 percent in Beni Arouss. Overall, 34.5 percent of individuals were misclassified.

Discussion

The results on body measurements showed that Hamra goats have a medium size. These measurements were higher than those reported by Okpeku *et al.* (2011) on Red Sokoto goats (62.3 cm for HaW, 31.4 cm for ChD, 12.1 cm for HeW and 69.8 cm for HG) and Ibnelbachyr, Boujenane and Chikhi (2015a) on Draa breed in South of Morocco (61.5 cm for Haw, 74.4 cm for HG and 23.1 cm for HL). However, these measurements were lower than those found by Rodero *et al.* (2003) on Payoya Spanish dairy goats (77.7 cm for HaW and 87.7 cm for HG). Higher values for body measurements were also reported by Martinez *et al.* (2014) for Blanca Andaluza, Blanca Celtibérica, Negra Serrana, Pirenaica, Payoya, Murciano-Granadina and Malagueña Spanish goat populations. Further, the Hamra goats had a HaW similar to the value reported by Pires *et al.* (2013) for Rhâali goats (64.04 cm), but lower than those reported for Zagora (65.74 cm) and Drâa (71.21 cm) goats. Moreover, it had a ChD greater than those found by Pires *et al.* (2013) for Rhâali, Zagora and Drâa (30.85, 30.53 and 29.73 cm, respectively) goats. The morphological measurements of Hamra goats may be due to the varied raising environment.

The effect of region was not significant on HS, CW, ChD, HG, BL and CC, but significant on the other traits, suggesting that morphological measurements of Beni Arouss and Rommani goat populations are closely similar to each other. This result is not in agreement with that of Hagan *et al.* (2012) who reported that BL and HaW of the indigenous Ghana goats significantly differ in various locations and with that of Dekhili, Bounechada and Mannalah (2013) who confirmed that the raising area had a great impact on the morphological measurements of Algerian goat populations.

The percentage of the variability between regions ranged from 0 percent for HS, CW, ChD and CC to 18.5 percent for HeL. This result is lower than that reported by Aziz and Al-Hur (2013) for the proportion of variability between three Saudi goat types Ardi, Line1 and Line2 (0.01 percent for CC to 45.7 percent for HG).

As expected, correlations among morphological measurements of these goat populations were in general high, indicating a good association between these measurements. These results are in the range of those reported by Yakubu (2009), Okpeku *et al.* (2011) and Hassen *et al.* (2012).

Also, the high correlations among traits found in the current study highlighted the appropriateness of multivariate analyses.

The factor analysis showed that four factor explained 73.5 percent of total variance in Hamra goat breed. These

Table 4. Eigenvalues and % of total variance along with factor loadings and communalities of the body measurements of the two goat populations[1].

Body measurement	Factor1	Factor2	Factor3	Factor4	Communality
BL	0.86	0.08	0.09	−0.22	0.81
PW	0.75	0.08	0.14	0.23	0.65
HeL	0.68	0.27	0.34	−0.09	0.66
ChD	0.62	0.46	0.36	−0.25	0.79
CC	0.48	0.09	0.40	0.42	0.57
HS	0.29	0.89	−0.15	0.11	0.91
HaW	0.26	0.81	0.23	0.14	0.80
HL	0.23	0.20	0.79	0.05	0.73
CW	0.22	−0.19	0.71	0.23	0.64
HG	0.12	0.46	0.67	−0.06	0.67
HeW	−0.42	0.63	0.42	−0.08	0.76
Eigenvalue	4.51	1.76	1.46	1.08	
% of total variance	37.63	14.68	12.16	8.99	

[1]BL, body length; HaW, height at withers; HL, horn length; ChD, chest depth; CW, chest width; HG, heart girth; HeL, head length; HeW, head width; HS, rump height; PW, pelvis width; CC, cannon circumference.

Fig. 3 - B/W online

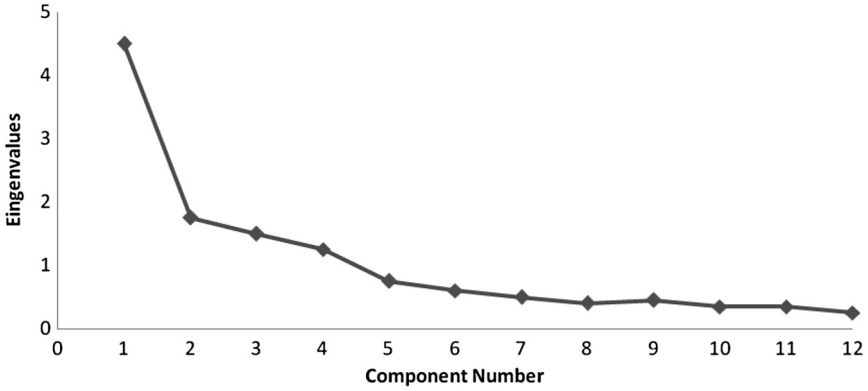

Figure 3. Scree plot of factor analysis for body measurements of Hamra goats.

proportions are lower than those reported by Rodero *et al.* (2003) (82.95 and 80.88 percent in Florida and Payoya Spanish dairy goats, respectively). Salako (2006) reduced the linear body measurements of immature Uda sheep in Nigeria to two factors that accounted for 75 percent of total variance. The communalities found in the present study indicated that all the traits had high loadings on factor 1, which is a good descriptor of general body size, while factors 2, 3 and 4 seemed to reflect the body shape and head size for the Hamra goat population.

The most discriminant variables selected through the stepwise discriminant analysis were HeL, HeW, PW and CW. These four measurements can be useful to differentiate between Beni Arouss and Rommani goat populations. As a result, the seven other body measurements had limited differentiation effects and hence might not be trusted in studying morphological studies to define the racial characterization of these two populations. Some of the discriminating variables obtained in the present study were similar to those reported by Herrera *et al.* (1996) in Andalusian breeds and Rodero *et al.* (2003) in Florida and Payoya Spanish dairy goats. Dekhili, Bounechada and Mannalah (2013) reported that BL, CW, ear length, rump height, HeL, rump width, tail length, BL and side depth would be more important in differentiating the Algerian goats. Herrera *et al.* (1996) concluded that two cephalic variables (length and width of the head) had the largest discriminant value.

Only one canonical variable was generated ($P < 0.001$) from the canonical discriminant analysis. A similar result was also reported by Yadav *et al.* (2013) for India sheep. The Mahalanobis distance between the two populations was very low (1.197), indicating a morphological similarity between the two goat populations. This similarity may be due to two distinct factors: the genetic exchange that might took place between the two populations in the past or the presence of a common or close origin. Yadav *et al.* (2013) reported that differences in Mahalanobis distances between four sheep breeds of southern peninsular zone of India were associated with differences in management practices, agro-climatic conditions and biophysical resources. Likewise, Dekhili, Bounechada and Mannalah (2013) reported a large Mahalanobis distance (8.6) between southern and northern goat populations in Algeria.

Fig. 4 - B/W online

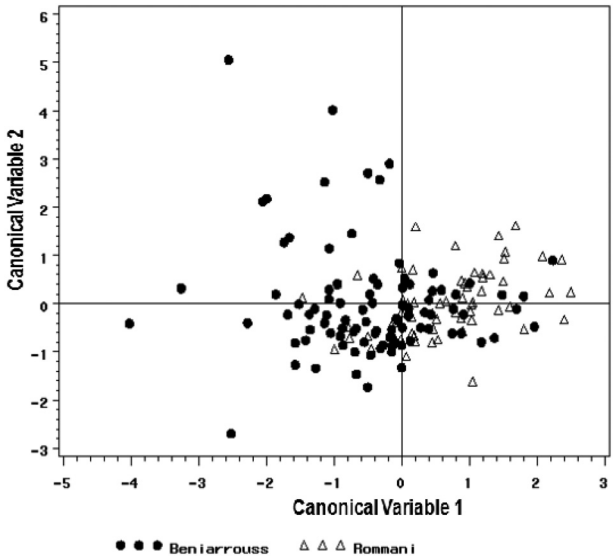

Figure 4. Canonical discriminant analysis for individual body measurements of Beni Arouss and Rommani goat populations.

Additionally, Pires *et al.* (2013) reported, in a Cluster evaluation of Brazilian and Moroccan goat populations, that the Euclidean distances between Drâa and Zagora, Drâa and Rhâali and between Zagora and Rhâali Moroccan populations were 1.12, 1.46 and 0.50, respectively.

The discriminant analysis showed a high misclassification error of individuals belonging to Rommani and Beni Arouss goat populations (34.5 percent). This indicates that on the basis of morphological measurements, there was similarity between the two goat populations. Rodero *et al.* (2003) reported that the misclassification error of Payoya and Florida breeds in Spain was almost nil. Dossa, Wollny and Gauly (2007) and Martinez *et al.* (2014) concluded that the degree of correct classification of goats in their zones depended on the high discriminating power of morphological measurements.

Conclusions

The results of this study showed that although Beni Arouss and Rommani goat populations are located far from each other, they are not morphologically so different, and may be considered as belonging to the same Hamra population. This information will help in developing improvement and preservation strategies for the Hamra goat population. Nevertheless, this morphological characterization should be confirmed by the molecular characterization.

Acknowledgements

We sincerely thank all the field officers and extension staff who assisted us in the survey work. We are also grateful to all the Beni Arouss and Rommani goat farmers who willingly provided unrestricted access to their farms and animals.

Conflict of interest

The authors have no conflict of interest regarding the research reported in this manuscript.

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Animal Genetic Resources, 2016, 59, 63–72. © Food and Agriculture Organization of the United Nations, 2016
doi:10.1017/S2078633616000187

Primary phenotypical characterization of the Pirot sheep from Stara Planina, Republic of Serbia: can we save the forgotten zackel?

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Summary

The Pirot sheep is a small Zackel that has been developed in the region of Pirot and the neighbouring municipalities in Serbia. Pirot sheep population has been reduced to only 60 animals in the Republic of Serbia. An overview of qualitative phenotypical and morphometrical characteristics of Pirot sheep from the Stara Planina is presented in this paper. The sheep included in this study belong to the last flock of the breed. The evaluation aims to obtain the phenotypical description of this indigenous breed as a phase of preservation strategy. Therefore, a total of 51 ewes and two rams were measured to obtain the detailed data concerning conformation. The phenotypical characteristics of animals included were also described. Based on the results, the Pirot sheep is a small breed with compact, slightly rectangular body frame (body length 115.40 percent of height at withers). The investigated sheep population was homogeneous, and morphological variations were limited to the data obtained in our research. The differences detected among different age groups were significant and reflected late maturing and slow growth of individuals. The comparison of the data determined by the evaluation of the modern population of Pirot sheep with the description from the older literature did not reveal that many significant changes of the morphological characteristics have occurred during the last 30 years. The small effective population and increasing inbreeding can threaten the efforts to preserve this sheep. The cultural heritage of the local community is also in danger due to the fact that the cornerstones of rural tradition in the area have been production of the three nationally important agricultural brands in Serbia – Pirot kilim (Pirot rug), Pirot/Stara Planina lamb and Pirot/Stara Planina Kachkaval cheese, all of which are depending on the Pirot sheep breeding. Additionally, some problems affecting the preservation of animal genetic resources in Serbia are reviewed with the focus on the Stara Planina. The research indicated that *ex situ* conservation should also be considered in the case of the Pirot sheep.

Keywords: *conservation, Pirot sheep, phenotypical characterization, Serbia*

Résumé

Le mouton Pirot est un petit mouton de type Zackel, qui a été développé dans la région de Pirot et dans des communes voisines en Serbie. La population de moutons Pirot s'est réduite à seulement 60 animaux dans la République de Serbie. Dans cet article, une présentation générale des caractères phénotypiques qualitatifs et des traits morphométriques des ovins Pirot du Grand Balkan est faite. Les moutons compris dans cet article appartiennent au dernier troupeau de la race. Dans le cadre de la stratégie de conservation, l'évaluation vise à obtenir la description phénotypique de cette race indigène. Ainsi, un total de 51 brebis et deux béliers ont été mesurés afin d'obtenir des données précises sur la conformation. Par ailleurs, une description des caractéristiques phénotypiques des animaux a aussi été faite. D'après les résultats, la race ovine Pirot est une race compacte de petite taille avec un format corporel plutôt rectangulaire (longueur du corps : 115,40 pour cent de la hauteur au garrot). La population ovine étudiée a été homogène et les variations morphologiques ont été rares dans les données obtenues dans cette recherche. Les différences décelées entre les différents groupes d'âge ont été significatives et ont reflété la maturité tardive et la croissance lente des individus. La comparaison des données générées en évaluant la population moderne des ovins Pirot avec des publications plus anciennes a révélé qu'il n'y a pas eu de nombreux changements significatifs dans les trente dernières années. La petite population efficace et l'accroissement de la consanguinité peuvent menacer les efforts pour conserver cette race ovine. L'héritage culturel de la communauté locale est également à risque en raison du fait que, dans cette zone, les piliers de la tradition rurale ont été les trois produits agricoles les plus importants en Serbie: le kilim de Pirot (tapis de Pirot), l'agneau de Pirot ou du Grand Balkan et le fromage Kashkaval, qui dépendent tous de l'élevage de moutons Pirot. En outre, certains problèmes qui affectent la conservation des ressources zoogénétiques en Serbie ont été examinés, avec l'accent particulièrement mis sur la région du Grand Balkan. L'étude a indiqué que la conservation *ex situ* devrait aussi être considérée dans le cas des ovins Pirot.

Mots-clés: *mouton Pirot, caractérisation phénotypique, conservation, Serbie*

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Resumen
La oveja Pirot es una oveja pequeña de tipo Zackel que se ha desarrollado en la región de Pirot y en municipios colindantes en Serbia. La población de ovejas Pirot se ha visto reducida a sólo 60 animales en la República de Serbia. En este artículo se hace una presentación general de las características fenotípicas cualitativas y de los rasgos morfométricos del ganado ovino Pirot de los Montes Balcanes. Las ovejas incluidas en este estudio pertenecen al último rebaño de la raza. Como parte de la estrategia de conservación, la evaluación pretende servir para obtener la descripción fenotípica de esta raza autóctona. Así, un total de 51 ovejas y dos carneros fueron medidos para conseguir información detallada sobre la conformación. También se llevó a cabo una descripción de las características fenotípicas de los animales considerados. De acuerdo con los resultados, la raza ovina Pirot es una raza compacta de pequeño formato, con un marco corporal ligeramente rectangular (longitud corporal: 115,40 por ciento de la altura a la cruz). La población ovina estudiada fue homogénea y las variaciones morfológicas fueron escasas en los datos obtenidos en esta investigación. Las diferencias detectadas entre los distintos grupos de edad fueron significativas y reflejaron la maduración tardía y el lento crecimiento de los individuos. La comparación de los datos generados mediante la evaluación de la población moderna de ganado ovino Pirot con respecto a publicaciones más antiguas desveló que no se han producido muchos cambios significativos durante los últimos treinta años. La pequeña población efectiva y el aumento de la consanguinidad pueden amenazar los esfuerzos por conservar esta raza ovina. El legado cultural de la comunidad local también está en peligro debido al hecho de que, en esta zona, los pilares de la tradición rural han sido los tres productos agrícolas más importantes en Serbia: Pirot kilim (alfombra de Pirot), el cordero de Pirot o de los Balcanes y el queso Kashkaval, todos ellos dependientes de la cría de ganado ovino Pirot. Asimismo, se han revisado algunos problemas que afectan a la conservación de los recursos zoogenéticos en Serbia, con especial atención a la región de los Balcanes. La investigación señala que la conservación *ex situ* también debería ser considerada en el caso del ganado ovino Pirot.

Palabras clave: *oveja Pirot, caracterización fenotípica, conservación, Serbia*

Submitted 13 January 2016; accepted 10 June 2016

Introduction

The Pirot sheep or Pirot pramenka is an indigenous Zackel traditionally reared/raised for centuries in the region of the South–Eastern Serbia. The geographic origin and breeding area of the Pirot sheep is directly associated with the following Serbian municipalities: Pirot, Knjazevac, Nis, Dimitrovgrad, Bela Palanka, Babusnica and even Crna Trava, Bosilegrad and Trgoviste or, more precisely, with the mountainous area of Suva Planina and Stara Planina. The most important historical breeding centre of the Pirot sheep was on the Stara Planina where, even today, the remaining population of this breed is located. Stara Planina – also known as the Balkan Mountains – occupies the central part of the Balkan Peninsula and represents the southern part of the Carpathian mountain range. It extends with its smaller, western part into the Republic of Serbia, while its eastern, larger part is in the Republic of Bulgaria. Mountain Stara Planina has been placed under protection as a nature park since 1997 ("Official Gazette of the RS" No. 19/97). This Act is a result of the recognition of the unique diversity of the local flora and fauna as well as the geological and hydrological characteristics of the mountain. Due to its agrobiodiversity and heritage, the area applied for the UNESCO Man and the Biosphere Reserve programme (MAB programme).

The Pirot area is famous for its unique traditional sheep herding, which over time became an integral part of the cultural and ethnic heritage that influenced the development of the rural community in this part of Serbia. Generally speaking, sheep breeding in this part of the country is rather extensive, and is focused on nomadic grazing during the summer and supplementary feeding of sheep with grains and forages during the winter. In the Pirot region, pastures (Figure 1) cover approximately 52.76 percent of the total land area, and are situated at the altitude of 600–2 000 m (Radoicic and Trkulja, 2012). Pastures are very poor in legumes (about 15 percent). The common grasses on the pastures of Mountain Stara Planina area are: *Agrostis vulgaris, Agrostis alba, Cynosurus cristatus, Anthoxanthum odoratum, Poa pratensis*, *Poa trivialis, Danthonia calyicina, Festuca vellesiaca*, so that the total digestive protein content available to the grazing sheep is very low and ranges from 4.18 to 13.57 percent (Ruzic-Muslic *et al*., 2006). In the Stara Planina and Suva Planina mountains, sheep graze from April to late November. The winter housing is modest (small wooden sheep pens) and confined animals consequently lose weight and decrease production due to poor winter nutrition. However, for many years this type of traditional sheep herding was economically justified due to the market demand of Kachkaval cheese production and famous tradition of Pirot carpet manufacturing. The Pirot lamb was the third local signature brand. The lamb meat was famous for its juiciness and tenderness, with its exceptional and well-recognized flavour. The sheep products from Pirot region were famous and exported worldwide at the beginning of the twentieth century.

The historical data concerning the characteristics of the Pirot sheep are scarce. The environmental conditions and poor pastures were the main factors that influenced the formation of Pirot sheep. The frame of Pirot sheep was small with the average withers height of 60 cm, harmoniously built, with the firm skeleton and sturdy constitution (Mitic, 1984), compact as body length (BL) was 108.7

Fig. 1 - Colour online

Figure 1. A typical pasture on the Stara Planina.

percent of the withers height, and with moderately developed chest. According to Mitic (1984), the body mass of an adult sheep was about 41 kg. Ewes of Pirot Zackel are polled. The lack of detailed description of the breed reflected the widespread policy that autochthonous animal breeds were "primitive", low in productivity and the signature of poverty. The selection of "improved animals" through crossbreeding with imported animals high in productivity was implemented as a compulsory measure of animal breeding and sheep reproduction practice in Socialist Yugoslavia.

Even so, the Pirot sheep was highly appreciated for its good milk and wool quality. The length of lactation was around 190 days, and the average milk yield per lactation was 77.5 kg (Mitic, 1984). The wool yield was very low – 1.4 kg per ewe, and 1.8 kg per ram (Mitic, 1984). The mandatory crossbreedings with Merino Precos and Wurtenbergin aiming to select larger animals for meat, increase the production of finer wool and preserve the milk production started in 1954, and the so-called "improved Pirot sheep" type was developed. However, certain important characteristics such as sturdiness were lost and, and at the same time, recognizable characteristics, i.e. milk taste, meat taste and suitability of wool for carpet manufacture were changed. The original sheep were bred in secrecy and obscurity in the very remote areas. The public awareness of the importance of traditional sheep production in the region was only recently recognized. During the last decade "Staroplaninski kachkavalj" and "Pirotski kachkavalj" have been certified as autochthonous hard cheese brands with protected geographic origin (The Intellectual Property Office of RS, 2010). According to the definition the "Staroplaninski kachkavalj" is a processed cheese made from fresh milk obtained from autochthonous Zackel sheep reared traditionally on Stara Planina and Suva Planina presuming that animals mentioned are supplied with the feed obtained in the defined geographic region and are kept grazing on high mountain pasture for at least 180 days/year. The "Pirot kachkavalj" can be dairy, sheep and/or goat hard cheese prepared from the milk of locally adopted animals reared under the same conditions as defined in case of Staroplaninski kachkavalj.

Since the selection strategy was compulsory and disregarded the sustainability of crossbred sheep both in regard to susceptibility to numerous diseases and the economic impact on small farmers, local production of sheep declined. Furthermore, the industrialization of the area attracted young people as a result of which the negative demographic trend has severely affected rural communities, and the local population abandoning villages caused a decrease in the number of sheep in the Pirot region. The original Pirot sheep population became gravely endangered. The demographic catastrophe of South–East rural areas in Serbia has started at the end of the Second

World War due to the social politics implemented throughout former Yugoslavia. Nowadays, mountain villages are mostly abandoned and only remnants of small sheep farms exist (Figure 2).

The conservation measures were introduced in the last decade of the twentieth century and included also the rural communities on the Stara Planina. At the same time local breeders have recognized the importance of indigenous sheep and identified the remaining examples of the original Pirot sheep. Moreover, with the support of the National AnGR authorities the remaining animals were registered and the condition of the population was rated as critical. Conservation authorities supported organized breeding and prevented the complete breed extinction. Nowadays around 60 individual Pirot sheep has been identified and included in one flock bred, controlled by the national authorities and according to the Serbian Plan for Animal Biodiversity Preservation for the period of 2011–2018 ("Official Gazette of RS" no. 13/2011; Stancic and Stancic, 2013). The risk of extinction of original Pirot sheep was the main reason for urgent inclusion of this population into the list of Serbian endangered AnGR. The original Pirot sheep flock has been identified on the territory of municipalities Dimitrovgrad and Pirot on Stara Planina, and together with the other autochthonous sheep breeds from Stara Planina, have been the subject of several studies hoping to obtain the data for genetic resources preservation (Stevanovic *et al.*, 2015; Stojiljkovic *et al.*, 2015). The site of Kamenica is actually located 30 km north of Dimitrovgrad and some 25 km from Pirot, near the border with the Republic of Bulgaria. It is a representative example of typical rural settlement on the Stara Planina. The remaining rural population is engaged exclusively in extensive animal breeding. In addition to the agriculture, some households are involved in the development of rural tourism. At the same time, milk and meat from the autochthonous sheep from this region are being evaluated for certification of autochthonous products of protected geographic origin.

Fig. 2 - Colour online

Figure 2. Remains of a sheep farm in Mount Stara Planina (local name: "pojata").

The aim of this study is to determine the phenotypical characteristics of the Pirot sheep from Stara Planina in Serbia. According to the current strategy recommended by FAO, the phenotypical characterization (FAO, 2012) is a prerequisite for the initiation of *in vivo* conservation of indigenous breeds. An overview of the greatest obstacles influencing the animal genetic resources preservation in Republic of Serbia, with the focus on Stara Planina, is also included in this paper.

Material and methods

The phenotypical investigation of the Pirot sheep was performed on the only flock of this breed that is under controlled breeding situated on the Stara Planina, the Kamenica village, municipality of Dimitrovgrad (Figures 3 and 4).

The phenotypical analysis included complete morphometrical investigation and qualitative body description performed upon the examination of 51 sheep and two breeding rams. The included individual sheep were divided into two age categories: category I (1–3 years) and category II (3–6 years) (FAO, 2012). The sheep were kept in a semi-intensive farming system. The nutrition was based on the fresh grass from regularly fertilized pastures with the addition of forages (hay) and grains. Body measurements were performed by the use of the zootechnic ribbon

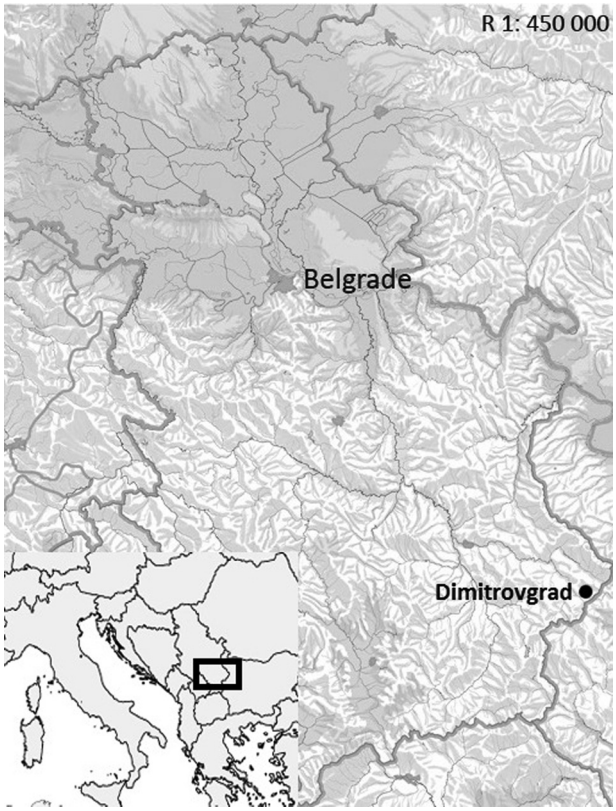

Fig. 3 - B/W online

Figure 3. Geographic location of Dimitrovgrad.

Fig. 4 - Colour online

Figure 4. Pirot sheep flock from Kamenica, Stara Planina.

(250 cm), and pelvimeter was used for cranial, pelvic and thoracic width and depth measurements. The following morphological parameters were measured: BL, from the point of the shoulder to the caudal margin of the pin bone; height at withers (HWi), the distance from the ground to the top of the withers; height at tuber coxae (HTC), the distance from the ground to the top of the *tuber coxae*; height at tuber ischii (HTI), the distance from the ground to the top of the *tuber ischii*; chest girth (CG), the circumference of the chest just behind the withers and shoulders; thoracic depth (TD), distance from the ventral edge of the sternum to the top of the withers; chest width (CW), the width between the lateral edges of the shoulder joints; head length (HdL), from the tip of the nose to the top of the occipital crest; front length (FL), from the imaginary line, which connects the medial eye corners to the top of the occipital crest; head width (HdW), the distance between two zygomatic arches; base of the ear height (BEH), from incisura vasorum to the ventral ear base; cannon circumference (CC), at the central of the front cannon; ear length (EL), lateral aspect of the ear from base to the tip; hip width (HpW), the distance between the coxal tubercles; coxo-femoral width (CfW), the distance between the coxo-femoral joints; bi-ischial width (BiW), the distance between the pin bones (*tuber ischii*); pelvic length (PL), from the hip to the pin bone; base of the tail-pelvic simphysis diameter; tail length (TL), from the base to the tip of the tail. Based on the measured parameters, the following conformational indices were calculated: body frame index – HWi/BL × 100; body compactness index – TGi/BL × 100; body massiveness index – TGi/Hwi × 100; over increase index – HTC/Hwi × 100; thoracic wide index – CW/TD × 100; thoracic depth index – TD/HWi × 100; head index – HdW/HdL × 100; Dactilo-thoracic index CC/TGi × 100; Cannon circumference index – CC/HWi × 100 and pelvic index – HpW/PL × 100 (Stojiljkovic *et al*., 2015). A total of 13 descriptive parameters were obtained: type of wool coloration, wool colour, type of fleece, ear orientation, head colour, head profile, hair/wool type on limbs, colour of limb wool/hair, pigmentation of the hoof, tail form, back profile, rump profile and udder type.

The statistical analysis of the parameters measured was performed by GraphPad Prism 5.0 program. In addition to the basic descriptive statistics and Student's *t*-test, the Pearson correlation coefficient was established to determine correlations between body parameters in the Pirot sheep population.

Results

The morphometrical parameters and statistical data determined in Pirot sheep were presented in Tables 1–4.

The data presented in Table 1 show that the average BL is 15.40 percent higher than HWi, thus reflecting compact

Table 1. Variability of morphometrical parameters in ewes of the Pirot sheep breed.

Parameter	*N*	$\bar{x} \pm SD$	SEM	CV (%)	Variance (VI)	90 percent percentile interval (90% PI)
Body length	51	69.61 ± 3.27	0.46	4.70	61.0–77.0	65.2–74.8
Height at withers	51	60.35 ± 2.64	0.37	4.38	55.0–67.0	57.0–63.8
Height at tuber coxae	51	61.71 ± 2.46	0.34	3.99	56.0–68.0	58.2–64.8
Height at *tuber ischii*	51	50.90 ± 2.27	0.32	4.45	45.0–56.0	48.0–54.0
Thorax girth	51	79.59 ± 4.17	0.58	5.24	70.0–91.0	73.4–85.0
Thorax depth	51	29.57 ± 2.53	0.35	8.56	23.0–35.0	26.2–33.0
Chest width	51	20.25 ± 1.07	0.15	5.31	18.0–23.0	19.0–21.8
Cannon circumference	51	7.20 ± 0.35	0.35	4.83	7.0–8.0	7.0–8.0
Tail length	51	27.65 ± 3.95	0.55	14.29	14.0–35.0	22.6–32.0
Body frame index	51	115.40 ± 4.88	0.68	4.23	105.2–125.0	109.6–123.0
Body compactness index	51	114.50 ± 6.40	0.90	5.59	100.0–128.1	105.4–123.1
Body massiveness index	51	132.00 ± 7.56	1.06	5.72	119.7–156.9	122.8–141.7
Overincrease index	51	102.3 ± 3.14	0.44	3.08	96.6–110.7	98.4–107.9
Thoracic wide index	51	68.93 ± 6.73	0.94	9.76	55.9–95.7	61.4–76.5
Thoracic depth index	51	48.96 ± 3.16	0.44	6.45	39.7–55.2	45.0–53.3
Dactyl-thoracic index	51	9.05 ± 0.44	0.06	4.82	8.2–10.0	8.5–9.6
Cannon circumference index	51	11.94 ± 0.61	0.09	5.09	10.9–13.8	11.2–12.7

Table 2. Variability of craniometrical parameters in Pirot sheep.

Parameter	*N*	$\bar{x} \pm SD$	SEM	CV	Variance (VI)	90 percent percentile interval (90% PI)
Head length	51	28.10 ± 1.15	0.16	4.09	26.0–30.0	26.0–29.9
Front length	51	15.71 ± 0.78	0.11	4.94	14.0–17.5	14.6–16.9
Head width	51	12.49 ± 0.70	0.10	5.63	11.0–14.0	11.5–13.4
Base of the ear height	51	9.64 ± 0.66	0.09	6.81	8.5–11.5	9.0–10.4
Ear length	51	10.44 ± 1.63	0.23	15.64	6.0–13.0	7.2–12.0
Head width index	51	44.48 ± 2.32	0.33	5.23	39.3–50.0	41.4–48.1
Skull width index	51	79.64 ± 4.83	0.68	6.06	68.8–92.9	73.7–86.1

and slightly rectangular body. The average HTC was 2.3 percent higher than HWi. The TD was 48.96 percent of HWi, This investigation revealed that the average CW was 33.55 percent of HWi, The average tail length was 27.65 cm. Body compactness and body massiveness indices suggest that the Pirot sheep has a strong and compact trunk. Low values of SD, SEM and coefficient of variation (CV) showed that the population studied was uniform.

According to the results presented in Table 2, it can be observed that FL is 55.91 percent, and HdW is 44.48 percent of HdL, on average. These data show that the Pirot sheep has relatively elongated and light head. The data presented in Table 3 shows that the pelvis in Pirot sheep is well developed, slightly elongated and relatively wide enough. The Pirot sheep has exclusively white wool (Table 5), although exceptional individual sheep with grey and black wool may occur. The colour of hair on the head is white – spotted with small and large pigmented black and brown patches around the eyes, mouth and nose. The craniometrical parameters show that the Pirot sheep has well developed head with slightly convex profile. Ewes are pooled, while rams are horned. Other distinctive breed characteristics are: short hair on the legs with pigmented black or brown spots, long cylindrical tail, nice, well-developed udder and pigmented hooves (Figures 5 and 6).

The body measures in ram 1 (2 years) and ram 2 (4 years) were the following: BL – 72.0 and 71.0 cm, HWi – 62.0 and 63.0 cm, CD – 33.0 and 33.0 cm, CW – 22.0 and 23.0 cm, CG – 82.0 and 84.0, HdL – 28.0 and 28.0 cm, FL – 15.0 and 15.0 cm, HdW – 13.0 and 14.0 cm, HTC – 61.0 and 62.0, HTI – 51.0 and 52.0, BEH – 10.5 and 10.5 cm, CC – 9.0 and 9.0 cm, horn length (HLr) – 45.0 and 65.0 cm, EL – 11.0 and 10.5 cm, HpW – 30.0 and 27.0 cm, coxo-femoral diameter (CfD) – 19.0 and 22.0 cm, BiW – 22.0 and 24.5 cm, PL – 25.0 and 25.0 cm and TL – 16.0 and 16.0 cm, respectively. Both rams are offspring of a single ram, which was identified when the preservation of Pirot sheep has been established. The lack of male reproductive individual can be a serious threat for the preservation of Pirot sheep.

The exterior characteristics of the modern Pirot sheep are similar to the historical data described by Mitic (1984). The Pirot sheep has exclusively white wool (Table 5), although exceptional individual sheep with grey and black wool may occur. The colour of hair on the head is white – spotted with small and large pigmented black and brown patches around the eyes, mouth and nose. The craniometrical parameters show that the Pirot sheep has well-developed head with slightly convex profile. Ewes are pooled, while rams are horned. Other distinctive breed characteristics are: short hair on the legs with pigmented black or brown spots, long cylindrical tail, nice, well-developed udder and pigmented hooves (Figures 5 and 6).

Discussion

The comparison of the data presented in Table 1 with historical descriptions of the Pirot sheep revealed some changes in morphometrical characteristics. The determined data showed that the average BL is 15.40 percent longer than HWi. This is slightly different from the literature data (8.70 percent, Mitic 1984). The HWi as estimated did not differ from the earlier description of Pirot sheep (Mitic, 1984), but both differed from the data obtained by Gutic *et al.* (2006), who suggested that the average

Table 3. Pelvimetrical variables in Pirot sheep.

Parameter	*N*	$\bar{x} \pm SD$	SEM	CV	Variance (VI)	90 percent percentile interval (90% PI)
Pelvic length	51	22.67 ± 1.29	0.18	5.68	20.0–27.0	21.0–24.0
Hip width	51	19.36 ± 1.02	0.14	5.27	18.0–21.0	18.0–21.0
Coxo-femoral width	51	21.43 ± 0.95	0.13	4.45	19.5–23.5	20.0–23.0
Bi/ischiadic width	51	8.25 ± 0.72	0.10	8.69	7.0–10.0	7.5–9.0
Tail-pelvic simphysis diameter base	51	22.67 ± 1.29	0.18	5.68	20.0–27.0	21.0–24.0
Pelvic index	51	85.57 ± 4.73	0.66	5.53	74.1–95.5	79.2–91.3
Pelvic slope index	51	42.62 ± 3.52	0.49	8.25	35.7–52.8	39.0–47.4

Table 4. Pearson's correlation between the selected morphometrical parameters in the Pirot sheep population.

Parameter	BL	HWi	TGi	TD	CW	HdL	CC	HpW
HWi	0.580***							
TGi	0.371**	0.327*						
TD	0.276	0.689***	0.149					
CW	0.321*	0.138	0.489***	0.172				
HdL	0.377**	0.433**	0.572***	0.383**	0.507***			
CC	0.359**	0.391**	0.540***	0.121	0.378**	0.477***		
HpW	0.385**	0.215	0.468***	0.306*	0.465***	0.344*	0.303*	
PL	0.474***	0.376**	0.358**	0.296*	0.379**	0.263	0.328*	0.459***

HWi of Pirot sheep was about 55 cm and that the average HTC was 2.3 percent higher than HWi. The TD of investigated animals was 48.96 percent of HWi, which is in accordance with the data by Mitic (1984). This investigation revealed that the average CW was 33.55 percent in relation to HWi, and this finding was slightly different from the literature data (29.42 percent, Mitic, 1984), probably due to improved breeding practice. The TL was in average about 4–5 cm shorter compared with the data by Mitic (1984). Body compactness and body massiveness indices suggest that the Pirot sheep has a strong and compact trunk. Low values of SD, SEM and CV showed that the studied population was uniform.

According to the results presented in Table 2, it can be observed that FL is 55.91 percent, and HdW is 44.48 percent of HdL, on average. These data show that the Pirot sheep has relatively elongated and light head. The data presented in Table 3 showed that the pelvis in Pirot sheep is well developed, elongated and relatively wide enough, considering the average mass of the lambs at birth (3.4–3.9 kg, Mitic, 1984). The craniometrical and pelvimetrical variability in the Pirot sheep has not been investigated

Table 5. Qualitative phenotypic traits of Pirot sheep from Stara Planina.

Parameter	Result	*N*	%
Type of wool colouration	Unicoloured	51	100.00
Wool colour	White	51	100.00
Type of fleece	Open	51	100.00
Ear orientation	Horizontal	36	70.59
	Slightly lowered	15	29.41
Head colour	White	16	31.37
	White with black patches around ears and eyes	8	15.69
	White with black spots	6	11.76
	White with black patches around eyes	5	9.80
	White with black patches around nose	5	9.80
	White with brown patches around eyes	4	7.84
	White with brown patches around eyes and ears	2	3.92
	White with brown patches around eyes and nose	2	3.92
	White with brown spots	1	1.96
	Black and white	1	1.96
	Brown	1	1.96
Head profile	Slightly convex	47	92.16
	Convex	4	7.84
Hair/wool on limbs	Short hair[1]	51	100.00
Colour of limb wool/hair	White	26	50.98
	Black and white	10	19.61
	White with black spots	5	9.80
	Brown and white	5	9.80
	White with brown spots	2	3.92
	Brown	2	3.92
	Black	1	1.96
Pigmentation of the hoof	Partially pigmented	36	70.59
	Pigmented	11	21.57
	Non-pigmented	4	7.84
Tail form	Cylindrical	51	100.00
Back profile	Flat	51	100.00
Rump profile	Slightly curved	51	100.00
Udder type	Properly developed	51	100.00

[1]Short hair up to the elbow joint on front, and slightly above tarsal joint on rear limbs in majority of studied individual sheep.

Fig. 5 - Colour online

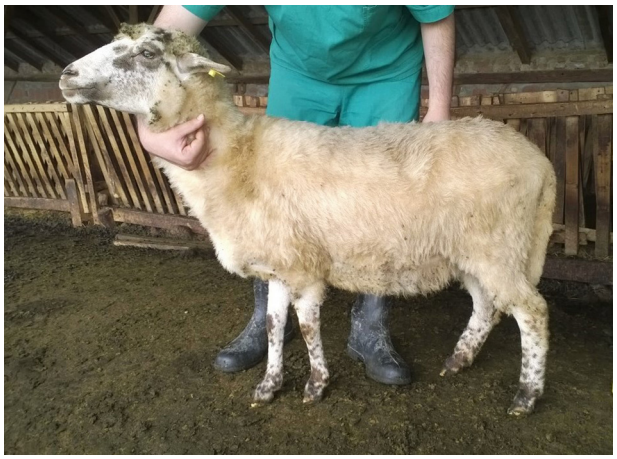

Figure 5. Pirot sheep ewes in the examined population – white head with black patches around eyes and nose.

before. The results obtained from craniometry and pelvimetry were similar to those determined for the Karakachan sheep (Stojiljkovic *et al.*, 2015), another Zackel from Mount Stara Planina (Table 6).

A statistically significant difference between the different age categories was established for the following parameters: BL ($P < 0.001$), CW ($P < 0.001$), front length ($P < 0.001$), thoracic girth ($P < 0.01$), bi/ischiadic width ($P < 0.01$), CfW ($P < 0.01$), tail length ($P < 0.01$), base of the tail-pelvic simphysis diameter ($P < 0.01$), HWi ($P < 0.05$), head width ($P < 0.05$) and hip width ($P < 0.05$). Body indices between age groups did not differ significantly. The Pirot sheep is slow-growing and late-maturing breed, as well as the other Zackel sheep in the region.

The results obtained by analysing the correlations between some morphometrical parameters evaluated in Pirot sheep are shown in Table 4. The existence of significant positive correlations was established between a large numbers of the studied parameters. The high correlation coefficients between selected parameters indicate that the Pirot sheep

Fig. 6 - Colour online

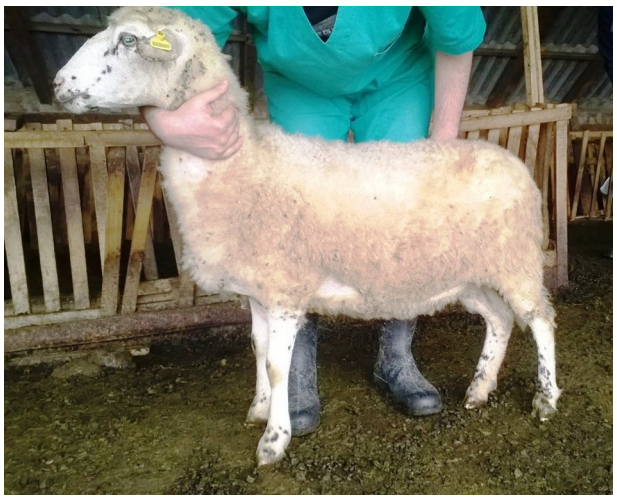

Figure 6. Pirot sheep with white head and black spots.

Table 6. Comparison of selected morphometrical parameters between Pirot and Karakachan sheeps.

Body parameter	Pirot sheep ($N = 51$)	Karakachan sheep (Stojiljkovic *et al.*, 2015, $N = 97$)	*t*
Body length	69.61 ± 3.27	70.60 ± 4.16	0.67
Height at withers	60.35 ± 2.64	61.26 ± 2.74	0.47
Thorax girth	79.59 ± 4.17	79.12 ± 4.23	0.64
Thorax depth	29.57 ± 2.53	30.40 ± 2.07	2.14*
Chest width	20.25 ± 1.07	21.58 ± 1.51	5.59***
Tail length	27.65 ± 3.95	28.95 ± 4.25	1.81
Head length	28.10 ± 1.15	27.64 ± 1.21	2.24*
Head width	12.49 ± 0.70	13.03 ± 0.68	4.55***
Pelvic length	22.67 ± 1.29	23.54 ± 1.17	4.15***
Cannon circumference	7.20 ± 0.35	8.18 ± 0.33	16.81***
Body compactness index	114.50 ± 6.40	112.30 ± 5.95	2.08*
Thoracic wide index	68.93 ± 6.73	71.17 ± 4.44	2.43*
Dactyl-thoracic index	9.05 ± 0.44	10.36 ± 0.55	14.71***
Cannon circumference index	11.94 ± 0.61	13.36 ± 0.57	14.05***
Head width index	44.48 ± 2.32	47.19 ± 2.43	6.55***
Pelvic index	85.57 ± 4.73	88.29 ± 4.04	3.67***

*$P < 0.05$ – significant **$P < 0.01$ – very significant ***$P < 0.001$ – extremely significant.

is very harmoniously conformed, reflecting balanced physical development (growth) and also revealing that the breed has adapted to the environmental conditions through the history of the breed. The lack of variation and differences in some morphometrical parameters studied could arise problems sequential to inbreeding that cannot be avoided due to extremely small number of breeding animals included in a single flock. The population status did not allow the comparison with other Pirot sheep.

The comparison (Table 6) of the morphometrical parameters obtained for Pirot sheep has revealed significant differences from the data established for Karakachan sheep, another local small Zackel from Stara Planina (Stojiljkovic *et al.*, 2015). There were no significant morphometrical variations observed for body parameters described within each breed, which means that both tested sheep populations were morphologically uniform and homogeneous but significantly different in regard to morphological characteristics and phenotype.

The phenotype of modern Pirot sheep is similar to the original breed described by Mitic (1984). The facial spots, leg hair and spotting, together with very light convexity of the head and the type of fleece and wool were among the important traits that have been used for identification of original Pirot sheep when the preservation programme was established.

According to the descriptive phenotypical data obtained in the investigation of the modern Pirot sheep population, we can say that no major differences between this Zackel and Karakachan sheep, another important autochthonous sheep population from Stara Planina, were observed (except

colour of wool). Over 90 percent of the sheep in Serbia is concentrated in small farms, with the variable structure and size of the flock, mainly in rural mountainous areas (Petrovic *et al*., 2011). The agriculture and animal breeding management strategy of the Republic of Serbia is facing a huge challenge: how to ensure the connection between the endangered breeds and sustainable sheep production in the future? Among future tasks, the conservation and revival of rural areas is the most important process that can influence the development of animal breeding and agriculture together with the biodiversity preservation. The previous experience in the selection did not bring the expected results, mostly due to depopulation of the rural area which affected the number of total domestic animals and also the number of indigenous animals and traditional production in all the rural areas. The question: "Can we preserve Pramenka?" can be rephrased: "Can we save Mountain Stara Planina?". The future strategy can be effective only after the project sustainability and financial investment are ensured. Rural development policies in Serbia have several tasks to preserve autochthonous animal populations (including Pirot sheep), i.e. changes in the people's behaviour, promotion of sustainable agriculture in accordance with local natural resources, further improvement of traditional products made from autochthonous breeds and efficient marketing campaigns, promotion of rural tourism, protection of nature and landscape, and strategies of decentralization (Stojanovic, 2008). Several centres and individual farms were formed to allow preserving the last herds of indigenous domestic animal breeds. Such centre is located on Stara Planina (Stojanovic and Pavlovic, 2003). Shortly after the foundation of the conservation farms, the arising problem was the financing and sustainability of those farms. Disinterest of the local population in traditional animal farming and bad product marketing were the main reasons for economic difficulties that occurred on farms. The Ministry of Agriculture and Environmental Protection has developed a strategy for the period from 2011 to 2018 in the Republic of Serbia (Official Gazette of RS, no. 13/2011) with well-defined strategic areas, objectives and activities for animal biodiversity preservation. According to this national document, the animal resources play an important role in the Serbian biodiversity plan. Furthermore, only "*in situ"* method was introduced for the preservation of autochthonous and indigenous breeds of mammalian and avian species (Stancic and Stancic, 2013).

There are two certified autochthonous animal products with protected geographic origin derived from the Pirot sheep – Stara Planina Kachkaval cheese and Pirot rug/kilim. These products are registered in the Serbian Intellectual Property Institute, and their basic source is the Pirot sheep. Unfortunately, this advantage of the Pirot sheep is not used in an efficient way because of poor marketing.

A possible success in preserving the Pirot sheep would be to promote the organic production system. According to Petrovic *et al*. (2011), sustainable approach to sheep production in Serbia and agriculture in general seeks to strengthen family farms, as well as to protect and exploit natural resources. This can be achieved with the Pirot sheep because of its triple-purpose production potential (milk–wool–meat), acclimatization capacity and breeding tradition, as well as the high-quality autochthonous wool, milk and meat products. Also a project by the Animal Husbandry Institute in Zemun, Serbia, proved that Pirot sheep could be the genetic basis for new, modern, highly productive breeds. MIS sheep has been developed at the Institute as a product of 20-year-long research in which the crossbreeding of the following two breeds were used Pirot sheep and Ile de France (Petrovic *et al*., 2014). This is an example of selective breeding for intensive modern sheep farming with the use of new technologies in lowland parts of Serbia.

Conclusion

The phenotypical characterization of the modern Pirot sheep population described in this paper provides new data necessary for future preservation programs and policies. In addition to that a survey among experienced agrotechnical specialists has drawn attention to the Pirot sheep's capacity and potential for future breeding and preservation of rural tradition in Serbia, especially in the Stara Planina and similar rural areas rich in mountain pastures, but with poor housing and winter care.

The established phenotypical data of the modern population of Pirot sheep in the area of Stara Planina did not differ from the description of the original sheep population mentioned in older literature, which means that the population examined may serve as the foundation for the programmes of preservation and can be the nucleus for the stabilization of the population on Stara Planina.

There is also the need for the implementation of breed preservation program as an important part of biological and traditional heritage of human rural population in Stara Planina and the whole Pirot region.

Although "*In situ"* conservation is an ongoing process and the Pirot sheep are carefully bred by the farmers devoted to preservation of biodiversity, the importance of "*Ex situ*" conservation should also be accentuated in the case of Pirot sheep, considering the small number of individuals, lack of the breeding rams and also bearing in mind that the remaining flock can be put in danger if an outbreak of infectious diseases came to be.

The depopulation of rural areas will remain the main obstacle in the progress of animal breeding, and therefore the socio-economic changes in the form of repopulation and local region revival must precede the rural development. Hence, the development of animal breeding as a priority must be taken into account as a long-lasting strategy. Short-term projects will only delay the extinction of endangered animal breeds and final demographic degradation of rural regions in Serbia for a short period of time.

Acknowledgements

This research was supported by the Ministry of Education, Science and Technological Development of Serbia (Grant number III 46002) and Public Veterinary Institute "Dr Vaso Butozan" Banja Luka, Bosnia and Herzegovina.

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Animal Genetic Resources, 2016, 59, 73–80. © Food and Agriculture Organization of the United Nations, 2016
doi:10.1017/S2078633616000217

Genetic relationships of indigenous goats reared by pastoralists in Kenya based on mitochondria D-loop sequence

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Summary
Kenya indigenous goat breeds (*Capra hircus*) have not been accurately described. Therefore, there is threat of erosion of unique genotypes such as those associated with adaptability and disease resistance, through indiscriminate crossbreeding. The Kenyan goats classification based on phenotype/morphology identifies three breeds: Small East African (SEA) goats, the Galla goat and crosses of SEA and the Galla. In the present study, we sampled goats from two main geographic regions of Kenya with pastoralist communities, the Maasai and Somali/Boran. DNA was extracted from whole blood and polymerase chain reaction amplified using primers flanking a fragment of Cytocrome-b and D-loop regions of mitochondria DNA. The sequences derived were analysed both within Kenya goat populations and also compared with phylogeographic-related datasets. These data show that the majority of Kenyan indigenous goats are not distinct and their genetic structure is very diverse; however, distinct haplogroups were present. Genetic diversity showed weak positive in Tajima D test for Kenyan indigenous goats, while the Iberian/Mediterranean/Middle-East dataset had a more pronounced negative value indicating that the two populations are under different selection pressure. These analyses enabled phylogenetic relationships between and within species and the comparisons of local goats to related breeds geographically. The information can be applied management of conservation-guided breeding programmes by crossing the indigenous breed's unique genes with high productivity traits from another source.

Keywords: *Indigenous goats, Kenya, mitochondrial DNA, phylogenetics*

Résumé
Les races caprines (*Capra hircus*) indigènes du Kenya n'ont pas été décrites avec précision. Ainsi, il existe une menace d'érosion de génotypes uniques, tels que ceux en rapport avec l'adaptabilité et la résistance aux maladies, du fait des croisements incontrôlés. Le classement des caprins kényans selon le phénotype/morphologie identifie trois races: Naine d'Afrique Orientale (NAO), Galla et les croisements entre NAO et Galla. Dans cette étude, les populations caprines de deux des principales régions géographiques du Kenya présentant des communautés pastorales (les Maasaï et les Somalis/Boran) ont été échantillonnées. De l'ADN a été extrait du sang et amplifié par PCR avec des amorces encadrant un fragment du Cytochrome b et les régions des boucles D de l'ADN mitochondrial. Les séquences obtenues ont été analysées au sein des populations caprines du Kenya, ainsi que comparées avec des ensembles de données phylogéographiques connexes. Les données ont montré que, dans l'ensemble, les populations caprines indigènes du Kenya ne sont pas distinctes et que leur structure génétique est très variée. Pourtant, la présence de différents haplogroupes a été décelée. La diversité génétique a présenté un résultat légèrement positif au test D de Tajima pour ce qui est des caprins indigènes du Kenya, alors que l'ensemble de données Ibérique/Méditerranéen/Proche-Orient a obtenu une valeur négative plus marquée, ce qui indique que les deux populations se trouvent soumises à différentes pressions de sélection. Ces analyses ont permis d'établir les relations phylogénétiques inter- et intra-espèce et de comparer les caprins locaux avec des races géographiquement connexes. Ces informations peuvent être appliquées à la gestion de programmes de conservation cherchant l'amélioration génétique par le croisement de gènes uniques des races indigènes avec des caractères de haute productivité en provenance d'autres sources.

Mots-clés: *caprins indigènes, Kenya, ADN mitochondrial, phylogénie*

Resumen
Las razas caprinas (*Capra hircus*) autóctonas de Kenya no han sido descritas con precisión. Por ello, existe un riesgo de erosión de genotipos únicos, tales como los relacionados con la capacidad de adaptación y la resistencia a enfermedades, debido a los cruzamientos indiscriminados. La clasificación de las cabras kenianas en base al fenotipo/morfología distingue tres razas: cabra Enana de África Oriental (EAO), cabra Galla y cruces entre EAO y Galla. En el presente estudio, se muestrearon cabras de dos de las principales regiones geográficas de Kenya con comunidades pastoriles, los Masáis y los Somalíes/Boran. Se extrajo ADN de la sangre y se

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amplificó mediante PCR usando cebadores contiguos a un fragmento de Citocromo b y a las regiones de los bucles D del ADN mitocondrial. Las secuencias derivadas fueron analizadas en el seno de las poblaciones caprinas de Kenya y comparadas con conjuntos de datos filogeográficos relacionados. Los datos muestran que la mayoría de las cabras autóctonas de Kenya no son distintas y que su estructura genética es muy diversa. Aun así, se detectó la presencia de diferentes haplogrupos. La diversidad genética arrojó un resultado levemente positivo en el test D de Tajima en el caso de las cabras autóctonas kenianas, mientras que el conjunto de datos Ibérico/Mediterráneo/Oriente Próximo dio un valor negativo más pronunciado, lo que indica que las dos poblaciones se hallan bajo distintas presiones selectivas. Estos análisis permitieron establecer las relaciones filogenéticas inter- e intra-especie y comparar las cabras locales con razas geográficamente relacionadas. Esta información puede ser aplicada a la gestión de programas, con la vista puesta en la conservación, que persiguen la mejora genética mediante el cruzamiento de genes únicos de las razas autóctonas con caracteres de alta productividad procedentes de otras fuentes.

Palabras clave: *cabras autóctonas, Kenya, ADN mitocondrial, filogenia*

Submitted 14 December 2015; accepted 21 July 2016

Introduction

The domestic goat is one of the most important livestock species most adaptable and geographically wide-spread, ranging from the mountains of Siberia to the deserts and tropics of Africa (Porter, 1996; Zeder and Hesse, 2000; Luikart *et al*., 2001; Pereira and Amorim, 2010). Their inherent characteristics such as resistance to dehydration, preference for browse and wide-ranging feeding habits, enable them to thrive in regions that receive <750 mm of rainfall (Devendra and McLeroy, 1982). Goats are valued primarily for the production of meat, milk, skins and fibre and have fulfilled agricultural, economic, cultural and religious roles from very early times in human civilization (Joshi *et al*., 2004; Dong *et al*., 2013).

Studies on domestic goat (*Capra hircus*) origins and genetic diversity are still not conclusive. Archaeological evidences indicate domestication of goats in the Fertile Crescent region of the Near East 10 000 years ago (Pringle, 1998; Luckert *et al*., 2001; MacHugh and Bradley, 2001). Using highly variable mitochondrial DNA segments, various authors have shown that the maternal lines of the current breeds comes from three founder lineages A, B and C (Bradley *et al*., 1996; Luikart *et al*., 2001). Lineage A is the most common in all continents. Lineage B was found in the Indian subcontinent, Mongolia and Southeast Asia. Lineage C was observed in a few samples from Mongolia, Switzerland and Slovenia. Further increase in local sample sizes have prompted re-definition of these three lineages and the number of haplogroups has increased to six (A, B, C, D, F and G) (Bradley *et al*., 1996; Manceau *et al*., 1999; Naderi *et al*., 2007, 2008). These studies found close matches in the alleged ancestor of the domestic goat, the bezoar, *Capra aegagrus* (Hughes *et al*., 2012; Doro *et al*., 2014).

Kenya Indigenous goat breeds have never been accurately described. Therefore, there is threat of erosion of unique genotypes such as those associated with adaptability to certain habitat and disease resistance, through indiscriminate crossbreeding. The current classification based on phenotype/morphology identifies three breeds: Small East African (SEA) goats, the Galla goat and crosses of SEA and the Galla (Abate *et al*., 1989). SEA goat has compact body, with short-haired coat of no definite colour though varied mixtures of black, brown and white are common. They have short erect ears and small horns. Galla goats have smooth short-haired coat that is white but the skin is black. Ears are long and dropping and the goat frequently have dewlap. Their horns are short and bent backwards (Devendra and Mcleroy, 1982).

In this study, we sampled DNA from indigenous goats from remote locations of pastoralists in Kenya, mainly, the Maasai and Somali/Boran communities and utilized mitochondria DNA (mtDNA) cytochrome-b/D-loop sequence to analyse their phylogenetic relationships and genetic diversity.

Materials and methods

Population description

Sampling regions were determined based regional and pastoral communities demographic distribution within Kenya. SEA goats were sampled from pastoralists in Narok and Kajiado (Southern Rift valley) while the Galla populations were sampled from Garissa and Isiolo, Northern Rift Valley). Other sampling was done at Kenyan coast (Kwale) and the Nyika plateau in Eastern Kenya.

Ethical clearance

This study was passed as a Master of Science dissertation project at University of Nairobi Veterinary School, Kabette and by National Commission of Science and Technology, Kenya (Nacosti). Ethical guidelines in the humane treatment of animals were followed.

Blood sample collection

Fifty blood samples representative of each of the various Kenyan indigenous goat breeds/regions were collected by

a veterinarian. Approximately 2 ml of peripheral blood was obtained by jugular vein puncture into a 10 ml EDTA Vacutainer tubes. The samples were stored on ice, transported to the laboratory within 24 h, and preserved at −20 °C until use.

DNA extraction

DNA was extracted from the whole blood (Sambrook, Maniatis and Fritsch, 1987). The blood was incubated in 50 µg/ml proteinase K, 1 percent SDS in STE buffer (150 mM NaCl, 100 mM EDTA, 10 mM Tris–HCl, pH 7.4) at 55 °C for 3 h. The DNA was extracted from the lysate by the phenol: chloroform method and precipitated from the aqueous phase by adding 2–3 volumes of absolute ethanol. The pellet was suspended in 50–100 µl TE (10 mM Tris–HCl, 1 mM EDTA, pH 8.0). DNA concentration was measured by absorbance at 260/280 nm and the quality analysed by electrophoresis in 1 percent agarose gel in 1× TAE buffer (40 mM Tris acetate, 1 mM EDTA, pH 8.0). The respective tubes with DNA were appropriately labelled and stored at –20 °C.

Polymerase chain reaction (PCR) and gene-clean procedures

In this study, 60 DNA samples representing SEA and Galla goats from Kenyan indigenous communities were analysed. PCR was carried out using the following parameters: denaturation at 94 °C, 1 min annealing at 56 °C, 1 min and extension at 72 °C for 1.5 min. The primers flanking region of cytochrome *b* and D-loop region were applied. The amplification product was verified on 1percent agarose gels and the fragment excised from the gel, solubilized in sodium iodide solution then bound to (silica) column in the gene clean procedure. Bound DNA was eluted in 30 µl nuclease-free ddH_2O.

Sequencing

Gene cleaned DNA of the amplified fragments were sequenced at Beckman Coulter Genomics Inc., France using the AppliedBiosystems ABI dye terminator method. Each of the analysed samples was independently sequenced three times and the raw sequences with non-ambiguous consensus selected. Representative consensus sequences were deposited in NCBI nucleotide database (GenBank Accession ID: 683928–683967).

Phylogenetic and diversity analysis

Sequences were aligned utilizing the Clustal-W program in BioEdit (Version 7.05) and the phylogenetic relationships inferred from the aligned nucleotide sequences by the neighbour-joining method at Boostrap 1 000 replicates using Phylip program (Felsentein, 1993) as implemented in the MEGA6 version suite (Tamura *et al.*, 2013). Multiple monophyletic lineages were deleted to simplify data presentation. Analysis of allele diversity and population divergence was done in DNA sequence polymorphism statistics pakages implemented in MEGA 6 and dnaSP V5 software (Librado and Rozas, 2009).

Results

Phylogenetics of indigenous goats within Kenya

Neighbour-joining tree analysis of Kenya's indigenous goats show a distance relationship to the root *Capra aegagrus*, extinct ancestral goats (Figure 1). Within Kenya population, two major clusters were observed but did not associate with any morphological trait description of Kenyan indigenous goats.

Relationship of Kenyan goats to Iberian Peninsula and Mediterranean Islands isolates

Databases DNA on mitochondrial D-loop from goats of phylogeographic origins was analysed against same region of D-loop of sampled Kenyan indigenous goats. Boostrap phylogenetic trees were constructed by Neighbour-joining analysis and edited to determine closest relationships to Kenya goats. This analysis of mtDNA defines Iberian Peninsula/Mediterranean islands goats as the dominant stock related to Kenyan indigenous goats (Figure 2) but there are a few lineages that cluster with Lehri and Pak Angora isolates from Pakistan.

Different phylogeographic goat isolates in relation the ancestral goats

DNA (D-loop) databases of goats from phylogeographic origins were analysed using un-rooted tree to determine phylogeographic clusters in relationship to Kenyan indigenous goats in relation to different ancestral goat lineages that determines today's goat breeds. Kenyan indigenous stock (thick lines) include an old lineage and a diverse group of isolates that clusters with goats lineage A, found in Southern Europe and parts of Middle East (Figure 3).

Population nucleotide diversity

Partial cytchrome b/D-loop region nucleotide sequences diversity in phylogeographic datasets and similar sequences derived from Kenya's indigenous goats were subjected Tajima-*D* test (Tables 1 and 2) to assess departure from neutrality. Data showed weak positive values for Kenyan dataset and slightly pronounced negative value for the Iberian/Mediterranean/Middle-East dataset. Divergence between the Kenyan indigenous goats and the Iberia/Mediterranean/Middle-East isolates showed no fixed differences and 35 shared mutations (Table 3).

Discussion

Determination of origins and phylogeographic relationships of goat breeds in sub-Saharan Africa is a necessary

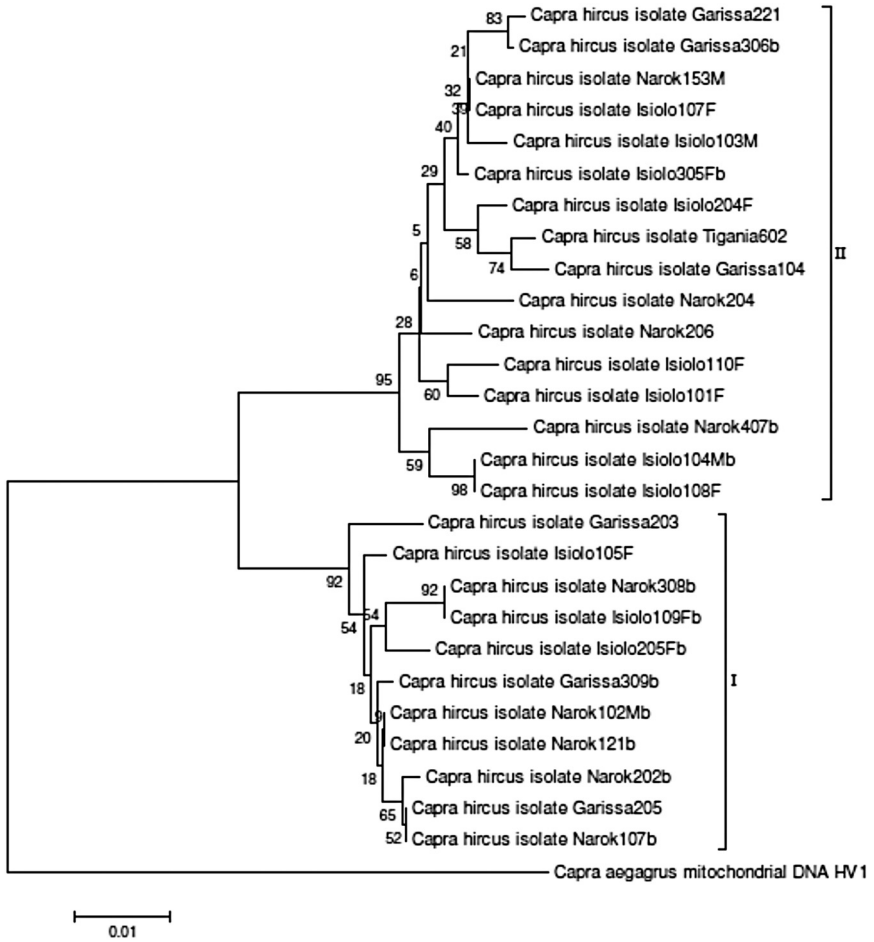

Fig. 1 - B/W online

Figure 1. Kenyan indigenous goats dendograph rooted against *C. aegagrus: Capra hircus* isolates from Kenya's main pastoralist communities. Observed clusters are indicated in brackets. The tree is drawn to scale with branch lengths proportional to evolutionary distances.

step in conservation of genetic diversity and their upgrading to higher productivity phenotypes Ajmone-Marsan *et al*. (2014). These goat populations are managed traditionally and preferred trait selection has maintained flow of important genes in high phenotype diversity (Taberlet *et al*., 2008). In this study, we sampled indigenous goats from traditional pastoralist communities in remote locations within Kenya. These goats are classified: (i) SEA goat and its crosses, reared by Maasai communities in South and South Eastern rangelands and (ii) the Galla goat reared by Boran and Somali communities in North and North Eastern rangelands and semi-deserts of Kenya (Abate *et al.* 1989; Kibegwa *et al*., 2015). The phylogenetic structure (Tamura, Nei and Kumar, 2004) based on mitochondria DNA sequences (Figure 1) show no distinct breed types since goats from different traditional communities cluster together and their diversity is uniformly spread. Mitochondria are maternally inherited and the inferred genetic relationship apply to the founder female goats, while introgression to these gene pools by the male goat is not accounted for. However, most studies on ancient lineages use mtDNA because there is high chance to get full-length genes in the preserved old specimen (Luikart *et al*., 2001; Fernandez *et al*., 2006; Naderi *et al*., 2007) and many comparative studies apply mtDNA to infer population phylogenetic structure. The phylogenetic data observed in the Kenyan indigenous goats (Figure 1) indicate that the mtDNA lineages are spread across preferred phenotypes shared among the different communities. These goats are adapted to the dry rangeland habitats and selection of goat weight for meat rather than daily goat is the preferred trait. This implies that the pastoralist communities exchange or trade in preferred male or female for breeding and that the founder female population(s) are spread among the Kenya indigenous goats.

The origin of indigenous goat breeds in Kenya is not documented and available details are not evidence-based but rather, speculation. Nucleotide sequence data will enable estimation of genetic distance in the phylogenetic relationships between and within species, and the comparisons of local databases to those in geographically similar landscapes. We analysed partial mitochondria D-loop/cytochrome b sequences from sequence data we generated (GenBank Accession ID: 683928 to 683967) in relation to similar dataset of phylogeographic distribution. The goat breeds in Kenya mainly clustered together with isolates: *Blceltiberica, Pirenaica, Blandaluza, Moncaina, Azpi-Gorri, Nserrana and Malta* (*MAL*) from Iberia

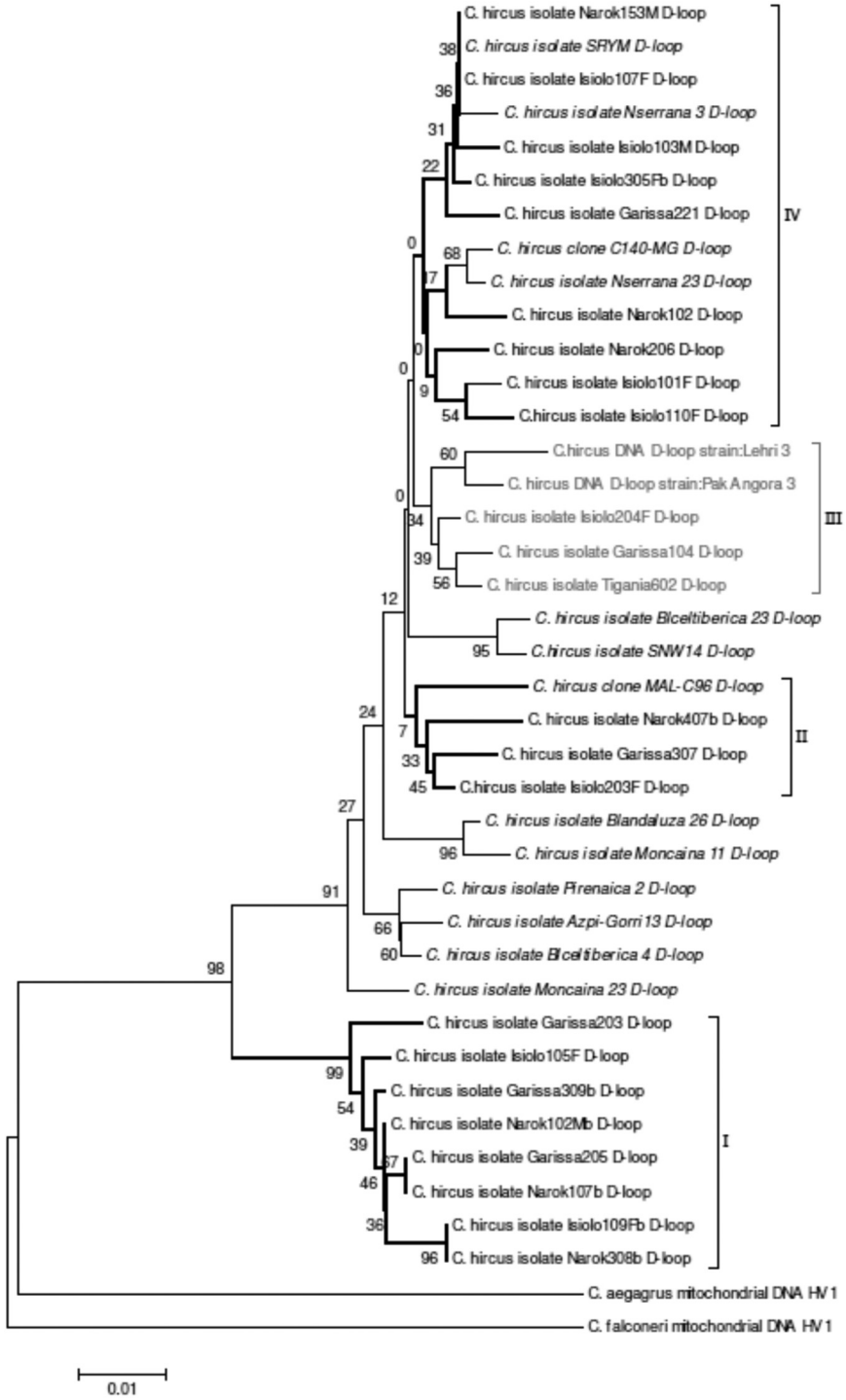

Fig. 2 - B/W online

Figure 2. Relationship of Kenyan indigenous goats to isolates of phylogeographic origin. The dendograph is rooted to two ancestral goats, *C. aegagrus* and *C. falconeri*. Different clusters of interest are indicated by bracket and the Iberia Peninsula/Mediterranean isolates are italized. The tree is drawn to scale with branch lengths proportional to evolutionary distances.

peninsula and Mediterranean coastal regions and islands belonging to the haplogroup A while a few Kenyan isolates clustered with *Pak Angorra* and *Lehri* from Pakistan goats (Figure 2) that belong to haplogroup C (Luikart *et al*., 2001; Fernandez *et al*., 2006; Naderi *et al*., 2007). This shows that Kenya indigenous goat breeds are more related to the Mediterranian region haplogroups and may suggest migration routes, initially by Berbers and Bedouins and then via Sahel region by Fulani tribesmen pastoralists and/or Portuguese sailors (Pereira and Amorim, 2010; Pieter, 2013). While this analysis relates to female lineages it is presumed that such migrations or/and trade include both sexes. The data also show Kenya's indigenous goats to be diverse stock, since their clusters are widespread over different phylogeographic isolates indicating diversity of founder stocks. Notably, there appears an early branching stock (Figures 2 and 3) that may represent a distinct haplogroup in this region

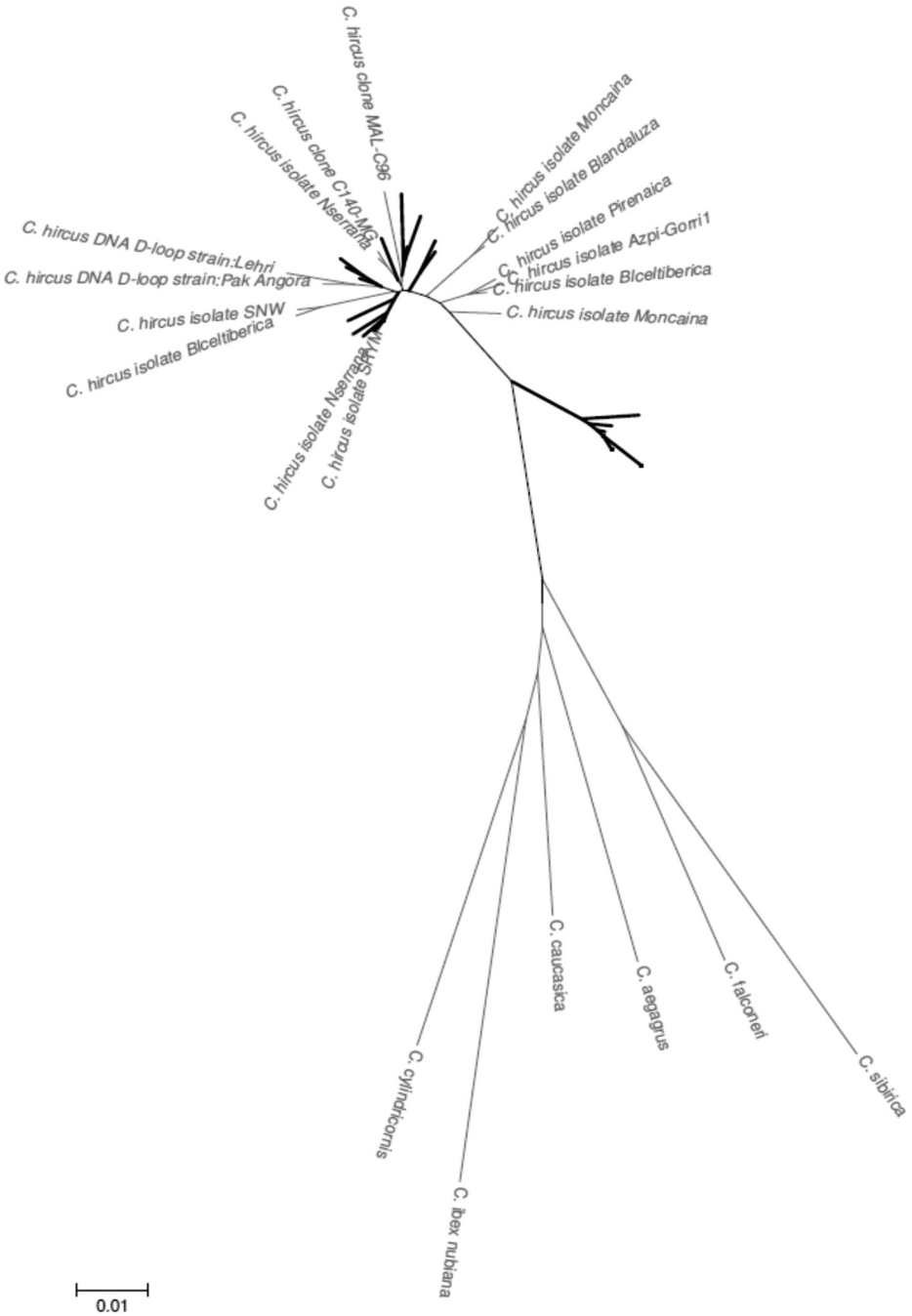

Fig. 3 - B/W online

Figure 3. Phylogeographic goat isolates in relation to lineages of the ancestral wild goats: *C. sibirica, C. falconeri, C. aegagrus, C. caucasica, C. ibex nubiana* and *C. cylindricornis* (Unrooted dendograph). Kenya's indigenous goat clusters lineages are shown in thick lines. The tree is drawn to scale with branch lengths proportional to evolutionary distances.

Table 1. Results from Tajima's neutrality test.

m	*S*	p_s	Θ	π	*D*
39	54	0.112735	0.026664	0.027278	0.082581

The analysis involved 39 nucleotide sequences of Kenyan indigenous goats. There were a total of 479 positions in the final dataset. Evolutionary analyses were conducted in MEGA6. m = number of sequences, n = total number of sites, S = number of segregating sites, $p_s = S/n$, $\Theta = p_s/a_1$, π = nucleotide diversity, and D is the Tajima test statistic (π and S/a_1 both estimate Θ, where E (expected) $E[\pi] = \Theta$, $E[S] = a_1\Theta$), software default significant at $P < 0.10$.

Table 2. Results from Tajima's neutrality test.

m	*S*	p_s	Θ	π	*D*
38	91	0.205882	0.049001	0.034506	−1.087796

The analysis involved 38 nucleotide sequences from Iberian/Mediterranean/Middle-East group. There were a total of 442 positions in the final dataset. Evolutionary analyses were conducted in MEGA6. m = number of sequences, n = total number of sites, S = number of segregating sites, $p_s = S/n$, $\Theta = p_s/a_1$, π = nucleotide diversity, and D is the Tajima test statistic (π and S/a_1 both estimate Θ, where E (expected) $E[\pi] = \Theta$, $E[S] = a_1\Theta$), software default significant at $P < 0.10$.

Table 3. DNA divergence between populations.

Population 1: Kenyan Indigenous group	**Population 2: Iberian/Mediterranean/Middle-East group**
Number of sequences: 38	Number of sequences: 38
Number of polymorphic sites: 52	Number of polymorphic sites: 90
Nucleotide diversity, Pi(1): 0.02963	Nucleotide diversity, Pi(2): 0.03354
Between populations:	
Number of fixed differences: 0	
Mutations polymorphic in population 1, but monomorphic in population 2:17	
Mutations polymorphic in population 2, but monomorphic in population 1:56	
Shared mutations: 35	
$P < 0.10$	

Genetic divergence between Kenyan indigenous goats and Iberian/Mediterranean region isolates (significant at $P < 0.10$) conducted using dnaSP V5 software.

and derived from one of ancestral wild goats and now has gene introgressions with haplogroup A. Further elaborate sampling and phylogenetic analysis can shed light on possible geographic Eastern African haplogroup related to the ancestral goats.

Studies (Hanotte *et al*., 2006) have shown that genetic diversity found in today's indigenous livestock populations and breeds greatly exceeds that found in their commercial counterparts therefore breeding programmes should include utilization of these genetic resources as long-term conservation strategies. The most significant threat to genetic diversity of indigenous breeds is the marginalization of traditional production systems and the associated local breeds, driven mainly by the rapid spread of intensive livestock production, often large-scale and utilizing a narrow range of breeds (FAO, 2007). In this study, genetic diversity was assessed on sequence dataset using Tajima-D neutrality test (Tajima, 1989; Nei and Kumar, 2000). Analysed data output gave weak positive value (0.082581) for Kenya indigenous goats and slightly pronounced negative value (−1.087796) for the Iberian/Mediterranean/Middle Eastern dataset (Tables 1 and 2). Values close to zero indicate that the nucleotide diversity is near neutrality and the population from which we sampled was almost in equilibrium with respect to drift and mutation. Kenyan indigenous goat population show positive value, though small, indicating that the population may have undergone a recent bottleneck or there is overdominant selection of a trait that is linked to analysed locus. Iberian/Mediterranean/Middle-East breeds dataset showed negative Tajima-D value implying purifying selection or population expansion resulting in low heterozygozity. Nucleotides divergence between the two goat populations revealed great variation in polymorphic sites with 35 shared mutations but no fixed differences (Table 3). The two datasets may indicate different selection pressure due to different management systems.

Indigenous livestock breeds are the result of thousands of years of natural and human selection that allows productivity in different ecological zones (FAO, 2007, 2012). Goats in arid/semiarid regions of Kenya form an integral part of a fragile livelihood systems and the pastoralist communities are endowed with indigenous knowledge on management of these ecosystems. The goats they stock have inherent characteristics such as resistance to dehydration, preference for browse and wide-ranging feeding habits, enabling them to thrive in regions that receive <750 mm of rainfall (Devendra and McLeroy, 1982; Georgoudis, 1995, 1998). This study has provided further information on genetic diversity of Kenya indigenous goats as a conservation interest in order to avoid erosion of unique genotypes due to uncontrolled breeding. Investment in animal weight over agility may limit tolerance to heat/drought and can limit pastoralists' long-distances migratory nature but the communities can adopt ecological improvement by intensifying shrubs conservation and growth in the browsing regions. Breeding programmes should select for tolerance to local diseases because large scale loss of goats and sheep reared by Kenya's pastoralist communities is attributed to disease outbreaks that cannot be managed by traditional methods. Government investment in epidemiological research to define etiological pathogens and apply interventions using alternatives in veterinary medicine is a plausible supplementary strategy that may not necessarily discourage approaches based on traditional medicines.

Conclusions

Based on genetic characterization data, Kenyan Indigenous goats phenotypic/morphological description as, (i) Galla goat and (ii) SEA goat would better be classified as mixed genotype of the two defined populations. Kenya Indigenous goats have an early lineage genetic stock that indicates distinct cluster and there has been introduction of other goats defining the introgression signatures mainly from Iberian Peninsula/Mediterranean stock. Kenyan indigenous goats are customarily rated on meat/weight and can be upgraded using related phylogeographic stocks.

Acknowledgements

This study was supported by National Commission for Science Technology and Innovation (Nacosti), Kenya. The project was facilitated by Molecular Genetics Laboratory at National Museums of Kenya/Institute of Primate Research.

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Animal Genetic Resources, 2016, 59, 81–95. © Food and Agriculture Organization of the United Nations, 2016
doi:10.1017/S2078633616000096

Dairy production systems and the adoption of genetic and breeding technologies in Tanzania, Kenya, India and Nicaragua

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Summary
Development of the livestock industry and its role in poverty alleviation in developing countries depends on how adaptive the production systems are to changing global environmental and economic trends. This paper characterizes dairy production systems in India, Tanzania, Kenya and Nicaragua, and describes the genetic and breeding technologies that hold promise for the advancement of global development goals. The dairy value chain has been prioritized for development under the CGIAR research programme on Livestock and Fish in Tanzania (East Africa), India (South Asia) and Nicaragua (Latin America), while ILRI is involved in research on dairy development in Kenya. In all the countries, a large number of smallholder farmers operating mixed crop–livestock production systems play a significant role in dairy production. In Tanzania, Kenya and Nicaragua, milk is predominantly produced by cattle of genotypes that differ both across countries and among production systems within the same country. In India, buffaloes contribute to a larger proportion of the national milk than cattle. Information on productivity per animal and on optimal genotypes to utilize within the smallholder production systems of all the countries is however limited. Crossbreeding and artificial insemination were identified as the most widely utilized breeding and reproductive technologies. Only in Kenya is there a national organization conducting livestock recording and monitoring productivity, however, the proportion of the dairy cattle population enrolled in the recording system is small (<2.5 percent). In all the countries, enhanced and adequately planned use of breeding and reproductive technologies, complemented with the relevant infrastructure, is needed to sustainably increase dairy productivity. The capacities of actors in the dairy value chain need to be developed in order to properly implement and manage improvements.

Keywords: *breeding technologies, dairy production, developing countries*

Résumé
Le développement du secteur de l'élevage et son rôle dans la réduction de la pauvreté dans les pays en développement dépendent de l'adaptabilité des systèmes de production à l'évolution des contextes environnementaux et économiques. Cet article caractérise les systèmes de production laitière en Inde, Tanzanie, Kenya et Nicaragua et décrit la génétique et les méthodes de sélection avec lesquelles l'on cherche à atteindre les objectifs mondiaux de développement. La chaîne de valeur du lait a été une priorité pour le développement dans le cadre du programme de recherche du CGIAR sur l'Élevage et la Pêche en Tanzanie (Afrique Orientale), Inde (Asie du Sud) et Nicaragua (Amérique Latine), alors qu'au Kenya c'est l'ILRI qui a pris en charge la recherche sur le développement du secteur laitier. Dans tous les pays, un grand nombre de petits éleveurs exploitant des systèmes agropastoraux mixtes jouent un rôle important dans la production de lait. En Tanzanie, Kenya et Nicaragua, le lait est principalement produit par des bovins de génotypes qui diffèrent à la fois entre les pays et entre les systèmes de production dans le même pays. En Inde, les bufflonnes contribuent plus que les bovins à la production nationale de lait. Cependant, il existe un manque d'information sur la productivité par animal et sur les génotypes optimaux à utiliser dans les systèmes de production des petits exploitants de ces pays. Les croisements et l'insémination artificielle ont été identifiés comme les stratégies reproductives et de sélection les plus amplement utilisées. Seulement au Kenya il existe une organisation nationale qui procède à l'enregistrement des animaux et qui fait le suivi de la productivité, bien que le pourcentage de bovins laitiers inscrits dans ce registre est faible (<2.5 pour cent). Dans tous les pays, il s'avère nécessaire d'améliorer et de planifier adéquatement l'utilisation des techniques de reproduction et de sélection, ceci complété par l'infrastructure pertinente, afin d'accroître de façon durable la productivité laitière. Les capacités des acteurs de la chaîne de valeur du lait doivent être renforcées afin que les progrès soient convenablement mis en œuvre et gérés.

Mots-clés: *production laitière, méthodes de sélection, pays en développement*

Resumen
El desarrollo del sector ganadero y su papel en la mitigación de la pobreza en países en desarrollo dependen de la capacidad de adaptación de los sistemas de producción a contextos ambientales y económicos cambiantes. Este artículo caracteriza los sistemas de producción lechera en India, Tanzania, Kenya y Nicaragua y describe la genética y las técnicas de selección con las que se pretende alcanzar los objetivos mundiales de desarrollo. Con vistas al desarrollo, se ha dado prioridad a la cadena de valor de la leche en el marco del programa de investigación CGIAR sobre Ganadería y Pesca en Tanzania (África Oriental), India (Asia Meridional) y

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Nicaragua (América Latina), mientras que en Kenya ha sido el ILRI quien ha asumido la investigación sobre el desarrollo del sector lechero. En todos los países, un gran número de pequeños ganaderos, que operan sistemas agropecuarios mixtos, juegan un papel destacado en la producción lechera. En Tanzania, Kenya y Nicaragua, la leche es producida principalmente por ganado bovino de genotipos que difieren entre países y de unos sistemas de producción a otros dentro del mismo país. En India, las búfalas contribuyen en mayor proporción que el ganado bovino a la producción nacional de leche. Sin embargo, es escasa la información sobre la productividad por animal y sobre los genotipos óptimos a utilizar en los sistemas de producción de los pequeños ganaderos de estos países. Los cruzamientos y la inseminación artificial fueron identificados como las estrategias reproductivas y de selección más ampliamente utilizadas. Únicamente en Kenya existe una organización nacional que lleva a cabo el registro del ganado y el seguimiento de la productividad, si bien el porcentaje de ganado bovino lechero inscrito en este registro es bajo (<2.5 por ciento). En todos los países, se necesita mejorar y planificar adecuadamente el uso de las tecnologías reproductivas y de selección, todo ello complementado por la infraestructura pertinente, para incrementar de manera sostenible la productividad lechera. Las capacidades de los actores en la cadena de valor de la leche deben ser desarrolladas con el fin de que las mejoras se implementen y se gestionen convenientemente.

Palabras clave: *producción lechera, técnicas de selección, países en desarrollo*

Submitted 22 July 2015; accepted 4 April 2016

Introduction

Rapid changes are taking place in the livestock sector of developing countries in response to globalization and an increasing demand for animal-product based diets, owing primarily to the combination of population growth, increasing consumer affluence and urbanization (Seré *et al.*, 2008; Robinson *et al.*, 2011; Mpofu, 2014). In these countries, the increasing consumption of livestock products is projected to continue beyond the year 2050 (Thornton, 2010; Table 1). However, livestock development faces increased threats from the growing competition for natural resources (such as land, water and fossil fuels), human conflicts and socio-political instability, weak institutions and market failures, and environmental effects of climate change. Changing climates are foreseen to have the greatest effect on food insecure areas in Africa and South Asia where hunger is a persistent problem and these changes in climate will present new challenges that may stifle rural development and livestock production (Thornton *et al.*, 2007; Global Harvest Initiative, 2013).

At a national level, livestock is a major contributor to the gross domestic product (GDP) of many developing country economies, both directly and indirectly. A large proportion of the rural households in developing countries own livestock, which are quite valuable financially and play significant social and economic roles in the communities (World Bank, 2008; Herrero *et al.*, 2013; Mpofu, 2014). Development of the livestock industry and its future role in alleviation of household poverty largely depends on how adaptive the livestock production systems will be to the changing global environment (Thornton *et al.*, 2007). According to the 2013 GAP report (Global Harvest Initiative, 2013), adoption of advanced agricultural technologies and better production practices are critical for realizing significant productivity gains in both industrialized and developing countries. In order to catalyse livestock producers in developing countries to be more efficient, take advantage of the rising demand for animal products, adapt to a changing climate, minimize disease risk and spread, and mitigate undesirable environmental impacts of livestock, a good understanding of the differences across livestock production systems is necessary (Robinson *et al.*, 2011). Characterization studies that elucidate the differences in the way livestock are produced in different places with regard to use of locally available production resources are critical for planning and targeting interventions.

In 2012, the Consultative Group on International Agricultural Research (CGIAR) implemented a number of collaborative research programmes to tackle cross-cutting issues in agricultural development (CGIAR, 2012). One of the programmes aimed to increase the productivity of small-scale livestock and fish systems in sustainable ways, thereby making meat, milk and fish more available and affordable to poor consumers across the developing world (ILRI *et al.*, 2011). The first phase of this Livestock and Fish Programme (L&F) had a focus on a small number of carefully selected animal source food value chains in multiple developing countries building on pre-existing work by ILRI in other countries. Inclusion of countries in multiple regions was to allow

Table 1. Consumption of meat and milk in developing countries and projected trends.

Year	Annual per capita consumption		Total consumption	
	Meat (kg)	Milk (kg)	Meat (MT)	Milk (MT)
1980	14	34	47	114
1990	18	38	73	152
2002	28	44	137	222
2015	32	55	184	323
2030	38	67	252	452
2050	44	78	326	585

Source: Thornton (2010).

comparisons and cross-system learning that would support development of lessons, methodologies and technologies of wide applicability (ILRI *et al.*, 2011). The dairy value chain, focusing on a commodity produced by small-scale farmers across Africa, Asia and Latin America was one area identified to have a high potential for transformational improvement. Analysis of this value chain, however, showed significant productivity gaps, and supply constraints that needed addressing (ILRI *et al.*, 2011). This paper presents information on dairy production systems and requisite genetic and breeding technologies that hold promise for the advancement of global development goals in countries prioritized for dairy improvement under the L&F programme, namely Tanzania (East Africa), India (South Asia), and Nicaragua (Latin America), in addition to Kenya (East Africa) where ILRI is involved in research on dairy development through other projects. Information compiled in this paper has more general application in the prioritization of long-term investments in scientific research for the development of appropriate genetic and breeding technologies to improve dairy livestock production in developing countries.

Materials and methods

A desk study was conducted to collate information on the dairy production systems of Kenya, Tanzania, India and Nicaragua. Information from published literature comprised papers spanning both field and experimental studies. Additional information on the current status of dairy production and utilization of breeding and genetic improvement technologies was obtained from other literature, including government reports, conference and symposia presentations, and from responses to a structured questionnaire administered to managers of the dairy and livestock sectors in the selected countries (listed in Acknowledgement section). The questionnaire was developed by the authors for the current context and included both closed and open-ended questions.

Data obtained from the literature and questionnaires was organized to fit into general classifications of dairy production systems in developing countries (following Robinson *et al.*, 2011), taking into consideration specific classifications used by the ministries responsible for livestock development in the respective countries. Production and related parameters were compared for dairy production systems found within a country, and, to the extent possible, for "similar" systems from one country to the other. The data assessed in this regard included the breeds and numbers of dairy animals raised within production systems, animal breeding technologies utilized in dairy production, and levels of milk productivity. Country-level information on the production and populations of milk-producing animals over the years was obtained from the statistical database of the Food and Agriculture Organization of the United Nations (FAOSTAT). To understand the context in which dairy genetic and breed improvement is occurring; information was also gathered on key organizations and institutions involved in providing support in the application and use of animal breeding technologies in the countries.

Results and discussion

Trends in animal populations and milk production

In all four countries, livestock play a significant role in people's lives, and positively contribute to the respective countries' GDP. Livestock contribute to the livelihoods of at least 70 percent of Eastern Africa's rural farmers in terms of income and diet (Cecchi *et al.*, 2010). In Tanzania, the livestock sector is estimated to provide livelihood support to 37 percent of households engaged in agricultural production. In 2013, the sector contributed 4.4 percent to the national GDP and accounted for 18 percent the agricultural GDP (National Bureau of Statistics Tanzania, 2014). From the 2014 national statistics of Kenya, agriculture is reported to have accounted for 27.3 percent of the GDP, while livestock accounted for 4.9 percent of the GDP (KNBS, 2014). Studies on the livestock sector in Kenya however indicate that livestock production is underestimated in the national GDP estimates and actually accounts for between 10 and 12 percent of the national GDP (IGAD, 2013; KEVEVAPI, 2014; KALRO, 2015). Kenya has the most developed dairy industry in East and Central Africa.

India's livestock sector accounts for 28 percent of the agriculture GDP and 3.9 percent of the national GDP (NDDB, 2013). India is the world's largest milk producing country in terms of volume, with a large proportion of the milk coming from buffaloes relative to cattle (Ahlawat and Singh, 2005; Gandhi and Sharma, 2005; Rao *et al.*, 2014). The dairy sector in India demonstrated steady growth during the different phases of "Operation Flood," a major initiative in dairy development first launched in 1970 by the National Dairy Development Board (NDDB). These initiatives over time have resulted in an increased per capita availability of milk. For example between 2010 and 2011, per capita availability of milk in the country increased from 128 to 267 g/day (Rao *et al.*, 2014). The average milk productivity per animal is however reported to be low (Gautam, Dalal and Pathak, 2010; Rao *et al.*, 2014).

Agriculture contributes 20 percent of the GDP of Nicaragua, with livestock accounting for 45 percent of the agriculture GDP (IFAD, 2014). The Ministry of Agriculture and Livestock (MAGFOR, 2012) in Nicaragua estimates that up to 75 percent of the income for cattle keepers comes from the sale of milk. Table 2 gives a summary of the number of bovine dairy animals in 2013, and the milk produced within the four countries in 2012.

Table 2. Bovine population and milk production statistics within four developing countries[1].

Country	Bovine species	Number of animals (1 000)	Milk production (tonnes)
Tanzania	Cattle	21 500	1 853
Kenya	Cattle	19 500	3 733
India	Cattle	214 350	54 000
	Buffaloes	115 420	66 000
Nicaragua	Cattle	3 740	765

Source: FAOSTAT (2014).
[1]The animal population estimates are for the year 2013; milk production estimates are for the year 2012.

A general overview of the dairy sectors and the key existing challenges with an economic focus is presented for Tanzania, India and Nicaragua at http://livestock-fish.wikispaces.com/Situational+Analysis+Report.

Cattle and domestic Buffalo population trends in the target countries

Statistics on animal populations and related parameters as reported by public sector departments responsible for livestock production in the various countries were generated from the Food and Agriculture Organization (FAO) of the United Nations' database (FAOSTAT, 2014). Trends in dairy cattle and buffalo populations are presented for India, Tanzania, Kenya and Nicaragua in Figures 1 and 2. India is home to the world's second largest population of cattle and half of the world's buffalo population, while Nicaragua has the largest cattle population in Central America (FAOSTAT, 2014). Tanzania has the second largest cattle population in Eastern Africa (behind Ethiopia), followed by Kenya (FAOSTAT, 2014). Kenya has the largest number of dairy animals in East Africa, estimated at 3.58 million (Muriuki, 2011).

Over the last decade, animal numbers have increased in all the countries, but at different rates. The slowest increase occurred in Nicaragua (Figure 2). Tanzania has experienced a fairly constant increase in its livestock population over the years, while the population of cattle in Kenya drastically increased between 2006 and 2008 then slowed down thereafter.

Milk production trends in the target countries

Trends in milk production from 2000 to 2013 are presented for the four countries in Figures 3 and 4. India recorded the largest volume of milk in all the years, with the milk

Fig. 1 - B/W online

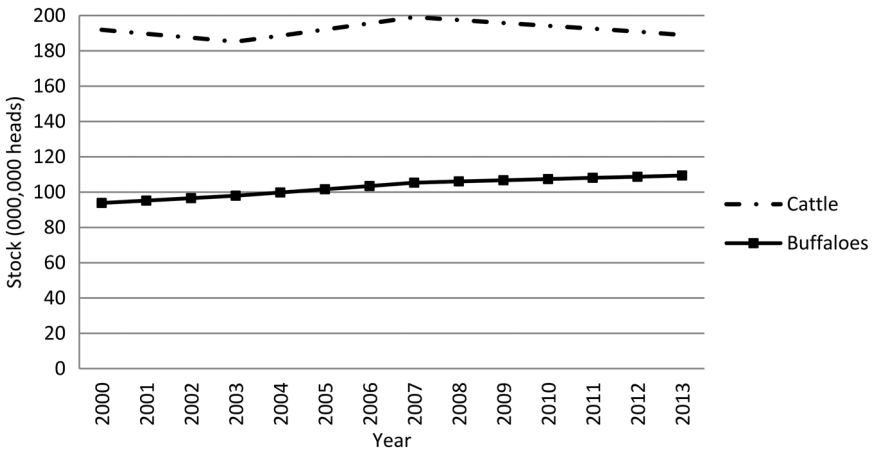


Figure 1. Trends in cattle and buffalo population in India (Source: FAOSTAT, 2014).

Fig. 2 - B/W online

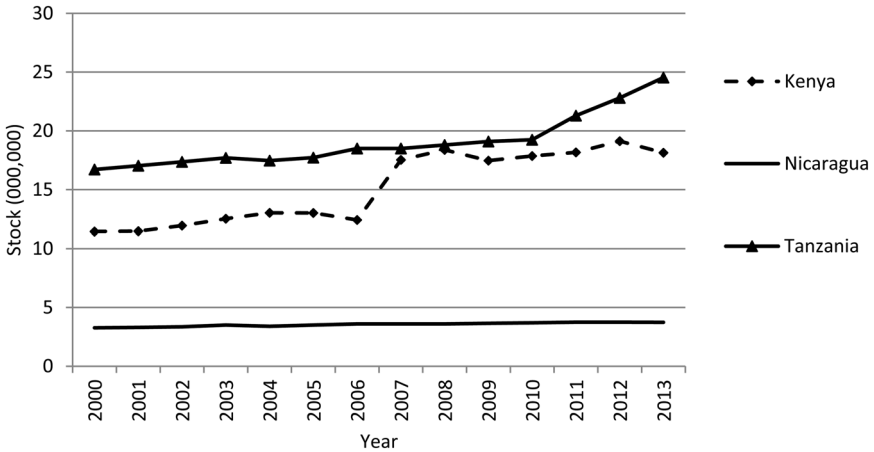


Figure 2. Trend in the cattle populations of Tanzania, Kenya and Nicaragua (Source: FAOSTAT, 2014).

Fig. 3 - B/W online

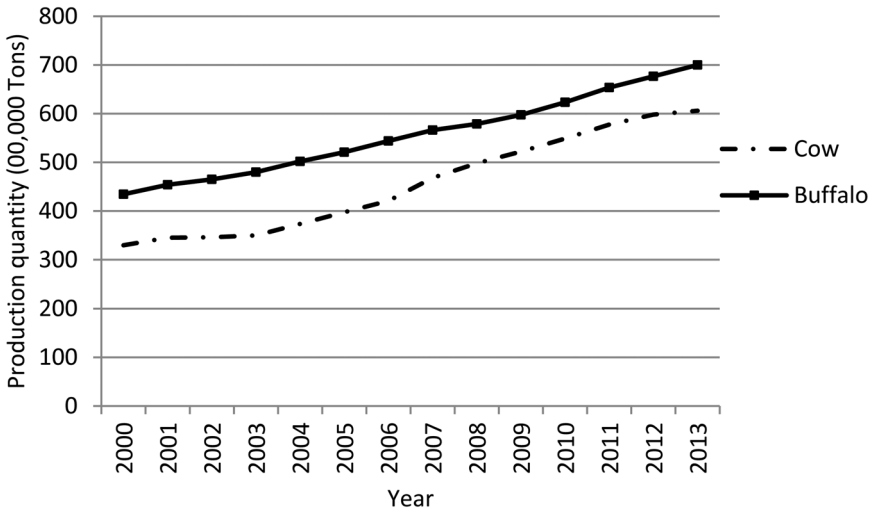


Figure 3. Amount of milk produced annually in India from 2000 to 2012 (Source: FAOSTAT, 2014).

Fig. 4 - B/W online

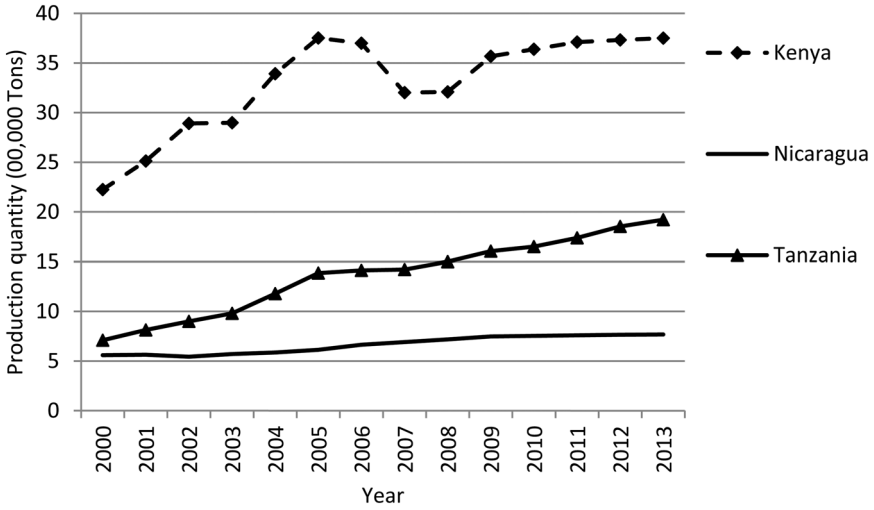


Figure 4. Annual milk production trends from cattle in Tanzania, Kenya and Nicaragua (Source: FAOSTAT, 2014).

produced from cows increasing from 35 million tonnes in 2001 to 55 million tonnes in 2010 (Figure 3), and that from buffalos increasing from 45 million tonnes in 2001 to 65 million tonnes in 2010. Total milk production from cows increased at a greater rate than the increase in the number of cattle, suggesting an increase in dairy cattle productivity in the country.

The quantity of milk produced in Nicaragua gradually increased from 2005 (614 000 tonnes) to 2010 (753 000 tonnes) then remained at the same level to 2013 (Figure 4). Over the same period, the cattle population increased, but by a smaller proportion – from 3.5 million animals in 2005 to 3.7 million animals in 2010, implying a slight increase in milk production per animal.

Figure 4 presents a scenario of increased milk production in Tanzania. This increase largely resulted from growth in the livestock population (Figure 2), rather than from an increase in production per dairy animal. In Kenya, milk production increased from 2000 to 2005, while the cattle population size remained practically the same (Figures 2 and 4). There was however a marked increase in the cattle population after 2006 (Figure 2), which did not result in increased milk production (Figure 4).

Systems of dairy production and the main dairy genotypes

Systems of livestock production vary with climates, availability of production resources, the economic ability and market orientation of producers, and consumer demands (Peeler and Omore, 1997; Thornton *et al.*, 2007). Attempts have been made to define more unified criteria for production systems classification, including providing spatial mapping of the systems (Robinson *et al.*, 2011). Table 3 presents dairy production systems found in the four countries of this study, and the types of dairy animals reared within the systems.

The main genotype reared in each system depends on the scale of operation and the level of economic investment. In the large-scale commercial dairy systems, the exotic dairy breeds are the main breed types reared due to their

Table 3. Dairy production systems reported in literature and breeds and genotypes of livestock used in the various systems of the four countries.

Country	Production system	Genotype	Breed	Reference
Tanzania	Mixed crop–livestock system	Crossbreed, purebred exotic, purebred indigenous, synthetic	50–75% Holstein crosses, unspecified exotics, Zebu, Mpwapwa	Msanga *et al.* (2000)
	Medium scale and Smallholder systems	Crossbreed/purebred	*B. indicus* × *B. taurus*, Ayrshire, Friesian	Chenyambuga and Mseleko (2009), Gillah, Kifaro and Madsen (2013), Ogutu, Kurwijila and Omore (2014)
	Extensive grass/rangeland-based (Traditional pastoral system)	Purebred/crossbreed	Tanzania Shorthorn Zebu (TSZ), Boran, Ankole, Tanzania shorthorn zebu × Exotic crosses	Kanuya *et al.* (2006), Msanga and Bee (2006)
	Intensive urban/peri-urban dairy production system	Purebred/crossbreed	Friesian, Ayrshire, Jersey and crosses indigenous × Exotics (Tanzania shorthorn zebu × exotic crosses)	Gillah, Kifaro and Madsen (2013)
Kenya	Mixed crop–livestock systems	Crossbreds:	Ayrshire x Sahiwal/Holstein × Zebu	Kahi (2000), Mwacharo *et al.* (2009), Muasya (2013)
	Large-scale commercial dairy	Purebred	Holstein/Jersey/Guernsey/Ayrshire/Brown Swiss/ Sahiwal	Ojango and Pollott (2001), Muasya (2013)
	Intensive smallholder farms (urban/ peri-urban systems)	Purebred/crossbreed	exotic breeds/zebu × exotic crosses	Mwacharo *et al.* (2009), Muriuki (2011)
India	Mixed crop–livestock systems (semi-intensive)	Purebred Cows/Buffaloes Crossbreds-among indigenous	*Buffalo*: Murrah, Nili Ravi, Surti and Jaffarabadi Buffalo *Cattle*: Local breeds (42 indigenous breeds)	Rao *et al.* (2014), Valsalan *et al.* (2014)
	Extensive grass/rangeland-based system	Purebred indigenous	Local breeds (both Buffalo and cattle)	Gandhi and Sharma (2005), Hegde (2006a), NDDB (2014)
	Small-holder low-input (traditional housed)	Purebred and crossbred indigenous cattle and buffalo	Local breeds and their crosses	Kumaresan *et al.* (2009)
	Intensive urban/peri-urban system	Cows and buffaloes : Purebred/crossbred (cattle-indigenous × exotic)	*Buffalo*: Nili Ravi, Surti and Jaffarabadi Murrah *Cattle*: Crossbreds of Jersey and Holstein Friesians with local indigenous breeds Sahiwal	Shekhar, Thakur and Shelke (2010), Rao *et al.* (2014)
Nicaragua	Extensive grass/rangeland-based dual-purpose system	Crossbreed	Crosses between *B. taurus* (Holstein and Brown Swiss) and *B. indicus* (Brahman)	Corrales (2011), Galetto and Berra (2011)
	Mixed crop–livestock dual-purpose system (medium and small scale)	Crossbreed/purebred	Crosses between *B. taurus* (Holstein and Brown Swiss) and *B. indicus* (Brahman), Reyna Creole Cattle	Holmann *et al.* (2014)

high milk production potential. In the mixed crop–livestock systems, either pure indigenous breeds or their crosses with the exotic milk breeds tend to be reared (Table 4). The differences among countries are outlined below.

Tanzania: In Tanzania, both traditional pastoral and urban dairy production systems exist (Msanga and Bee, 2006; Gillah, Kifaro and Madsen, 2013; Ogutu, Kurwijila and Omore, 2014). Although typically associated with meat production elsewhere, the traditional pastoral system is included as a dairy production system in the country because more than 75 percent of the milk in Tanzania, is produced by indigenous cattle in these systems (Msanga and Bee, 2006; National Bureau of Statistics Tanzania, 2014). The main breed-type reared within these systems is the indigenous Zebu (Musanga, Questionnaire response). Mixed crop–livestock systems are mainly found in the highland, sub-humid and less-humid areas (Kaijage, 2011). Within the intensive urban and peri-urban systems, crossbreds of the exotic (*Bos taurus*) breeds and the indigenous (*Bos indicus*) breeds, with some limited numbers of pure bred exotic breeds are reared.

Kenya: Mixed crop–livestock systems are common in Kenya. These systems are found in highlands, sub-humid and less-humid areas with good potential for agricultural production (Kahi, 2000). The systems are highly varied in terms of level of inputs. Animals reared in the smallholder mixed crop and livestock production systems comprise a mixture of exotic (*B. taurus*) and indigenous (*B. indicus*) breed-types. These account for more than 70 percent of milk consumed in areas not classified as milk shed areas (Staal *et al.*, 2001; Muriuki, 2011). Smallholder systems of dairy production in Kenya have been extensively characterized under the Smallholder Dairy Development Programme (ILRI, 2004). Large-scale commercial farms operating in the country as either dairy or dual-purpose units have also been described (Kahi, 2000; Ojango and Pollott, 2001). These systems are highly mechanized, tend to raise pure-bred exotic (*B. taurus*) breeds, and produce large quantities of dairy products to which they add value. They produce diverse products that are mainly targeted for urban consumers. The large-scale commercial dairy systems also serve as a source of replacement animals for smallholder farmers, and carry out the bulk of the selection and improvement of dairy cattle in the country (Kahi, Nitter and Gall, 2004; Makoni *et al.*, 2013).

There have been concerted efforts over the past two decades to improve dairy production in Eastern Africa.

Table 4. Milk production performance of animals used for dairy production in, India, Kenya, Nicaragua and Tanzania.

Country	Production system	Genotype	Ave. DMY[1]	Ave. LMY[2]	Ave. LL[3]	Reference
Tanzania	Mixed crop–Livestock	Crossbred cattle, Mpwapwa	5.42	1 626	300	Msanga *et al.* (2000)
	Extensive grass/rangeland based (traditional pastoral)	Tanzania shorthorn zebu × Exotic crosses	3.0	600	200	Mwambene *et al.* (2014)
	Medium-scale system	Exotic × indigenous crosses	7.0	–	–	Gillah, Kifaro and Madsen (2014)
	Smallholder system	Exotic × indigenous crosses, purebred exotic	5.6	–	–	Gillah, Kifaro and Madsen (2013, 2014)
				1 381	288	Chenyambuga and Mseleko (2009)
Kenya	Mixed crop–livestock (semi-arid lowland)	Crossbred (Ayrshire, Sahiwal, Friesian, Brown Swiss)	5.2	1 485	286	Kahi (2000)
	Mixed crop–livestock (sub-humid tropics)	Crossbred (Ayrshire, Sahiwal, Friesian, Brown Swiss)	11.5	4 065	354	Kahi *et al.* (2000)
	Large-scale commercial dairy	Exotic (Holstein Friesian)	15.1	4 540	301	Ojango and Pollott (2001)
	Small-holder dairy	Crossbred	6.5	2 021	365	Muraguri, McLeod and Taylor (2004)
			5.4		365	Ojango *et al.* (2014)
India	Mixed crop–livestock system (Semi-Intensive)	Nili Ravi	–	1 941	286	Sethi and Kala (2005)
		Murrah Bufallo	9.0	2 080	–	Sethi and Kala (2005)
	Smallholder low-input (Traditional housed)	Crossbred cows, Sahiwal	7.0	2 064	285	Joshi and Singh (2005), Kumaresan *et al.* (2009)
		Crossbred (Holstein × indigenous		2 932	305	Duclos *et al.* (2008)
Nicaragua	Extensive grass dual-purpose system	Reyna Creole Cattle	4.8	1 321	274	Corrales (2011)
	Mixed crop–livestock dual-purpose medium scale	Brahman × Exotic dairy	3.7			Holmann *et al.* (2014)

[1]DMY, Daily Milk Yield.
[2]LMY, Lactation Milk Yield.
[3]LL, Lactation Length.

These include the Smallholder Dairy Development Programme in Kenya (ILRI, 2004), the East Africa Dairy Development Programme in Kenya, Uganda, Rwanda and Tanzania (ILRI, 2008) and the Livestock Sector Development Policy in 2011 by the government of Tanzania (MLFD, 2011a). Ogutu, Kurwijila and Omore (2014) summarized the impacts of ten projects implemented for dairy improvement in Tanzania starting in the 1980s. Though each of the projects impacted dairy production in a specific region of the country, the level of impact was greatly variable. In general, it was considered that improvement in infrastructure would be required for long-term sustainability of the interventions.

India: Most of dairy production in India is by a large number of livestock producers on small land holdings, typically organized into three main systems of production (Table 3). Dairying is also practiced by landless farmers (NDDB, 2014). More than 70 percent of the cattle reared in India are indigenous (*B. indicus*) breeds (Rao *et al.*, 2014). The dairy cattle among these comprise both pure and crossbred animals (Hegde, 2006a). An increased level of cross breeding between indigenous (*B. indicus*) and exotic (*B. taurus*) cattle has been encouraged in the country through various projects (Hegde, 2006a; NDDB, 2014; NPBBDD, 2014).

Among the 13 buffalo breeds used for milk production in the country, the Murrah breed is the most important for dairy (Valsalan *et al.*, 2014) and is the breed of choice for upgrading buffaloes for milk production (Rao *et al.*, 2014). A National dairy plan implemented since 2011 aims to develop more productive animals (both cattle and buffaloes) and to develop and expand production systems to increase milk supply in India over a 15 year horizon (NDDB, 2014).

Nicaragua: In Nicaragua, more than 95 percent of the milk is produced by dual-purpose (dairy and beef) cattle (MAGFOR, 2012). A large proportion of the farmers producing milk in the country are medium and small scale (CENAGRO, 2012). In addition to producing milk, these farmers raise male calves for sale at weaning to larger-scale farmers/feedlots where they are grown for beef production (Holmann *et al.*, 2014). Up to half of the milk produced in the country is processed and sold as cheese either for local consumption or for export to neighbouring countries. Dairy production in Nicaragua received a major boost from 2001 to 2010 following the creation of a network of dairy cooperatives through a joint project between the Ministry of Agriculture and Forestry and the Swedish Agency for agricultural development (FondeAgro). By providing milk cooling and storage facilities and other essential services, the cooperatives enabled more stable farm gate prices for milk and supported an expanded market for milk products and livestock production (Galetto and Berra, 2011).

In all the countries, farmer cooperative groups formed through an aggregation of smallholder farmers serve to promote services to farmers, and also organize the collection, handling and sale of milk from the farms. Cooperatives enable small holder farmers to improve their competitive edge in the open market economies (Devendra, 2001).

Milk production and the reproductive performance of dairy animals

Milk production performance

Most of the studies reviewed on the performance of dairy animals in the target countries addressed milk production, reproductive performance, and to a lesser extent survival ability of the animals (Muasya, 2005; Zambrano *et al.*, 2006; Chenyambuga and Mseleko, 2009; Kumaresan *et al.*, 2009; Corrales, 2011; Holmann *et al.*, 2014). Table 4 presents reported milk production performance of dairy animals in the countries.

The average daily milk yield (DMY) was slightly more than 5 kg in mixed crop–livestock systems in semi-arid areas, and in the smallholder systems in Kenya and Tanzania. Large-scale commercial dairy systems in Kenya had the highest recorded production in the literature for systems in developing countries, with an average DMY per animal of 15 kg (Table 4). This high level of production could be attributed to the high genetic potential of the animals for milk production as well as good nutritional management. Crop–livestock systems in the semi-arid lands of Kenya, and Tanzania had the lowest milk yield, understandably due to the breeds and types of cattle kept in such systems.

In India, indigenous cattle, which comprise more than 75 percent of the total cattle population, are reported to produce on average 1.83 kg milk/day, crossbred cattle produce on average 6.36 kg/day, while buffaloes on average produce 3.83 kg/day (Gandhi and Sharma, 2005; Joshi and Singh, 2005). Higher yields are reported for animals raised under more intensive management systems (Table 4). It should be noted that a large amount of published information on productivity of different breed-types in India is based on animals reared in research stations rather than on the small holder farms (Gaur, Garg and Singh, 2005; Joshi and Singh, 2005; Birthal, Taneja and Thorpe, 2006; Mwacharo *et al.*, 2009).

The quantity of milk produced by animals in the dual-purpose production systems of Nicaragua is reported to be greatly variable depending on the season, and ranges from 3 to 5 kg/animal/day (Holmann *et al.*, 2014). Information on individual animal productivity within the country is, however, limited; reports from the country tended to present data on bulk milk production only.

Lactation length in dairy cattle is usually standardized to 305 days for purposes of performance comparison. Animals in the mixed crop–livestock systems, however, were not reported to have lactation lengths of 305 days. The longest lactation was 365 days in Kenya, and the

Table 5. Age at first calving (AFC, in months) and calving interval (CI, in days) of animals used for dairy production in India, Kenya, Nicaragua and Tanzania.

Country	Production system	Genotype	Ave. AFC (Months)	Ave. CI (days)	Source
Tanzania	Mixed crop–livestock	Crossbred	33	498	Msanga *et al.* (2000), Kaijage (2011)
	Extensive grass/rangeland based (traditional pastoral)	Tanzania shorthorn zebu × Exotic crosses, purebreds	51	476	Swai, Kyakaisho and Ole-Kawanara (2007)
	Medium-scale systems	Crossbred		412	Gillah, Kifaro and Madsen (2013)
	Smallholder dairy systems	Exotic × Indigenous crosses		432	Kanuya *et al.* (2000)
	Intensive urban/peri-urban dairy production	Crossbred/purebred	33	506	Gillah, Kifaro and Madsen (2013)
Kenya	Mixed crop–livestock (semi-arid lowland)	Crossbred	33	454	Thorpe *et al.* (1993)
	Mixed crop–livestock (sub-humid tropics)	Crossbred		412	Kahi (2000)
	Large-scale commercial dairy	Crossbred/purebred exotic	30	409	Ojango and Pollott (2004)
			36	412	Kahi (2000)
	Smallholder dairy	Crossbred		451	Mujibi *et al.* (2014)
India	Smallholder low input (traditional housed)	Crossbred cows	41	538	Kumaresan *et al.* (2009)
		Sahiwal	36	420	Joshi and Singh (2005)
Nicaragua	Extensive grass dual-purpose system	Reyna Creole	37	424	Corrales (2011)

shortest was reported in Tanzania (200 days). Tanzania relies mostly on indigenous *B. indicus* breeds that are associated with short lactation length (Katjiuongua and Nelgen, 2014).

Reproductive performance

Reproductive traits reported in the literature were age at first calving (AFC) and calving interval (CI). These traits are easily recorded as they occur following major events in an animal's life. Information is more difficult to obtain on other reproductive traits like conception rates and services per conception. These two indicators may only be accurately captured in systems where artificial insemination (AI) or hand mating is practiced and followed by pregnancy diagnosis. Pregnancy diagnosis generally requires more specialized personnel who may not be readily available; consequently, producers carry out insemination/mating of their animals and wait to see the progress of pregnancy hoping that their animals have conceived. This leads to poor capturing and reporting of information on animal conception in the low-input production systems. Table 5 presents AFC and CI for dairy animals in the countries.

In India, AFC was greater in more traditional low-input smallholder systems than in the mixed crop–livestock semi-intensive smallholder systems. The Kenyan large-scale dairy systems had the earliest AFC (30 months), while the extensive grazing systems in Tanzania reported the latest AFC (51 months) (Table 5). Large variations in AFC have been reported between production systems in the different countries (Table 5). These differences could be due to variation in breeds used within systems, differences in management practices across systems, and differences in climatic conditions. In systems where exotic breeds were reared, the AFC was generally younger than observed in systems utilizing mostly indigenous *B. indicus* breeds.

The average CI across the systems was 449 days. The longest CIs (more than 500 days) were reported in the traditional small holder systems of India, and in the urban dairy systems of Tanzania (Table 5). The large-scale commercial systems in Kenya had the shortest CIs. Improved and better informed management of dairy animals can greatly reduce the CIs and improve the overall herd productivity.

Reproductive and genetic technologies

Reproductive technologies

Dairy systems have experienced drastic transformation in the past few decades due to intensification and more extensive use of a wide range of technologies. Reproductive and genetic technologies (biotechnologies) are major avenues through which herd improvement has been achieved. The biotechnologies used in dairy production systems in India, Kenya, Nicaragua and Tanzania are presented in Table 6.

The most widely used reproductive technology in all four countries under review is AI, where it serves to introduce and disseminate desired dairy characteristics in populations. Large-scale commercial dairy systems in particular tend to use AI extensively, and opt for semen from commercial dairy *B. taurus* sires that is imported from more developed countries. Although available since the 1930s

Table 6. Biotechnologies used in dairy production in the target countries.

Country	Genetic technology	Reproductive technology	Source
Tanzania	Crossbreeding, upgrading	Artificial insemination (AI)	Msangi, Bryant and Thorne (2005), MLFD (2011b), Gillah, Kifaro and Madsen (2013), Katjiuongua and Nelgen (2014)
Kenya	Pedigree and milk recording, phenotypic and BLUP selection, progeny testing, pure breeding, crossbreeding and upgrading	AI, sexed semen, MOET (small extent) Cryopreservation	Ilatsia *et al.* (2007), Kosgey *et al.* (2011), Muriuki (2011), KALRO (2015)
	SNP assays to optimize choice of different crossbred dairy cattle genotypes		Ojango *et al.* (2014)
India	Crossbreeding, milk recording, progeny testing, genetic parameter and breeding value estimation, milk-based selection criteria, crossbreeding, pedigree selection	AI pregnancy diagnosis MOET, cryopreservation	Hegde (2006a), Kumaresan *et al.* (2009), NDDB (2014)
Nicaragua	Crossbreeding	AI, sexed semen, MOET (small extent)	Galetto and Berra (2011), Holmann *et al.* (2014)

and relatively cheap and easy to use, AI has been difficult to administer successfully in smallholder cattle production systems in developing countries. This difficulty is due mostly to logistical and institutional challenges (Okeyo *et al.*, 2000; Kosgey *et al.*, 2011; NDDB, 2014).

In India, the use of AI has risen since 2008 (Gautam, Dalal and Pathak, 2010; NDDB, 2014; Rao *et al.*, 2014) and has been applied to animal breeding in both cattle and buffalo. Although national coverage was low until 2005 (Ahlawat and Singh, 2005); India currently has the world's largest AI infrastructure, consisting of 49 semen stations producing 66.8 million doses of frozen semen annually (NDDB, 2014). Transformations have been induced in the dairy sector through new interests in the organization of services and markets such that dairy farming in India is rapidly evolving to a more professionally managed industry (NDDB, 2014).

Use of sexed semen and MOET has also been reported in India with an increasing demand for sexed semen (Hegde, 2006b). More extensive use of sexed semen alongside the ongoing expansion of AI infrastructure and adoption in the country could prove useful for improving the productivity of India's dairy sector by helping to reduce the number of male animals born into dairy herds that are not needed for reproduction.

Within East Africa, use of AI is most widespread in Kenya. However, national coverage in Kenya is quite low when compared with current AI use in India (Figure 5). Sexed semen and MOET are also reported to have been used by large-scale commercial farms in Kenya (Muriuki, 2011), although details are scanty on their adoption and use.

AI use in Tanzania is very low, as are conception rates following use of AI in the country (Ogutu, Kurwijila and Omore, 2014). Tanzania has a single national AI centre that produces about 150 000 doses annually, and also relies on limited amounts of semen imported through private companies (MLFD, 2011b; Katjiuongua and Nelgen, 2014; Ogutu, Kurwijila and Omore, 2014). Plans are now underway for substantial investment through public–private partnership arrangements between the government and its development partners to enhance the existing

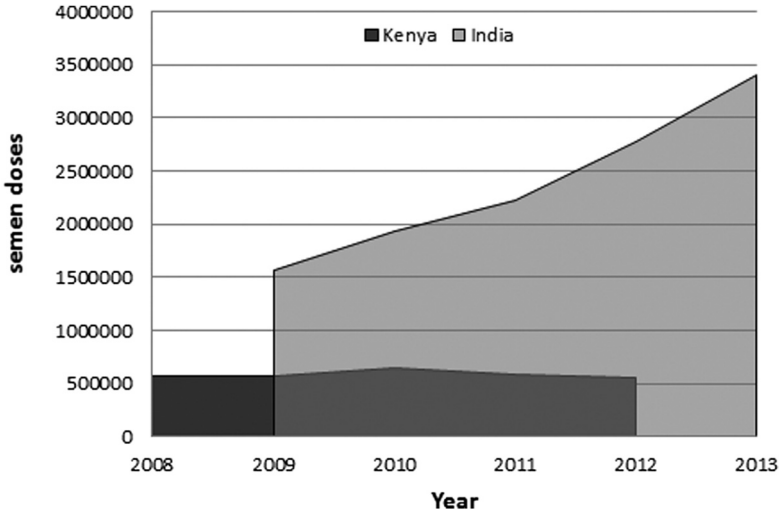


Fig. 5 - B/W online

Figure 5. Number of semen doses used for AI annually between 2008 and 2013 (Source: BAIF reports 2008–2013; KAGRC website (http://www.kagrc.co.ke).

semen production and to implement an increasingly private sector-driven delivery system (Ogutu, Kurwijila and Omore, 2014).

In more extensive systems of Eastern Africa, the use of bulls, mostly of the local *B. indicus* breeds, remains common practice (Kaimba, Njehia and Guliye, 2011).

Although promoted through projects and organizations supported by the government, AI is not widespread in the smallholder cattle systems in Nicaragua (Holmann *et al.*, 2014). Many of the smallholder farmers have a strong cultural attachment to having a bull (Toro) in their herds. Where adopted, AI is mainly carried out by private companies that import semen of various breed-types. Smallholder and mixed crop–livestock farmers with interest in upgrading their dairy animals for higher productivity do so using both AI and hand mating, with the semen typically coming from *B. taurus* bulls (MAGFOR, 2012). The government recently introduced an animal traceability system that allows for monitoring of the movement of animals. This system is applied mainly to animals for export and its adoption remains limited across the country (Holmann *et al.*, 2014).

Use of other reproductive technologies such as in-vitro fertilization and embryo sexing is limited in all the countries, as these technologies tend to be more expensive, are logistically more demanding and require administration by more technically skilled manpower. Statistics are generally not available on the use of these technologies in dairy production in the study countries.

Genetic improvement technologies

The use of genetic improvement technologies (Table 6) is not widespread in the target countries. However, crossbreeding and upgrading are commonly used in all the countries with the objective of improving the local stock (Hegde, 2006a; Mwacharo *et al.*, 2009; Holmann *et al.*, 2014; Katjiuongua and Nelgen, 2014; NDDB, 2014). Livestock keepers in the countries practice some form of selection of their heifers and bulls for breeding. This is usually based on either physical appraisal of the animals, or records on phenotypic performance available for the animals and their relatives. Use of molecular information and genomic selection technologies is limited. However, studies are ongoing on utilization of genomic information for selection and parentage determination (Ahlawat and Singh, 2005; Kios, van Marle-Köster and Visser, 2012; Mujibi *et al.*, 2014).

In India, structures are in place to facilitate milk recording and genetic evaluation (Hegde, 2006b; Duclos *et al.*, 2008; NDDB, 2013). A milk-based selection criteria has been adopted for the selection of breeding bulls using pedigree information as well as progeny testing.

In East Africa, Kenya is the only country that has a national animal recording system where pedigree and performance recording is carried out. Kenya also has a national contract mating scheme through which sire selection is done (Mukisira, 2002; Kosgey *et al.*, 2011). These schemes, though open to all producers, are primarily used by the large-scale dairy producers in high-input systems where pure-breeding is common (Kosgey *et al.*, 2011). To date, only an estimated 2.5 percent of the national dairy herd is accounted for in the national animal recording program (Kenya Livestock Breeders Organization, (http://www.klbo.co.ke/) personal communication). Given that the larger-scale producers are the major source of improved dairy animals for other production systems, the benefits of the animal recording infrastructure would be greatly improved if more of the smallholder farmers provided information on the performance of their animals. Crossbreeding and upgrading of local stock are more common practice within the smaller-scale livestock production enterprises in Kenya.

In Tanzania, many livestock keepers in the pastoral systems practice selection within their own herds, and use natural mating (Ogutu, Kurwijila and Omore, 2014). In the smaller-scale systems, crossbreeding of various breed-types is common, resulting in a broad mix of breeds (Msangi, Bryant and Thorne, 2005). Few farmers have a well-developed breeding strategy and many rely on inseminators marketing semen from different countries to inform them about available bulls, characteristics of those bulls and about AI. Use of genomic selection technologies is still in an experimental stage (ILRI, personal communication).

Many livestock keepers in Nicaragua practice crossbreeding using natural mating, and strive to maintain animals with no more than 50 percent exotic breed-types (Corrales, 2011; Holmann *et al.*, 2014).

Table 7. Average costs of artificial insemination (AI) offered by various service providers in the four countries.

Country	AI service providers	Country currency[1]	Average costs of AI in US$	
			Local semen	Imported Semen
Tanzania	Government	TzS	11.2	67.04
Kenya[2]	Government	KES	3.0 (at source)	
	Private sector	KES	16.64	33.3
India	Government/private farms, NGO	INR	0.80	79.80
Nicaragua	Government/private	USD	10	15

Source: Ouma *et al.* (2014).
[1]Currency exchange rates used: 1 US$ = 62.65 INR; 90.12 KES; 1790.15 TzS; 597 CFA.
[2]See an example AI supply chain in Box 1.

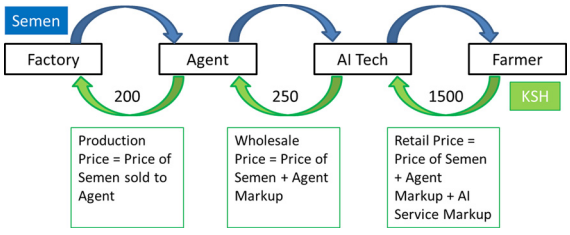
Costs of genetic and reproductive technologies and their influence on extent of usage

Compared with other reproductive technologies, AI has lower costs. The average costs vary greatly across the countries and are influenced by the population from which the sires were selected. This is illustrated in Table 7. In all the countries, semen imported from other countries is generally priced higher than that produced locally from the *B. taurus* breeds. It should however be noted that semen costs will also differ between countries based on national policies governing the use of imported semen, and depending on the prevailing exchange rates. The price of MOET was reported to be very high with no specific values given, and its use limited to high-input production systems (Questionnaire responses). In Nicaragua, the price per unit of sexed semen was reported to be US$30–40, while costs of embryos were reported to be higher than US$100 (Martin Mena Urbina, Questionnaire response).

The farm gate price of AI may vary greatly depending on the number of actors involved in the distribution chain (Ouma *et al.*, 2014). An example of variation pricing along one AI distribution chain in Kenya is presented in Box 1. Data were generally hard to find on current and projected costs of technology adoption, particularly at the level of dairy producer.

Box 1. Illustration of one AI Distribution chain in Kenya (adapted from (Ouma *et al.*, 2014)).

Semen is sold from the producer for Ksh 200 to the agent (price may vary depending on the bull and its breed), this represents 13 percent of the end price. The Agent sells to the AI service provider at 250, retaining 50 shillings representing 3 percent of the end price. The AI technician then sells the semen to a farmer at 1500 Shillings, retaining 1250 Shillings gross profit which represents 83 percent of the end price.



Conclusion and recommendations

The information collated in this study indicates that data on genetic characteristics and performance of the dairy populations in developing countries is becoming more readily available although still limited in scope. The countries presented are at different stages of developing national genetic improvement strategies that have clearly defined objectives, and are implementing genetic technologies that already exist, notably AI, to effect change. However, limited evidence on the current state and costs of the technologies in the different livestock populations makes it difficult to estimate the benefits to obtain from their adoption.

Crossbreeding and upgrading are common practices across the systems and countries but there is limited information on animal genetics and performance to guide selection towards the desired change. Changing a population without evidence of which animals to retain in the existing population could yield negative rather than positive results.

To bring about change in production practices that will lead to improved productivity of dairy systems within the countries, investments will be needed: to improve measurement and documentation of animal performance; to build technical capacity at different levels to better design and manage genetic improvement; for research to improve the uptake of genetic technologies in key production systems; and in the infrastructure and processes that will deliver appropriate technologies to target populations.

While there seems to be a case for public and private sector providers to offer services like AI that are already popular in some countries, in combination with other technologies currently adopted to a lesser extent, there is still much to understand about technology combinations that will bring about optimum benefits under the different management and agro-ecological systems. There also remains the larger questions on what models of policy, market and institution interventions and arrangements are needed to improve the adoption of animal genetic and reproductive technologies in the mostly smallholder dairy systems found in developing countries.

Acknowledgements

We wish to acknowledge the support of the Global Futures and Strategic Foresight Project (GFSF). This work was undertaken at the International Livestock Research Institute (ILRI) as part of the CGIAR Research Programme on Policies, Institutions and Markets (PIM), led by the International Food Policy Research Institute (IFPRI), and the CGIAR Research Programme on Livestock and Fish (L&F). Colleagues from various institutions in the countries studied shared documents and information on breeding technologies used in their countries. This information was provided by:

1. Dr. Y.N. Msanga National Coordinator AnGRs, Tanzania
2. Dr. H.A. Mruttu Principal Livestock Research Officer, Tanzania
3. Martin Mena Urbina Research Assistant on forages, CIAT, Nicaragua
4. Dr. Albert Soudre Scientist/Lecturer University of Koudougou, Burkina faso
5. Dr. S.K. Singh Principal Scientist, Head Genetics and Breeding Division, India

We gratefully acknowledge the support received from these institutions and individuals. However, the opinions expressed here are the full responsibility of the authors, as are the errors and omissions.

Statement of interest

No.

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Animal Genetic Resources, 2016, 59, 97–103. © Food and Agriculture Organization of the United Nations, 2016
doi:10.1017/S2078633616000229

Reproductive parameters of some native bovine breeds: Sanmartinero and Casanareño

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Resumen

En bovinos, la reducción de la eficiencia reproductiva de los sistemas de producción de carne y doble propósito se atribuye a factores nutricionales, sanitarios, climáticos y en última instancia a características genéticas de los animales. Sin embargo, en condiciones de trópico cálido húmedo, variaciones genéticas entre razas podrían reducir la edad al primer parto, el intervalo entre partos y aumentar la vida útil de las vacas. Las razas Sanmartinero y Casanareño podrían mejorar los sistemas de producción bovina debido al aporte de variantes genéticas que emergieron en el proceso de adaptación a las duras condiciones de la Orinoquía Colombiana. Actualmente, los genes con función biológica conocida se usan como marcadores moleculares para estimar parámetros de diversidad genética pecuaria facilitando la identificación y ubicación dentro del genoma de regiones que codifican o regulan la expresión de rasgos de interés económico. En ganado criollo colombiano Romosinuano se han identificado genes candidatos del eje Hormona de crecimiento/Factor de crecimiento similar a la insulina que se asocian positivamente con edad al primer parto, intervalo entre partos, longevidad y protección del embrión al estrés calórico. No obstante en las razas criollas Sanmartinero y Casanareño reconocidas empíricamente por estas características, no han sido sometidas a dichos análisis de genes candidatos que permitan promover un valor agregado a los animales. El objetivo de esta revisión es documentar algunos parámetros reproductivos y genéticos de las razas criollas Sanmartinero y Casanareño que soportan la necesidad de desarrollar estudios moleculares y justificar su uso en los sistemas de producción de carne y doble propósito de la Orinoquía colombiana.

Palabras clave: *edad primer parto, intervalo entre partos, genes candidatos, bovinos*

Summary

In cattle, reduced reproductive efficiency of beef and milk production systems is attributed to nutritional factors, health, climate changes and ultimately to genetic characteristics of animals. However, under warm humid tropics genetic variations between breeds could reduce age first calving, calving interval and increase the life of cows. Sanmartinero and Casanareño creole breeds can improve cattle production due to the contribution of genetic variants that emerged in the process of adaptation to the harsh conditions of the Colombian Orinoqia. Currently, genes with known biological function are used as molecular markers to estimate livestock genetic diversity parameters, facilitating the identification and location of genetic loci within the genome that encode or regulate the expression of traits of economic interest. In Colombia Romosinuano cattle candidate genes of the Growth hormone / Insulin growth factor axis have been identified and are positively associated with age at first calving, calving interval, longevity and protection of the embryo growth to heat stress. However, native Colombian bovine breeds such as Casanareño and Sanmartinero, which are empirically recognized by having those characteristic, have not been subjected to those genetic analysis for candidate genes that may allow to promote added value to animals. The aim of this review is to document some reproductive and genetic parameters of Sanmartinero and Casanareño bovine brees that may give support the need to conduct molecular studies and justify their use in beef and milk production systems in the Colombian Orinoquia.

Keywords: *age at first calving, calving interval, candidate genes, cattle*

Résumé

Chez bovins, réduit l'efficacité de la reproduction des bovins systèmes de production et à double objectif nutritionnel, la santé, le climat et, finalement, aux caractéristiques génétiques des animaux facteurs attribué. Cependant, dans des conditions tropicales chaudes et humides, les variations génétiques entre les races pourraient réduire l'âge au premier vêlage, l'intervalle de vêlage et d'augmenter la durée de vie des vaches. Les races Sanmartinero et Casanareño pourraient améliorer les systèmes de production de bovins en raison de la contribution des variants génétiques qui a émergé dans le processus d'adaptation aux conditions difficiles du Orinoquia colombien. Actuellement, les gènes ayant une fonction biologique connue sont utilisés comme marqueurs moléculaires pour estimer les paramètres de diversité génétique du bétail facilitant l'identification et la localisation des régions dans le génome qui codent ou réguler l'expression des caractères d'intérêt économique. Dans les gènes candidats colombiens Creole romosinuano ont été identifiés Croissance de l'axe hormone / facteur similaire à l'insuline qui est positivement associée à l'âge au premier vêlage, l'intervalle de vêlage, la longévité et la protection de l'embryon pour chauffer la croissance du stress. Cependant, dans Sanmartinero et Casanareño empiriquement reconnu par ces caractéristiques des variétés locales, ils ne sont pas soumis à une telle analyse des gènes candidats qui peuvent favoriser la valeur ajoutée aux animaux. L'objectif de cet examen est de documenter certains

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paramètres génétiques et de reproduction Sanmartinero et Casanareño variétés locales qui soutiennent la nécessité de développer des études moléculaires et de justifier leur utilisation dans les systèmes de production de viande et double objectif de la région colombienne Orénoque.

Mots-clés: *âge au premier vêlage, l'intervalle entre vêlages, bovins*

Presentado: 19 Octubre 2016; aceptado: 5 Septiembre 2016

1. Introducción

Los sistemas de producción de carne y doble propósito en el trópico húmedo colombiano poseen ventajas comparativas frente a otros sistemas productivos gracias a la eficiencia reproductiva de las hembras bovinas, sin embargo, se ha observado que en las ultimas 4 décadas, la tasa de fertilidad es cada vez menor, convirtiéndose en la principal causa de descartes y remplazos en los hatos a nivel mundial (Gómez, 1981; Hill, 2010; Cummins et al. 2012; Martinez-Rocha J. 2012; Minozzi et al. 2013; Nicolini et al. 2013). La reducción de la capacidad reproductiva se ha atribuido a factores nutricionales, sanitarios, climáticos y en última instancia a características genéticas del grupo racial.

En la Orinoquía Colombiana, las razas criollas Sanmartinero y Casanareño reconocidas localmente por su longevidad, productividad, adaptación a altas temperaturas y elevada humedad, resistencia a parásitos y enfermedades, gran capacidad de desplazamiento, excelente habilidad materna y aprovechamiento de pastos pobres en nutrientes (Sastre, 2003). Gracias a dichas características, éstas razas pueden ser introducidas en climas cálidos y húmedos para mejorar la eficiencia reproductiva.

En ganadería cebú, las altas temperaturas en época de sequía, la elevada humedad relativa en la época de lluvias y la baja nutrición de los forrajes retrasan en las hembras el inicio de la pubertad, la edad al primer parto, aumenta los intervalos entre partos y disminuye el número de crías durante la vida útil de los animales (Hernández et al. 2008; Martinez-Rocha J. 2012), aunque también se argumenta que la baja tasa productiva se debe a una falta de control reproductivo (Vergara et al. 2008), sin embargo, trabajos realizados por Grajales et al. (2006) en condiciones de trópico cálido húmedo sugieren la existencia de variantes genéticas entre y dentro de razas que pueden afectar la edad al primer parto (EPP), el intervalo entre partos (IEP), los días abiertos, reducir el número de descendientes, la vida útil de las vacas y el valor comercial de los animales.

Actualmente, análisis genómicos facilitan la identificación de regiones del genoma asociadas positivamente a características productivas de interés económico. En hembras de raza criolla Colombiana Romosinuano se han reconocido genes del eje Hormona de crecimiento/Factor de crecimiento similar a la insulina tipo I (GH/IGF-I) asociados positivamente como la reducción de la EPP, el IEP (Riley et al. 2010; Luna-Nevárez et al. 2012), y protección del embrión al estrés calórico (Hernández-Cerón, 2004). Estas características también han sido reconocidas empíricamente en el ganado criollo Sanmartinero y Casanareño, donde las hembras podrían ser utilizadas de manera racional en programas de mejoramiento genético para mejorar la tasa de natalidad al año y de forma práctica mejorar los complejos procesos de conservación de la raza (Bedoya et al. 2001). No obstante, los análisis de genes candidatos para corroborar dichas observaciones y promover un valor agregado a los animales, no se han llevado a cabo. El objetivo de esta revisión es describir las características reproductivas de las razas criollas Sanmartinero y Casanareño que soportan su uso en los sistemas de producción de cría y doble propósito de la Orinoquía colombiana.

2. Productividad de las razas criollas

Las condiciones anuales de temperatura, lluvias, oferta de forraje, el grupo racial y la orientación de la producción, influencian el desempeño reproductivo de las hembras en el hato como consecuencia de la interacción genotipo-ambiente (Lemka et al. 1973; Martin et al. 1992; Cerón-Muñoz et al. 2003; García et al. 2003; Grajales et al. 2006; Perotto et al. 2006; Vergara et al. 2009; Mejía-Bautista et al. 2010; O'Neill et al. 2010; Castillo-Badilla et al. 2013). No obstante, el comportamiento reproductivo de las razas criollas siempre ha sido superior, al comparar la eficiencia reproductiva de 2 razas *Bos indicus* de origen indio y dos razas *Bos taurus* de origen colombiano, las razas BON (blanco orejinegro) y CCC (costeño con cuernos) manejadas en un sistema de pastoreo tradicional con gramíneas nativas en Turipaná (Colombia), y las razas Hariana y Deshi alimentadas con forraje verde y paja de arroz , se demostró que las razas BON y CCC fueron más eficientes reproductivamente que las razas Indias, y alcanzaron edades al primer parto menores de 30 meses e intervalo entre partos de 399 días, frente a 36 meses y 463 días en las razas Hariana y Deshi (Lemka et al.1973). Los autores concluyeron que si bien la oferta de alimento a temprana edad influye en la edad al primer parto, el intervalo entre partos podría estar relacionado con los genotipos (Lemka et al.1973).

En condiciones de trópico cálido húmedo (Centro de investigaciones Turipaná-Cereté, Córdoba, Colombia) se ha demostrado que la raza criolla colombiana

Romosinuano, llega a la pubertad a los 601 días con 315 kg con una tasa de concepción al primer servicio de 95%, mientras que razas cebuinas alcanzan la pubertad a los 713 días con 400 kg de peso y una tasa de concepción del 81.8% en el primer servicio (Grajales et al. 2006). También advierten que el desempeño reproductivo de la Romosinuano es superior en cuanto a tasa de concepción e intervalo entre partos con respecto a hembras Simmental x Cebú y Holstein x Cebú. En este sentido, las diferencias en la edad de manifestación de la pubertad entre razas se puede atribuir a efectos aditivos de genes presentes en diferentes frecuencias (Martin et al.1992).

Una evaluación del efecto genético directo que posee la raza Romosinuano para reducir la EPP, llevada a cabo en la estación de Investigación en Agricultura subtropical (USDA-ARS) Brooksville-Florida (EU), demostró que el Romosinuano (R) presento edades más tempranas para primer parto frente al Angus (A) y Brahman (B) (489 días vs. 537 y 600 días, respectivamente). Adicionalmente, en los cruces RB y AR (primera letra raza paterna), las edades del primer parto disminuyeron significativamente (471 y 409 días, respectivamente) (Riley et al. 2010).

El análisis del F1 Sanmartinero x Cebú, este posee ventajas en natalidad del 12.6%, sobrevivencia de 6.9% y produjo 41.6% más carne que el Cebú puro en estudios llevados a cabo en el centro de investigaciones la Libertad (Villavicencio, Colombia). Con respecto a la EPP, la heterosis fue de -5.2% y para IEP de -6.6% (Sastre, 2003). En el mismo centro de investigaciones, se estimó una EPP de 2.71 años para la raza Sanmartinero, con heredabilidad de 0.34 (Martínez-Villate et al. 2009).

En la raza Casanareño, los parámetro zootécnicos reportados por Sastre (2003) fueron: Preñez 87.6%, natalidad 82.6%, mortalidad en neonatos 1.0%, mortalidad en jóvenes 0.7%, Edad al primer servicio 25 meses, Edad al primer parto entre 34 a 36 meses e Intervalo entre partos 353–425 días. Parámetros registrados por la raza en condiciones de sabana inundable, muy superiores a los reportados en Cebú en la misma ecorregión, que dejan en evidencia su superioridad reproductiva.

3. Importancia biológica del eje Hormona de crecimiento / Factor de crecimiento similar a la Insulina (GH/IGF-I)

El eje hormonal GH/IGF (*Growth Hormone – Insulin like Growth Factor*) a nivel celular, se ha relacionado con crecimiento, diferenciación y expresión genética (Sánchez de Gómez, 2006). A nivel reproductivo, interviene en procesos como foliculogénesis, estereidogénesis y desarrollo embrionario (Luna-Nevárez et al. 2009).

Características reproductivas como EPP e IEP han sido asociadas a QTLs (*quantitative trait loci*) localizados en los cromosomas 5 y 16 del bovino (BTA5 y BTA16), particularmente en hembras tipo carne adaptadas a condiciones tropicales y subtropicales: Brahman (Hawken et al. 2012), Brangus (Fortes et al. 2012) y Romosinuano (Luna-Nevárez et al. 2012). Estas características podrían estar reguladas por los genes *IGF-I*, el receptor de la hormona de crecimiento *GHR*, proteínas de enlace del IGF: *IGFBP*, el transductor de señal y activador de la transcripción *STAT* y el supresor de señales *SOCS* (Luna-Nevárez et al. 2012).

Desde el punto de vista fisiológico, la hormona de crecimiento estimula la síntesis hepática de IGF-I. Las IGFBP, regulan su biodisponibilidad, mientras que STAT, activan procesos enzimáticos para la síntesis y SOCS, inhibe su secreción en condiciones de estrés (Luna-Nevárez et al. 2012).

Bajo restricción nutricional proteica, ratones con silenciamiento específico del gen *IGF-I* LID (*Liver-specific IGF-I gene deficient*), pusieron en evidencia alteraciones en la producción de IGF-I en hígado. Las deficiencias nutricionales alteran la señalización de GH corriente abajo del receptor *GHR* que inhibe la actividad de JAK2-STAT5 por GH y la sobre expresión de *SOCS-3*, reduciendo los niveles de expresión de *IGF-I* en animales malnutridos (Sánchez de Gómez, 2006).

En el ovario, la expresión de RNA mensajero (RNAm) de receptores para hormona de crecimiento, la producción local de IGF y de IGFBP, tiene un importante papel en el desarrollo folicular y función del cuerpo lúteo (Schams et al. 1999; Rhoads et al. 2008). En estudios realizados en Fleckvieh-Alemania, indicaron que existe una correlación positiva entre la expresión de RNAm de *GHR* en células de la granulosa de folículos preovulatorios y en células luteales al inicio de la formación del cuerpo lúteo (entre el 5 y 7día). Mientras que el RNAm de *IGF-I* se evidencio en las células intersticiales de la teca hacia el final del crecimiento folicular, esto también indica la presencia de IGFBP en las células intersticiales de la teca y de la granulosa, llegando a ser muy fuerte la expresión de IGFBP 3, 4 y 6 en células de la teca cuando el estradiol alcanza una concentración entre 20 y 180 ng/ml en el fluido folicular y de IGFBP 4 en células de la granulosa cuando el estradiol sobrepasa los 20 ng/ml en el fluido folicular (Schams et al. 1999).

En útero, la expresión de RNAm de *GHR* y *IGF-I* no es tan marcada como en el cuerpo lúteo y folículo dominante (Rhodas et al. 2008), sin embargo, Walenkamp y Wit (2007) reportaron en humanos y en ratones, que las deficiencias en los niveles de expresión de *IGF-I* debidas a polimorfismos en la región promotora del gen están asociadas a la reducción del peso y la talla al nacimiento. En bovino criollos Sanmartinero, se ha reportado que los pesos al nacimiento son menores con respecto a otras razas europeas y cebuinas. Sin embargo, esta característica ha sido relacionada con una involución uterinas temprana que permite a las hembras disminuir días abiertos (Martínez, 2000).

4. Genes candidatos

Los genes candidatos son genes con función biológica conocida que regulan directa o indirectamente procesos de desarrollo de rasgos de interés (Parra y Sifuentes, 2012). Se usan como marcadores moleculares para estimar parámetros de diversidad genética pecuaria, lo que facilita el descubrimiento y la localización de una región dentro de un genoma que codifica para uno o más genes con efecto significativo sobre un carácter cuantitativo (Parra y Sifuentes, 2012). Estos análisis han demostrado ser extremadamente poderosos para el estudio de la arquitectura genética de rasgos complejos como fertilidad y resistencia a enfermedades (Zhu y Zhao, 2007).

4.1 Gen PAPP-A2

El gen *PAPP-A2*, transcribe para la *Pappalisina-2,* una metalo-proteína que degrada la proteína de enlace del IGF-5 (IGFBP-5), facilitando la liberación del IGF1, quien se encarga de regular múltiples funciones relacionadas con la reproducción y el crecimiento (Wickramasinghe et al. 2011; Luna-Nevárez et al. 2012).

En hembras Romosinuano, un polimorfismo (T) en el gen *PAPP-A2*, fue significativamente ($p < 0.05$) asociado con reducción de la EPP ($-37{,}1 \pm 14.4$ días) y edad al segundo parto ($-65{,}43 \pm 30.8$ días), demostrando un efecto de sustitución alélica y un efecto aditivo de los alelos (Luna-Nevárez et al. 2012).

En ganado Holstein, 19 SNP han sido encontrados en *PAPP-A2*. Los genotipos TT para SNP13, TT en SNP15 y GG en SNP16, han sido significativamente ($p < 0.05$) asociados a facilidad de parto, tasa de preñes, vida productiva, producción de leche y de grasa. El SNP13 está a 15 pb de un sitio donador de corte y empalme alternativo (*splice*), mientras que el SNP15 a 104 pb de una región *splice* receptora y la sustitución no sinónima A/G del SNP16 cambia la Glicina^{1254} por ácido aspártico (Wickramasinghe et al. 2011).

En humanos, esta metaloproteina *Pappalisina-2,* recientemente descubierta, tiene un homologo, la PAPP-A, pero exhibe la misma actividad proteolítica sobre IGFBP-5 y IGFBP-3. Aunque no sobre la IGFBP-4 que es el sustrato para PAPP-A. Ambas están relacionadas con la inhibición o estimulación de la actividad del IGF 1 y 2 (Wang et al. 2009; Conover et al. 2011). La proteína PAPP-A juega un papel importante en el desarrollo fetal y su deficiencia en humanos puede indicar riesgo de desórdenes genéticos como los síndromes de Down y Cornelia de Lange; mientras que en ratones, implica retardado y deficiente desarrollo fetal. La PAPP-A2 humana se ha relacionado con desordenes hipertensos durante la preñez, preclamsia, hemolisis, elevación de enzimas hepática y el síndrome de plaquetas bajas (Wang et al. 2009).

4.2 Gen *STAT2* Gene ID: 511023

Los factores de transducción y activadores de la transcripción están involucrados en eventos biológicos diversos como programación de la expresión génica, desarrollo embrionario, muerte celular programada, organogénesis, respuesta inmune innata y adaptativa. Luna-Nevárez et al. (2009 y 2012) hallaron en el promotor del gen *STAT2* un polimorfismo (A/T) asociado con los días de gestación e intervalo entre partos en *Bos taurus* y *Bos indicus*. *STAT2* (*signal transducer and activator of transcription 2*) (http://www.ensembl.org/Bos_taurus), de 24 exones, 23 transcriben para proteínas de la familia traductora de señales y activadora de la transcripción. *STAT2* estaría involucrado en el proceso de reconocimiento materno del embrión en la etapa de pre-implantación (Bauersachs et al. 2006; Luna-Nevárez et al. 2009), donde luego de la unión del Interferon-Tau a los receptores tipo I de IFN endometriales, el complejo STAT2 se une a elementos de respuesta ubicados en los promotores de genes regulados por el interferón, ISRE (*Interferon-stimulated response element*) (Bauersachs et al. 2006; Steen and Gamero, 2013).

4.3 Gen *IGF1* Gene ID: 281239

En bovinos, *IGF1* ha sido asociado con la edad al primer parto, reanudación de la actividad ovárica posparto, tasa de concepción al primer servicio, ovulación doble y pre-implantación embrionaria (Mullen et al. 2011).

En hembras no gestantes, los receptores para IGF1 se encuentran en las células de la granulosa del ovario, donde actúa como un mecanismo de amplificación local para acción de las gonadotropinas facilitando el desarrollo folicular. En bovinos, el IGF1 aumenta la liberación de LH sin modificar los receptores para GnRH (Lenz et al. 2007). Sin embargo, en vacas lecheras posparto la baja concentración de IGF1 circulante está relacionado con el desarrollo de ovarios inactivos, quistes foliculares y cuerpo lúteo persistente (Velázquez y Oropeza, 2007; Nicolini et al. 2013). Mientras que en ganado de carne, los estudios han demostrado que la ovulación se encuentra significativamente afectada por la concentración sanguínea de IGF1 independiente de la secreción de gonadotropinas (Velázquez y Oropeza, 2007).

La elevación plasmática del IGF-1 es importante no solamente para el desarrollo folicular, también promueve de forma directa la supervivencia de espermatozoides y del embrión precoz, o indirecta al aumentar las secreciones del oviducto y el útero (Lenz et al. 2007). En las vacas gestantes, el IGF1 aumenta la proporción de implantación embrionaria y modifica la expresión genética del blastocito, además puede proveer resistencia al choque térmico y al estrés oxidativo a embriones pre-implantados de 4 a 6 días. *In vitro*, mejora la supervivencia embrionaria luego de ser transferidos dentro de recipientes para medir resistencia a estrés por calor (Bonilla et al. 2011).

Uno de los polimorfismos más estudiado en ganado lechero es el *IGF1/SnaBI* que ha sido asociado a características de producción lechera. No obstante, también se ha considerado como un SNP marcador de

características de fertilidad en hembras Holstein-Friesian (Mullen et al. 2011; Nicolini et al. 2013).

En trabajos realizados por Nicolini et al. (2013), el cambio en la base 512 de T a C (512 T/C) que se encuentra en la región promotora del gen, genera 3 genotipos: AA (TT), AB (TC) y BB (CC). El genotipo AA, esta significativamente asociado con la reactivación de la actividad cíclica del ovario, independiente de la condición corporal y balance energético de las vacas posparto Holstein-Friesian en sistema de pastoreo.

4.4 Gen *GHR* Gene ID: 280805

La hormona de crecimiento (GH: *growth hormone*) es un péptido secretado por la glándula pituitaria y se reconoce como la mayor reguladora del crecimiento y metabolismo de carbohidratos y proteínas (Grace, 2012). En humanos, su exceso puede resultar en enfermedades como acromegalia o resistencia a la insulina. Su deficiencia en jóvenes termina en fallas en el crecimiento y corta estatura, mientras que en adultos en problemas como obesidad o disminución de la masa muscular (Grace, 2012). Estas respuestas son usualmente mediadas por su unión a los receptores de la GH (GHR) en el hígado (Shoba et al. 1999). El GHR (*growth hormone receptor*), es un miembro de la superfamilia de receptores citoquinas y es clasificado como un receptor de citokina tipo I (Grace, 2012).

En trabajos realizados por Rhoads et al. (2008) se evidencio que la expresión de los GHR en el cuerpo lúteo están positivamente relacionados con la expresión de IGF1 y esta expresión aumenta con el número de partos, encontrando mayor expresión en vacas de tercer parto que en vacas de segundo parto.

Luna-Neváres et al. (2012) encontraron un polimorfismo (A/G) en la región promotora del gen *GHR* que se asoció a disminución de EEP por su acción reguladora en la expresión del *IGF1*.

5. Conclusión

Las razas criollas colombianas como Sanmartinero y Casanareño se adaptaron a las altas temperaturas en época de sequía y elevada humedad relativa durante las lluvias en la Orinoquía, aprovechan mejor los recursos disponibles para producir más terneros por finca que en última instancia aumenta los kilogramos de carne producida por hectárea. Sin embargo, en Colombia, los trabajos genéticos sobre razas criollas, aun no incluyen el análisis de genes candidatos que soporten la superioridad genética en características de baja heredabilidad y alto interés económico como las reproductivas. La caracterización genética del comportamiento reproductivo de estas razas, solo por conservar el recurso, no es en sí mismo una razón para conservar, la estrategia es dar la oportunidad de optimizar los sistemas de producción de alimentos de origen animal y suplir las demandas de la creciente población humana, razón por la que se requiere caracterizar genéticamente las razas criollas Sanmartinero y Casanareño. El genoma bovino ya está en base de datos, los genes candidatos existen y los parámetros fenotípicos sobre la superioridad reproductiva de las razas criollas bajo condiciones ambientales colombianas también están reportadas, solo resta identificar genes candidatos del eje GH/IGF y su relación con el potencial genético y reproductivo de dichas razas.

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Animal Genetic Resources, 2016, 59, 105–112. © Food and Agriculture Organization of the United Nations, 2016
doi:10.1017/S2078633616000205

Population viability analysis on a native Danish cattle breed

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Summary
Many domestic breeds face challenges concerning genetic variability, because of their small population sizes along with a high risk of inbreeding. Therefore, it is important to obtain knowledge on their extinction risk, along with the possible benefits of certain breeding strategies. Since many domestic breeds face the same problems, results from such studies can be applied across breeds and species. Here a Population Viability Analysis (PVA) was implemented to simulate the future probability of extinction for a population of the endangered Danish Jutland cattle (*Bos taurus*), based on the software Vortex. A PVA evaluates the extinction risk of a population by including threats and demographic values. According to the results from the PVA the population will go extinct after 122 years with the current management. Four scenarios were created to investigate which changes in the breeding scheme would have the largest effect on the survival probabilities, including Scenario 1: More females in the breeding pool, scenario 2: More males in the breeding pool, scenario 3: Increased carrying capacity, and scenario 4: Supplementing males to the population through artificial insemination using semen from bulls used in the populations in past generations. All scenarios showed a positive effect on the population's probability of survival, and with a combination of the different scenarios, the population size seems to be stabilized.

Keywords: *Bos taurus, breeding simulation, genetic management, Population Viability Analysis, Vortex*

Résumé
De nombreuses races domestiques font face à des défis liés à la variabilité génétique en raison de leur petite taille de population qui s'accompagne d'un risque élevé de consanguinité. Par conséquent, il s'avère important de connaître leur risque d'extinction, ainsi que les avantages potentiels de certaines stratégies de sélection. Vu que beaucoup de races domestiques confrontent les mêmes problèmes, les résultats de ces études peuvent être appliqués sans distinction de race ou d'espèce. Ici une Analyse de Viabilité des Populations (AVP) a été menée pour simuler, en utilisant le logiciel Vortex, la probabilité future d'extinction d'une population de bovins menacés: les bovins danois du Jutland (*Bos taurus*). Une AVP évalue le risque d'extinction d'une population en tenant compte des menaces et des données démographiques. D'après les résultats de l'AVP, la population s'éteindra après 122 ans avec la gestion actuelle. Quatre scénarios ont été présentés pour examiner quels changements dans le schéma de sélection auraient le plus grand effet sur les probabilités de survie, y compris le scénario 1: Plus de femelles dans le pool de reproducteurs, le scénario 2: Plus de mâles dans le pool de reproducteurs, le scénario 3: Une plus grande capacité porteuse et le scénario 4: Fournir des mâles à la population par le biais de l'insémination artificielle avec du sperme de taureaux utilisés dans les populations dans les générations passées. Tous les scénarios ont présenté un effet positif sur la probabilité de survie de la population et, avec une combinaison des différents scénarios, la taille de la population semble se stabiliser.

Mots-clés: *Analyse de Viabilité des Populations, Vortex, gestion génétique, logiciel de simulation pour la sélection, Bos taurus*

Resumen
Muchas razas domésticas se enfrentan a desafíos relacionados con la variabilidad genética, debido a su pequeño tamaño de población que se acompaña de un elevado riesgo de endogamia. Por ello, resulta importante conocer su riesgo de extinción, así como las posibles ventajas de ciertas estrategias de selección. Puesto que muchas razas domésticas comparten los mismos problemas, los resultados de dichos estudios pueden ser aplicados independientemente de la raza o la especie. En este caso, se llevó a cabo un Análisis de Viabilidad de Poblaciones (AVP) para simular, basándose en el programa Vortex, la probabilidad futura de extinción de una población de ganado bovino amenazado: el ganado danés de Jutlandia (*Bos taurus*). Un AVP evalúa el riesgo de extinción de una población teniendo en cuenta las amenazas y los datos demográficos. De acuerdo con los resultados del AVP, la población se extinguirá al cabo de 122 años con el manejo actual. Se plantearon cuatro escenarios para investigar qué cambios en el esquema de selección tendrían el mayor efecto sobre las probabilidades de supervivencia, incluyendo el escenario 1: Más hembras en el núcleo reproductor, el escenario 2: Más machos en el núcleo reproductor, el escenario 3: Una mayor capacidad de carga y el escenario 4: Aportar machos a la población mediante inseminación artificial con semen de toros empleados en las poblaciones en generaciones pasadas. Todos los escenarios

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presentaron un efecto positivo sobre la probabilidad de supervivencia de la población y, con una combinación de los diferentes escenarios, el tamaño de la población parece estabilizarse.

Palabras clave: *Análisis de Viabilidad de Poblaciones, Vortex, manejo genético, programa de simulación para la mejora genética, Bos taurus*

Submitted 23 February 2016; accepted 21 July 2016

Introduction

Small populations have an increased risk of extinction due to genetic, environmental and demographic stochasticity (Frankham, Ballou and Briscoe, 2009; Allendorf, Luikart and Aitkin, 2012). Genetic challenges for small populations include increased homozygosity due to inbreeding, which may lead to inbreeding depression, and genetic drift which reduce genetic variation within populations (Demontis *et al.*, 2011; Kristensen *et al.*, 2015). Because genetic drift is a stochastic process, which is affected by demographic changes, catastrophes, and environmental variation, the outcome can only be simulated (Shaffer, 1987; Mills and Allendorf, 1996; Frankham, Ballou and Briscoe, 2009; Allendorf, Luikart and Aitkin, 2012). Livestock breeds often have low effective population sizes (N_E) and inbreeding depression is commonly observed (Kristensen and Sørensen, 2005; Leroy *et al.*, 2013). Developments in reproductive technologies, such as artificial insemination, have accelerated the reductions of N_E within many domestic breeds, e.g. because these technologies have led to highly skewed sex ratios with many more females than males in the populations (Hiemstra *et al.*, 2010). The purpose of this study is to use a population viability analysis (PVA) as a tool to predict the possible future of a population of an endangered native breed, the Jutland cattle (*Bos taurus*) from Denmark. The Jutland cattle breed originated from the Grey and Black Pied cattle, which was widespread in Jutland (Danish peninsula), Denmark, from the Middle ages until the 19th century (Brüniche-Olsen, Gravlund and Lorenzen, 2012). This breed has since experienced a dramatic decline in population size because other dairy cattle breeds, such as Jersey and Holstein–Friesian, are more productive and therefore preferred by farmers. Only a few farmers have kept the original Jutland cattle. Today the breed consists of a few populations, one named the Kortegaard population, which is investigated in this project. The impact of changes in the breeding scheme on the population will be investigated, and, based on the results, practices expected to increase the long-term survival probabilities of this, and likely other small populations of domestic animals, will be suggested.

Materials and methods

Population viability analysis

A database created by the Danish Veterinary and Food Administration (available at chr.fvst.dk) provided data on the individual cattle from the entire Kortegaard population. The demographic data were collected from the database and the farmers. The data include: the number of individuals, the age of living individuals, dates of birth and death, the age of females when calving, the average age of reproduction, the number of males in the breeding pool, the average number of offspring per male, and the level of allowed kinship between male and female when breeding. These data were used to construct a PVA.

Different software can be used for making a PVA and one is Vortex, a program developed by Chicago Zoological Society, and Vortex version 9.999 was employed in this project (Miller and Lacy, 2005). Vortex uses a stochastic model, and the demographic variables needed to run the simulation can be found in Vortex's user's manual and in Table 1 (Miller and Lacy, 2005).

Parameters

The age categories are denoted age 1, age 2, age 3 and so forth, which are equivalent to 0–1 year, 1–2 years and 2–3 years, respectively (Miller and Lacy, 2005). The inserted values represent the current population. The carrying capacity and initial population size were set to 104, equal to the population size of the Kortegaard population at the time of the study. In the simulations, the population is characterized by a polygynous mating system because males can mate with several females each year. Values for the different parameters can be found in Table 1.

Demographic parameters

A female typically becomes sexually mature when approximately 1 year old (Nowak, 1999). Though, based on data from cattle born in year 2001–2010, a calculated average showed that females of Jutland cattle calve for the first time at the age of 30 months after nine months of gestation. If allowed, females reproduce until around age 12 years; however, there are examples of Jutland cattle reproducing at age 15 (Nowak, 1999). Females active in the breeding pool normally produce one calf per year (twins are rare) after a successful mating. Abortions and twin births were not taken into consideration in the simulations. The sex ratio of born calves is approximately 50:50, but more females than males enter the breeding pool because most males are slaughtered or castrated before they reproduce. From 2001 to 2010 the average percentage of females being active in the breeding pool was

Table 1. Conditions and basic parameters for the simulations on the Kortegaard herd of Jutland cattle in Vortex.

	Baseline	Scenario 1	Scenario 2	Scenario 3	Scenario 4
Scenario settings					
Iterations	10 000	10 000	10 000	10 000	10 000
Number of populations	2	2	2	2	2
Species description					
Lethal equivalents	3.14	3.14	3.14	3.14	3.14
Percent due to recessive lethals	50%	50%	50%	50%	50%
Dispersal					
Age range: youngest	2	2	2	2	2
Age range: oldest	15	15	15	15	15
Dispersing sex	Males	Males	Males	Males	Males
Dispersal rates					
Population 1 → Population 2	100	100	100	100	100
Population 2 → Population 1	0	0	0	0	0
Reproductive system					
Polygynous	Yes	Yes	Yes	Yes	Yes
Age of first offspring (females)	2.6	2.6	2.6	2.6	2.6
Age of first offspring (males)	1	1	1	1	1
Max. age of reproduction	15	15	15	15	15
Max. number of broods per year	1	1	1	1	1
Max. number progeny per brood	1	1	1	1	1
Sex ratio birth in percent (males)	50	50	50	50	50
Reproductive rates					
% Adult females breeding	59.3	**70**	59.3	59.3	59.3
EV in %breeding	20.7	20.7	20.7	20.7	20.7
Distribution of broods per year					
1 brood	100	100	100	100	100
Mortality rates:					
Mortality of females as %:					
Mortality from age 0 to 1 (± SD)	1.8 ± 3.8	1.8 ± 3.8	1.8 ± 3.8	1.8 ± 3.8	1.8 ± 3.8
Mortality from age 1 to 2 (± SD)	2.0 ± 6.3	2.0 ± 6.3	2.0 ± 6.3	2.0 ± 6.3	2.0 ± 6.3
Annual mortality after age 2 (±SD)	29.8 ± 6.8	29.8 ± 6.8	29.8 ± 6.8	29.8 ± 6.8	29.8 ± 6.8
Mortality of males as %:					
Mortality from age 0 to 1 (± SD)	8.9 ± 9.0	8.9 ± 9.0	8.9 ± 9.0	8.9 ± 9.0	8.9 ± 9.0
Annual mortality after age 1 (± SD)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Mate monopolization					
% Males in breeding pool	46.7	46.7	**60**	46.7	46.7
Initial population size	104	104	104	104	104
Carrying capacity					
Carrying capacity (K) (± SD)	104 ± 10	104 ± 10	104 ± 10	104 ± 10	104 ± 10
Future change in K	No	No	No	**Yes**	No
Over how many years	–	–	–	**20**	–
% Annual increase	–	–	–	**4**	–
Supplementation					
Population supplemented?	–	–	–	–	**1**
First year of supplementation	–	–	–	–	**0**
Last year of supplementation	–	–	–	–	**100**
Interval between supplementations	–	–	–	–	**5**
Number of adult males supplemented	–	–	–	–	**1**
Genetic management					
Prevent matings with kinship greater than	0.25	0.25	0.25	0.25	0.25

Conditions and basic parameters for the Kortegaard herd of Jutland cattle for simulations in Vortex 9.999. "Baseline" is the continuation of the current way of breeding, "Scenario 1" includes increased percentage of the females in the breeding pool, "Scenario 2" includes increased percentage of the males in the breeding pool, "Scenario 3" includes increased carrying capacity, and "Scenario 4" includes supplementation of cryopreserved semen to the population.
The bold values are to highlight the values that are different in the scenarios compared to Baseline.

estimated to 59.3 percent. This corresponds to approximately 27 females out of a total of 46 sexually mature females in the current breeding scheme. Males enter the breeding pool when 1–2 years old. In theory, males can reproduce their whole life, but on average they only sire six to seven calves in the population. This was taken into account in the simulations by using the function "Dispersal" and letting all males older than 2 years migrate without the ability to return. A composed matrix forced males older than 2 years to migrate from populations 1 to 2 (a fictive population made for this purpose), but the probability of dispersal from populations 2 to 1 was set

to 0 percent (Miller and Lacy, 2005). Occasionally a male is used several years and is therefore older than 2 years before castration or slaughter, but this happens rarely and was thus not included in the simulations. At the time this study was performed the population consisted of 15 reproductively active males including four steers, and these make out the potential breeding pool. The steers were included in the calculations of the percentage of males active in the breeding pool, since they have reached the age of sexual maturity, and Vortex cannot differentiate between steers and bulls. Of the 15 males, seven were selected for breeding in 2013, and therefore it was estimated that only 46.7 percent of the males were active in the breeding pool.

Mortality rates for females were calculated from data gathered from females born from 2001 to 2010, by taking the numbers of deaths amongst the 0–1 and 1–2 years old females plus the annual percentage of deaths among individuals older than 2 years. The same method was used to estimate mortality rates for males, where the numbers of deaths amongst the 0–1-year-old males as well as the annual percentage of deaths among individuals older than 1 year were calculated.

Environmental variation

Vortex utilizes the environmental variation as the annual differences in the prospects of reproduction and survival due to stochastic changes in the environment (Miller and Lacy, 2005). Since this domestic population is reared in a controlled environment, "Environmental variation" is not a relevant parameter for these scenarios. The likelihood of a domestic cattle breed being struck by diseases or other catastrophes is low because of veterinary treatment and because natural disasters are rare in Denmark. Therefore "Catastrophes" were not taken into consideration.

Inbreeding and genetic management

The loss of genetic diversity is expressed as the rate of inbreeding in both wildlife and domestic populations (Hartl and Clark, 1989). This category in Vortex is primarily used for populations under management or in captivity, where the breeding can be controlled (Miller and Lacy, 2005). Lethal equivalents per diploid genome were set to 3.14, as this is the median value observed from a population study of 40 captive vertebrates (Ralls, Ballou and Templeton, 1988). The number of lethal equivalents determines the severity of inbreeding depression (Miller and Lacy, 2005).

Under "Genetic management" matings between closely related individuals can be prevented and the coefficient was set to 0.25, because offspring/parent and full sibling mating are avoided. It must be considered that livestock breeding often has a greater degree of inbreeding compared with wildlife populations (Lacy, 1993). Inbreeding may not have the same effect on domestic and wild populations, e.g. because the domestic populations may tolerate greater levels of inbreeding due to controlled and benign environmental conditions (Reed *et al.*, 2012; Kristensen *et al.*, 2015). Therefore, the inbreeding coefficient; $F = 0.25$ was used for this population, although lower values are normally used when investigating wild populations (Lacy, 1997; Allendorf and Ryman, 2002).

Scenarios

Four scenarios were made to simulate the future of the Kortegaard population under different breeding strategies.

Baseline: A continuation of the current breeding practice.

Scenario 1: The percentage of the total number of females active in the breeding pool was increased from 59.3 to 70 percent per year.

Scenario 2: The percentage of the total number of males active in the breeding pool was increased from 46.7 to 60 percent per year.

Scenario 3: The carrying capacity was set to increase with 4 percent per year over a period of 20 years.

Scenario 4: Supplementing one male to the population every 5 years representing use of cryopreserved semen from bulls from previous generations. By using "Optional criteria of supplementation" the supplemented individual will have a certain relation to the population. The allowed kinship between the supplemented males and the population was set to 0.125–0.25.

Furthermore, combinations of the different scenarios were investigated.

Results

The results of the study suggest that the Kortegaard population will go extinct after 122 years if the current breeding practice continues (represented as Baseline). The higher the level of inbreeding the lower the reproductive fitness, but the extinction is due to too few sufficiently unrelated individuals. However, the results also show that the chances of survival can be increased by changing aspects of the breeding practices (Figure 1a). With an increase of females active in the breeding pool from 59.3 to 70 percent of all available females (Scenario 1), the population reaches extinction after 126 years and the population size is more stable compared with Baseline. When increasing the number of males active in the breeding pool from 46.7 to 60 percent (Scenario 2) the population is predicted to go extinct after 136 years. Increasing the carrying capacity annually by 4 percent over the course of 20 years (Scenario 3) results in an increase in the population size for 25 years, after which the population size decreases and reaches extinction after 189 years. The investigated increases in the number of females and males in the breeding pool, as well as the carrying capacity, are selected based on trial simulations in which these percentages showed the best outcome compared with the farmers'

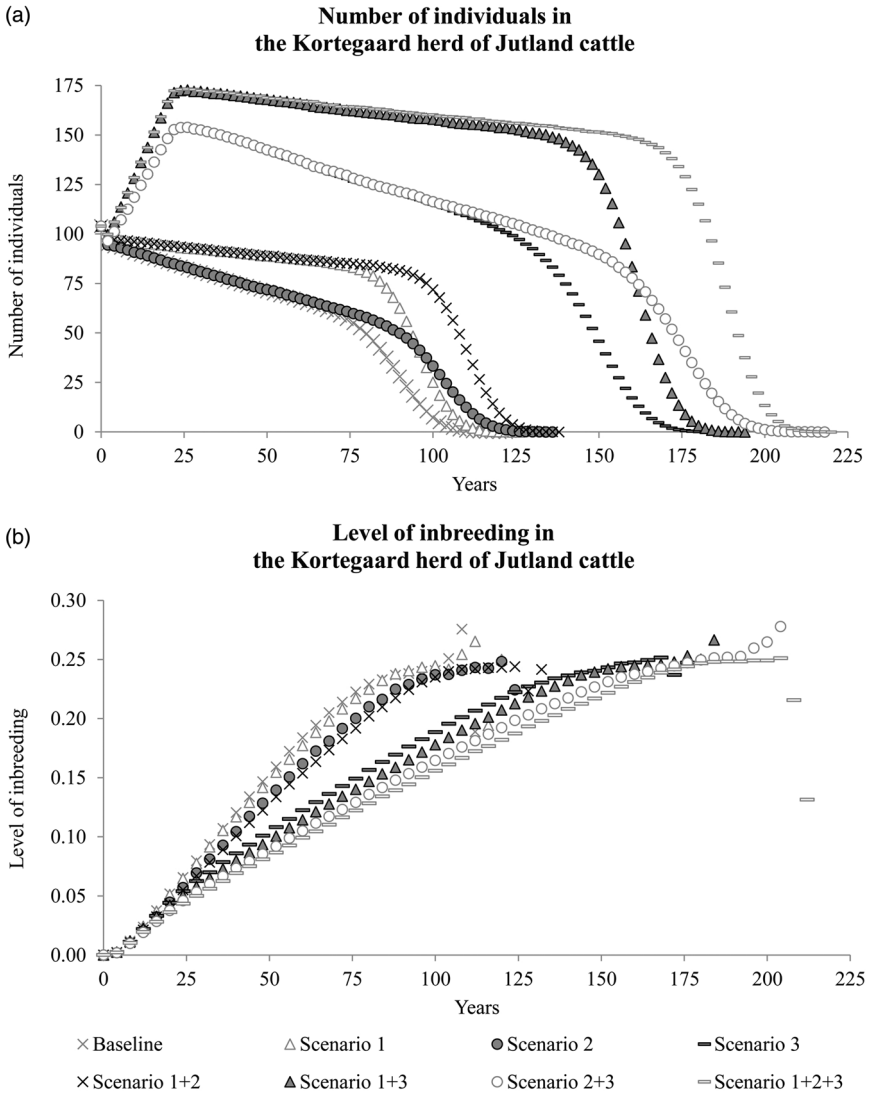

Fig. 1 - B/W online

Figure 1. The number of individuals (a) and the level of inbreeding (b) in the Kortegaard herd of Jutland cattle according to simulations in Vortex 9.999. "Baseline" is the continuation of the current way of breeding, "Scenario 1" includes increased percentage of the females in the breeding pool, "Scenario 2" includes increased percentage of the males in the breeding pool, and "Scenario 3" includes increased carrying capacity.

effort. The level of inbreeding shows the same tendencies; the lower the pace of increase, the later the population reaches extinction (Figure 1b). The population goes extinct when the level of inbreeding reaches 0.25 because that is the chosen limit for allowed kinship between mating individuals in Vortex. By supplementing one male to the population every 5 years (Scenario 4) the time of extinction is postponed considerably, but the population size is constantly decreasing (Figure 2a). Trial simulations with different intervals of supplementation of different numbers of males to the existing cattle population have been made and show a decrease in the level of inbreeding compared with Baseline with such practices. These simulations show that fewer supplementations with short intervals result in a slower increase in the level of inbreeding than many supplementations with longer intervals, which formed the foundation for the selected supplementation regime. Scenario 4 shows a different development in the level of inbreeding compared with the other scenarios because it never reaches the set limit of 0.25, and the population instead reaches extinction due to the birth rate being lower than the mortality rate (which both are based on the current breeding scheme), i.e. the number of individuals dying each year is greater than the number of individuals being born (Figure 2b).

By combining the different scenarios the population becomes more stable and the time of extinction is postponed (Figure 1a). The combination that results in the largest population size and longest survival, without supplementation, is the combination of Scenarios 1, 2 and 3. This combination results in the number of individuals increasing for 25 years, the population size being more stable compared with Baseline, and the population reaches extinction after 221 years (Figure 1a). The best outcome is achieved by combining Scenarios 1, 2 and 4, which results in stabilization of the population size, and after 2 000 years the population has only decreased from 104 to 77 individuals (Figure 2a).

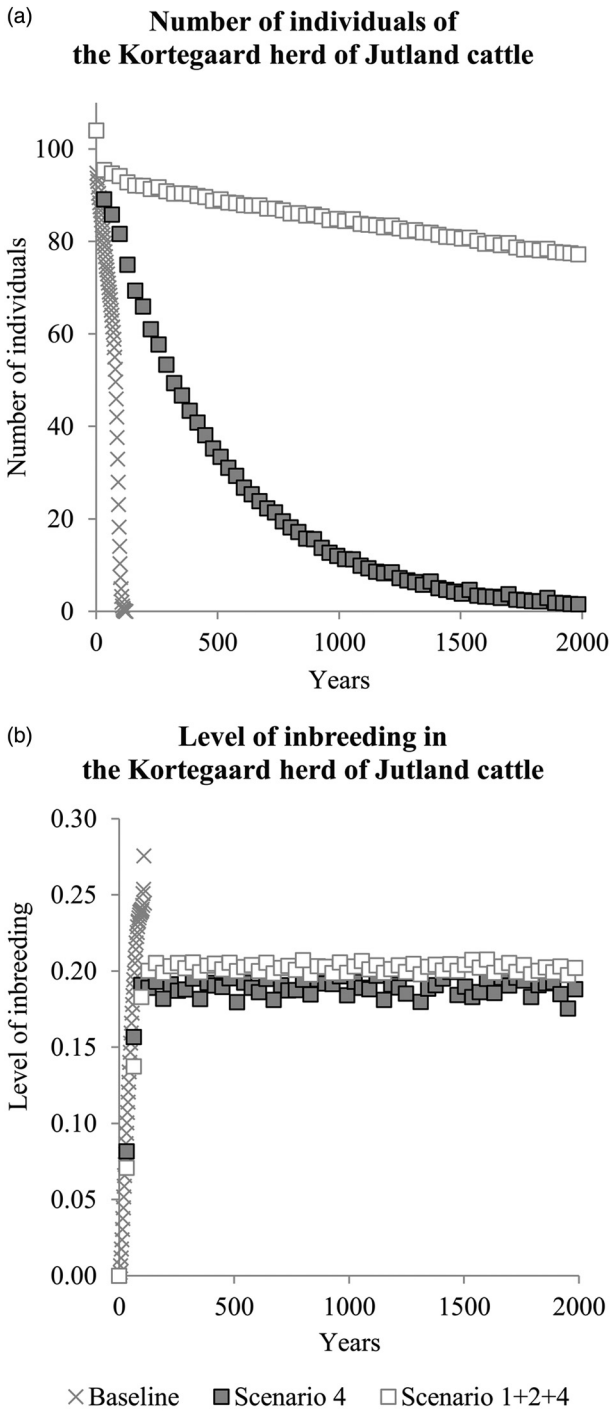

Fig. 2 - B/W online

Figure 2. The number of individuals (a) and the level of inbreeding (b) in the Kortegaard herd of Jutland cattle according to simulations in Vortex 9.999. "Baseline" is the continuation of the current way of breeding, "Scenario 1" includes increased percentage of the females in the breeding pool, "Scenario 2" includes increased percentage of the males in the breeding pool, and "Scenario 4" includes supplementation with cryopreserved semen.

Discussion

The results presented in this study suggest that long-term survival of a small, managed population can be improved even with relatively small changes to the current breeding practices. It seems that an increase in the number of females in the breeding pool (Scenario 1) makes no significant difference when looking at the level of inbreeding in the population. However, a small increase in the number of females in the breeding pool has a positive effect on the growth rate, which has also been observed in other studies on domestic animals (Thirstrup *et al.*, 2009).

The growth rate does not change significantly when adding more males to the breeding pool (Scenario 2), but more males do lead to a postponement of the year of extinction, as the limit of allowed kinship is reached later when more males are active in the breeding pool. When comparing the effect on inbreeding by increased number of females in the breeding pool to increased number of males, the effect is greater when changing the latter. Hence, it seems that the number of females in the breeding pool limits the number of individuals, and thus the population size, whereas males limit the genetic diversity.

Raising the carrying capacity each year over a course of 20 years (Scenario 3) results in an increase in population size over this period, and thereafter the population size declines at the same rate as seen in Baseline. Pilot simulations (data not presented) showed that there is a constant relation between the percentage change and the outcome, and, therefore, the farmers can decide to raise their carrying capacity with what fits their needs, accommodations and funds. Increasing the carrying capacity with 4 percent each year over a period of 20 years and also changing the number of either females or males in the breeding pool, results in the same development of population size as seen in the individual scenarios. However, there is an increase in the population size during the first 25 years, which results in a postponed extinction. Compared with Baseline, the combination of increasing the carrying capacity and the number of females in the breeding pool (Scenario 1 + 3) gains fewer years than the combination of increasing the carrying capacity and the number of males in the breeding pool (Scenario 2 + 3). This difference is, as previously mentioned, because the males are the limiting factor when finding suitable matings within the allowed limits of kinship. This is also reflected in the level of inbreeding because the rate at which the level of inbreeding increases is slower in the combination of Scenarios 2 and 3 than the combination of Scenarios 1 and 3. As the growth rate for Scenario 1 + 3 declines less rapidly, it can be argued that even though the population survives for a longer period in Scenario 2 + 3, Scenario 1 + 3 is more beneficial. A combination of Scenario 1, 2, and 3 shows a cumulative effect resulting in both a more stable population size and a greater postponement of the population's extinction.

To increase the chances of long-term survival of the population, we also suggest supplementing the gene pool with cryopreserved semen. It is possible to cryopreserve spermatozoa from cattle and store it in a gene bank until use (Curry, 2000). In this manner, farmers can raise the carrying capacity artificially, without actually expanding the *in*

situ population and still supply genetic material from less related earlier generations of animals. The simulations in this study are based on supplementing one male every 5 years, representing cryopreserved semen from related bulls from previous generations, but it may also be considered to utilize cryopreserved oocytes or embryos (Su *et al.*, 2012). According to the simulation (Scenario 4), the population survives for 2 000 years with supplementation of one male every 5 years, although the population size decreases over the years. If this supplementation is combined with Scenarios 1 and 2, then the population size can be almost stabilized. As prior males will be less related, the population's genetic diversity will be increased at each supplementation and thereby keep the inbreeding at a lower level.

For both Scenario 4 and the combination of Scenarios 1, 2 and 4 the level of inbreeding stabilizes around 0.20. The population size still decreases over the years and is not due to an insufficient number of suitable matings, but due to the birth rate being lower than the mortality rate, as previously mentioned. However, this might be avoided if the farmers are made aware of the problem. To lower the mortality rate it is necessary to increase the carrying capacity in order to avoid slaughtering as many individuals each year as is the case with the current breeding scheme. The increase should not be temporary as suggested with Scenario 3 nor artificial as suggested with Scenario 4. A possible approach to obtain a greater carrying capacity in the future is to use the cattle for sustainable nature management and restoration. The investigated breed, Jutland cattle, has been suggested to be suitable for nature management due to its unique ability to graze efficiently on vegetation often avoided by other breeds, and thereby preserving certain natural environments, e.g. heaths and meadows (Ejrnæs and Buttenschøn, 2012). This can increase the biodiversity of e.g. plants and insects significantly in the area in question (García *et al.*, 2013).

It is important to bear in mind that the presented results are simulations, and thus, do not necessarily depict reality (Beissinger and Westphal, 1998). It would have been ideal to compose a pedigree for the population, but it was not possible due to shortage of registrations. Complete pedigrees would have enabled Vortex to estimate a more accurate degree of inbreeding and thereby generate more precise results. However, even without a pedigree, a PVA can still be used to assess the optimal breeding scheme. The results of this study are too optimistic, because it was assumed that all the individuals are unrelated at the starting point of the simulation. A study by Pertoldi *et al.* (2014) showed that the population is indeed inbred and has a low effective population size. Furthermore, Vortex is created for simulations on wild populations, and therefore, lacks possibilities for advanced adjustments suitable when working with domesticated populations (Miller and Lacy, 2005). Because of these uncertainties and limitations, the simulations presented cannot be used for determining the precise time of extinction, but rather for the purpose of assessing effective and realistic ways to manage the Kortegaard population and other endangered breeds or species.

Conclusions

Based on the results it can be concluded that there are several possibilities to increase the chance of long-term survival of the Kortegaard population of Jutland cattle. All tested adjustments lead to postponement of extinction. The number and combination of changes applied to the current breeding practices can be selected from what is possible both economically and practically. Generally, the more parameters adjusted and the greater adjustments, the greater effect on the outcome. The results suggest that the most positive outcome of the simulations will secure the population for at least 2 000 years. Even if supplementation is not economically or practically possible, the population's chance of survival can be increased by almost 100 years by increasing the number of females and males active in the breeding pool from 59.3 to 70 percent and from 46.7 to 60 percent, respectively, and increasing the carrying capacity by 4 percent annually over a period of 20 years. Considering the fact that the Kortegaard population is a small population, this is a major improvement. Similar studies can be made for other small populations, both domestic and captive, to optimize their breeding scheme.

Acknowledgements

CP was financially supported by the Danish Natural Science Research Council (grant numbers: 11-103926, 09-065999 and 95095995), the Carlsberg Foundation (grant number: 2011-01-0059) and the Aalborg Zoo Conservation Foundation (AZCF). TNK was financially supported by the Ministry of Food, Agriculture and Fisheries (grant number: 14-32640-000017). AVS was financially supported by the Danish Natural Science Research Council (postdoctoral grant 1337-00007). A special thanks to two anonymous reviewers for their helpful comments on the manuscript. We thank the farmers Holger Jessen, Jakob Kortegaard, Ole Mols, Louise Lemche, Kirsten Larsen, Simon Wiesner and Randers Regnskov, for their cooperation and supply of data on their cattle.

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Animal Genetic Resources, 2016, 59, 113–121. © Food and Agriculture Organization of the United Nations, 2016
doi:10.1017/S2078633616000230

Impacts of climate variability on livestock population dynamics and breed distribution patterns in selected districts of Western Amhara, Ethiopia

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Summary

Climate change affects the livestock populations. As temperature increases, the rainfall distribution patterns shifts. These indirectly change the ecosystems like changes in crop yield, alter the distribution of animal diseases, geographically restriction of rare breed populations and increased competition for resources. Therefore, the objective of the study was to quantify impacts of climate variability on livestock population dynamics and breed distribution patterns. The study was conducted in Gondar Zuria, Farta and Bahir Dar Zuria districts. The sites were selected based on agro-ecology and livestock distribution potential. Data were collected through desk reviews of different documents and studies, focused group discussions, key informants interviews and different projection models. The results revealed that 70 percent of respondents believed that the trends of livestock breed distribution varied from year to year and from agro-ecology to agro-ecology. The number of cattle and equines are decreasing from year to year due to climate variability. Particularly, the crossbred cattle population decreased in 1998, 2002 and 2008 due to shortage of rainfall, increments of temperature and feed shortage. A correlation analysis was used to quantify impacts of temperature and rainfall on livestock population dynamics and breed distribution. The analyses revealed that sheep ($r = -0.535$, $P < 0.05$) and cattle ($r = -0.\ 512$, $P < 0.05$) were negatively affected by climate variability. Whereas goats were having positive relationship ($r = 0.345$, $P < 0.001$). As the average maximum temperature steadily increases, the population dynamics of ruminant livestock fluctuated after the year 1996. About 92.2, 78 and 83.3 percent respondents in Farta, Gondar Zuria and Bahir Dar Zuria districts, respectively, stated that there is a fluctuation in amount of rainfall distribution during the main rainy seasons. About 84.5 percent of respondent of the three districts also believed that climate change made variation in rainfall distribution. About 52 percent of the respondents also suggested that if livestock is to be protected from climate change and related effects, changing the farming system with appropriate breed is important and can be achieved with the zero-grazing system. The farmers also recommended with stocking climate change adaptive and productive breeds. In conclusion, climate variability affected livestock population dynamics and breed distribution pattern negatively.

Keywords: *breed, climate, livestock, variability*

Résumé

Le changement climatique affecte les populations d'animaux d'élevage. Au fur et à mesure que la température augmente, le patron de distribution des précipitations varie. Cela modifie indirectement les écosystèmes puisque le rendement des cultures change, la distribution des maladies animales est altérée, l'espace géographique des populations de races rares devient de plus en plus restreint et la concurrence pour les ressources s'accroît. Par conséquent, l'objectif de l'étude a été de quantifier les impacts de la variabilité climatique sur la dynamique des populations d'animaux d'élevage et sur le patron de distribution des races. L'étude a été menée dans les woredas de Gondar Zuria, Farta et Bahir Dar Zuria. Les lieux ont été sélectionnés sur la base du potentiel agro-écologique et en fonction de la distribution des animaux d'élevage. Les données ont été recueillies en examinant divers documents et études dans le bureau et au moyen de discussions de groupe ciblées, d'entretiens avec des informateurs clés et de différents modèles de prévision. Selon les résultats, 70 pour cent des personnes interrogées croient que l'évolution de la distribution des races d'animaux d'élevage varie d'année en année et entre les différentes zones agro-écologiques. Les effectifs bovins et équins sont en baisse année après année en raison de la variabilité du climat. En particulier, la population de bovins croisés a diminué en 1998, 2002 et 2008 à cause du manque de précipitations, de l'accroissement de la température et de la pénurie d'aliments. Une analyse de corrélation a été utilisée pour quantifier les impacts de la température et des précipitations sur la dynamique de la population d'animaux d'élevage et sur la distribution des races. Les analyses ont montré que la variabilité du climat affecte négativement les ovins ($r = -0{,}535$, $P < 0{,}05$) et les bovins ($r = -0{,}512$, $P < 0{,}05$). Par contre, dans le cas des caprins une corrélation positive ($r = 0{,}345$, $P < 0{,}001$) a été observée. En raison de l'augmentation continue de la température maximale moyenne, la dynamique de la population de ruminants a varié depuis l'année 1996. Environ 92,2 pour cent, 78 pour cent et 83,3 pour cent des personnes interrogées dans les woredas de Farta, Gondar Zuria et

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Bahir Dar Zuria, respectivement, ont signalé qu'une fluctuation quantitative de la distribution des précipitations a eu lieu pendant les principales saisons de pluie. À peu près 84,5 pour cent des personnes interrogées dans les trois woredas pensent aussi que la variation de la distribution des précipitations est due au changement climatique. Environ 52 pour cent des personnes interrogées ont de même suggéré que, s'il s'avère nécessaire de protéger les animaux d'élevage contre le changement climatique et les effets associés, la modification du système d'exploitation avec la race appropriée serait importante et pourrait être accomplie avec le système de zéro pâturage. Pour faire face au changement climatique, les éleveurs ont aussi recommandé des races productives et à grande capacité d'adaptation. En conclusion, la variabilité climatique a affecté négativement la dynamique des populations d'animaux d'élevage et le patron de distribution des races.

Mots-clés: *race, climat, bétail, variabilité*

Resumen
El cambio climático afecta a las poblaciones ganaderas. A medida que la temperatura aumenta, el patrón de distribución de las precipitaciones varía. Esto modifica indirectamente los ecosistemas, ya que se producen cambios en el rendimiento de los cultivos, se altera la distribución de las enfermedades animales, se acotan geográficamente las poblaciones de razas escasas y se incrementa la competencia por los recursos. En consecuencia, el objetivo del estudio fue cuantificar los efectos de la variabilidad climática sobre la dinámica de las poblaciones ganaderas y sobre los patrones de distribución de las razas. El estudio fue llevado a cabo en los distritos de Área del Gran Gondar, Farta y Área del Gran Bahir Dar. Los lugares fueron seleccionados en base al potencial agroecológico y a la distribución de la ganadería. Los datos fueron obtenidos mediante revisión en despacho de diferentes documentos y estudios, debates de grupo focalizados, entrevistas a informadores clave y diferentes modelos de predicción. De acuerdo con los resultados, el 70 por ciento de los encuestados creen que la tendencia en la distribución de las razas ganaderas varía de año en año y entre distintas zonas agroecológicas. Los censos bovinos y equinos están decayendo año tras año debido a la variabilidad climática. En concreto, la población de ganado bovino cruzado se redujo en 1998, 2002 y 2008 debido a la falta de precipitaciones, al incremento de la temperatura y a la carestía de alimentos. Se empleó un análisis de correlación para cuantificar los efectos de la temperatura y las precipitaciones sobre la dinámica de la población ganadera y la distribución de las razas. Los análisis mostraron que a las ovejas ($r = -0{,}535$, $P < 0{,}05$) y al ganado bovino ($r = -0{,}512$, $P < 0{,}05$) les afectó negativamente la variabilidad climática. Por el contrario, en el caso de las cabras se dio una relación positiva ($r = 0{,}345$, $P < 0{,}001$). Dado que la temperatura máxima media ha ido aumentando de manera continuada, la dinámica de la población de ganado rumiante ha variado después del año 1996. En torno al 92,2 por ciento, el 78 por ciento y el 83,3 por ciento de los encuestados en los distritos de Farta, Área del Gran Gondar y Área del Gran Bahir Dar, respectivamente, indicaron que se ha producido una fluctuación cuantitativa en la distribución de las precipitaciones durante las principales estaciones de lluvia. Alrededor del 84,5 por ciento de los encuestados de los tres distritos creen también que la variación en la distribución de las precipitaciones es debida al cambio climático. En torno al 52 por ciento de los encuestados sugirieron asimismo que, si se hace necesario proteger la ganadería frente al cambio climático y los efectos asociados, sería importante cambiar el sistema productivo con la raza apropiada, lo cual se podría alcanzar con el sistema de pastoreo cero. Frente al cambio climático, los ganaderos también recomendaron razas productivas y con capacidad de adaptación. En conclusión, la variabilidad climática afectó negativamente a la dinámica de las poblaciones ganaderas y al patrón de distribución de las razas.

Palabras clave: *raza, clima, ganado, variabilidad*

Submitted 15 October 2015; accepted 5 September 2016

Introduction

Livestock production systems may be affected in various ways and changes in productivity are inevitable (Thornton and Gerber, 2010). Livestock production both contributes to and is affected by climate change (Hoffmann, 2010). Livestock and environmental trade-offs are currently substantial and will increase significantly as a result of the increased demand for livestock products from the growing population (Herrero *et al.,* 2009a). Livestock in the pastoral systems depends to a great extent on the productivity of the rangelands, which is predicted to decline and become more erratic due to climate change (FAO, 2007b). Genetically diverse livestock populations are an important resource to be drawn upon as production systems change and develop. The prospect of future challenges such as adapting to global climate change underlines the importance of retaining a diverse portfolio of livestock breeds (FAO, 2007b). Climate change requires more efficient animal production systems, careful husbandry of natural resources and measures to reduce waste and environmental pollution (FAO, 2010).

In addition to the physiological effects of higher temperatures, climate change increased risk that geographically restricted rare breed populations will be badly affected by disturbances (Hoffmann, 2010). The IPCC (2007) predicts that by 2100 the increase in global average surface temperature may be between 1.8 and 4.0 °C. With global average temperature increases of only 1.5–2.5 °C, approximately 20–30 percent of plant and animal species are expected to be at risk of extinction (FAO, 2007a).

Climate change may have also significant impacts on the livestock number, emergence, spread and distribution of livestock diseases (IFAD, 2009). For example, the distribution and impacts of vector-borne diseases of animals such as Rift Valley fever, African horse sickness, and bluetongue vary considerably with seasonal and longer-term climatic variations (Baylis and Githeko, 2006; Middison, 2006). Moreover, genetic mechanisms influence fitness and adaptation (Hoffmann, 2010). Barker (2009) defined adaptedness as the state of being adapted, the ability of breeds to produce and reproduce in a given set of environments, or the choice of particular breeds for specific environments.

Breeding and selection aim to improve the use value of animal genetic diversity. Climate change projections suggest that further selection for breeds with effective thermoregulatory control may be needed (Hoffmann, 2010). Animal breeding indices should include traits associated with thermal tolerance, low-quality feed and disease resistance, and give more consideration of genotype-by-environment interactions (G × E) to identify animals most adapted to specific conditions (Hoffmann, 2010). NMSA (2001) indicated that mean temperature and precipitation in Ethiopia have been changing over time. Accordingly, the average annual minimum temperature over the country has been increasing by about 0.25 °C every 10 years, while the average annual maximum temperature has been increasing by about 0.1 °C every decade. The average annual rainfall of the country showed a very high level of variability over the past few years even though the trend remained more or less constant (NMS, 2007). Over the past 60 years, some of the years have been characterized by dry rainfall conditions resulting in drought and famine, whereas the others are characterized by wet conditions (Kefyalew and Tegegn, 2012). Droughts in Ethiopia can shrink household farm production by up to 90 percent of a normal year output (World Bank, 2003). The effect of climate change/variability Ethiopia in general and the Amhara regional state in particular expected to have significant effects on livestock population dynamics, genetic diversity and breed distribution patterns and as a result variability in diversity and breed distributions patterns were expected and needed investigations. Therefore, the objectives of the study were to assess the impact of climate variability livestock population dynamics, the perceptions of community about climate change impacts and to quantify the trends breed distribution as compared to climate variability of the region.

Materials and methods

Description of the study area

The study was conducted on three zones of the Amhara regional state, namely North Gondar, South Gondar and West Gojjam zones. From each zone one district was considered and hence a total of three districts were included in the study namely, Gondar Zuria from North Gondar, Farta from South Gondar and Bahir Dar Zuria from West Gojjam zones (Figure 1).

Farta district has the mean annual rainfall of 1 651 mm. The mean monthly average temperature is 18.4 °C. The altitudes range from 1 500 to 4 135 m above sea level. Gondar Zuria district is also found in ANRS, the North Gondar zone and has also tepid moist to cool mountains. The altitude ranges from 1 966 to 2 133 m above sea level. The mean annual rainfall is 1 161 mm. The average temperature is 19.1 °C. Bahir Dar Zuria district is found in West Gojam zone of ANRS, which is tepid moist to cool plain with altitude ranging from 1 786 to 1 969 m above sea level. Mean annual rainfall of 1 224 mm and the mean annual daily temperature recorded is 18.5 °C. The main crops produced in these three study areas are barely, wheat, teff and other pulse crops. The average length of growing period of the plants in all study sites ranges from 120 to 270 days.

Sites selection techniques

The study was conducted in the three agro-climatic zones. From each agro-climatic zone one district was selected based on agro-ecology and livestock potential. Similarly, from each district two Peasant Associations (PAs) were purposively selected.

Tools and design

The study was based on multi-approaches tools to gather important information on the climate change issues. Collection of secondary data, desk reviews of different documents and studies, focused group discussions, key informants interviews and different models were used.

Desk reviews and secondary data collection

Previous studies, guidelines, manuals and literatures reviewed were used to assess the effect of climate change on population dynamics and breed distribution pattern. Secondary data were collected pertaining to the investigated issues from different concerned bodies. Routine activities and reporting formats were developed and thoroughly assessed at different pertinent offices. Based on the assessments of secondary data, desk reviews were conducted with pertinent bodies.

Focused group discussion and interviews

The focus group discussions were formed by selecting communities of the targeted population. One focus group discussions were conducted at each selected PA. Therefore, a total of three focus group discussions were conducted during the study period. The in-depth information was collected using interviewing key informants who have deep knowledge on climate change and animal breed distribution

Fig. 1 - Colour online

Figure 1. Map of the study area: The study districts are indicated with triangular shape.

pattern in the area. The questionnaires were introduced to the farmers and completed by the sort of staff indicated for the farmers involved. Therefore, a total of 103 respondents, three districts and three grouped key informants including professionals were considered. The interview was considered livestock officers, animal sciences assistants and veterinarians/officers who are working in government agriculture offices and nongovernmental participates in animal science and veterinary services.

Climate data

The climatic data for the region were taken from regional metrological agency and livestock data were taken from Central Statistical Agency (CSA) of Ethiopia and regression model was be used to see the trends of the effect.

Empirical models for perceptions, trends of livestock and climate variability

Since the probability of an event must lie between 0 and 1, it is impractical to model probabilities with linear regression techniques. The linear regression model allows the dependent variable to take values >1 or <0. The probit analysis model is a type of generalized linear model that extends the linear regression model by linking the range of real numbers to the 0–1 range. Adaptation to climate change involves a two-stage process: first perceiving change and then deciding whether or not to adapt by taking a particular measure. This leads to sample selectivity problem since only those who perceive climate change will adapt, where as it need to infer about the adaptation by the population (Middison, 2006). The linear regression model was also used to show the trends of climate change and breed distribution pattern with the trends of climate variability treating livestock and breed distribution pattern as dependent variable and climate variability (maximum temperature and rainfall) as predictor. This relationship is described in the following formula.

$$Y_i = b_0 + b_{1\times1} + b_{2\times2} + b_{3\times3} + b_{4\times4} + \cdots + b_n x_n$$

where Y_i is the response variable (livestock trend); b_0, the intercept; $x_1, x_2, \ldots, x_4$ are the explanatory variables such as temperature, rainfall, humidity and sunshine, respectively; b_1, b_2 and $b4$ are regression coefficients of the variables x_1, x_4.

e_i, the residual random error.

Both for the qualitative and quantitative data analyses were undertaken at the field work such as descriptive statistics, ANOVA and other related using available version of SPSS (SPSS, 2011).

Table 1. Overall situation of the peoples.

Status	Farta ($N = 103$)	%	Gondar Zuria ($N = 104$)	%	Bahir Dar Zuria ($N = 100$)	%
Sex of respondents						
Male	87	84.5	79	76.7	72	69.9
Female	16	14.5	21	23.3	29	29.1
Marital status						
Married	78	75.7	91	87.5	72	72
Divorced	12	11.7	7	6.7	7	7
Widowed	13	12.6	6	5.7	22	22
Educational status						
Illiterate	43	41.8	49	47.6	69	69
Read and write	37	35.9	34	33.0	24	24
Literate	23	22.3	20	19.4	8	8

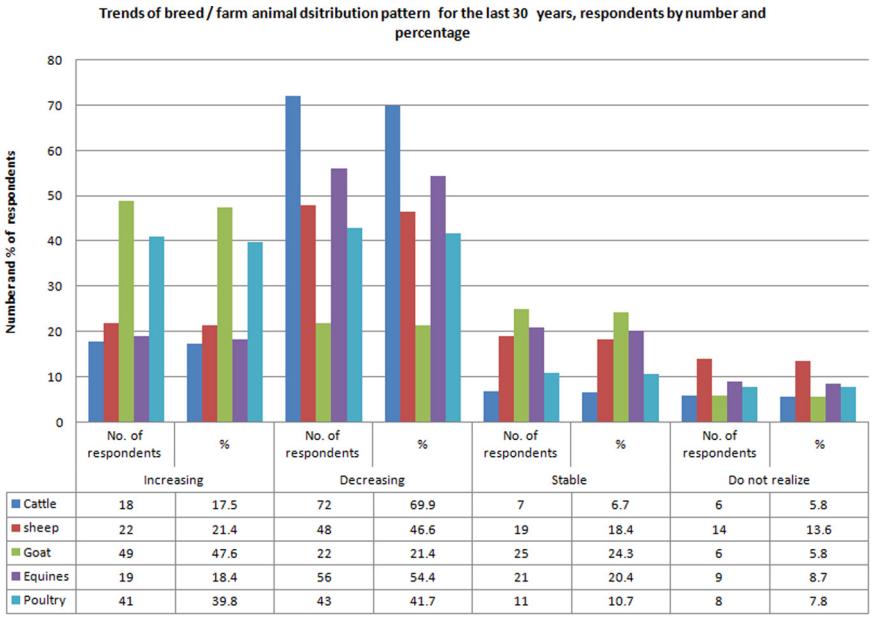

Fig. 2 - Colour online

Figure 2. Trends of breed distribution pattern for the last 30 years and number of respondents.

Results and Discussions

Socio-economic characteristics of the respondents

About 99 percent of interviewed households were fully involved in mixed crop–livestock traditional production systems. While, livestock production is the main sources of income for immediate expenses such as purchasing salt, coffee, cloth and animals' medicine. The majority of the respondents were males (Table 1). About 8, 19.4 and 22.3 percent respondents from Bahir Dar Zuria, Gondar Zuria and Farta districts were literate, respectively.

Impacts of climate variability on breed distribution pattern

The impact of climate variability on livestock population/breed/species distribution pattern in relation to maximum temperature and rain fall variation are presented in Figure 2. Respondents noted that when there is an increase in temperature, cattle stop grazing and walking for searching pasture and water, no interest for breeding and of course decrease in milk production. About 70 percent of respondents stated that trends of livestock breed distribution varied from year to year and from agro-ecology to agro-ecology. The respondents also stressed that number of cattle (69.9 percent) and equines (54.5 percent) are decreasing due to shortage of feeds and water that might be linked with climate variability (Figure 2). The farmer underlined that the contribution of groundwater and zero-grazing systems with a forestation are becoming more important in the future to tackle climate change. According to NRC (1981) the farmer water intake increases from 3 kg per kg DM intake at 10 °C ambient temperature to 5 kg at 30 °C and 10 kg at 35 °C.

About more than 80 percent of the interviewed farmer reported that that most evident and important effects of climate change on livestock production are mediated through changes in feed resources and livestock dynamics.

Fig. 3 - Colour online

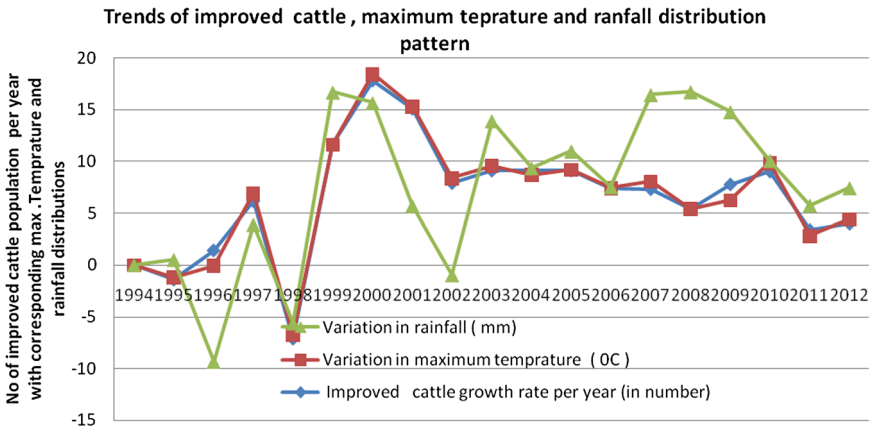

Figure 3. Trends of improved/crossbred cattle number in Amhara region from 1994 to 2012.

Fig. 4 - Colour online

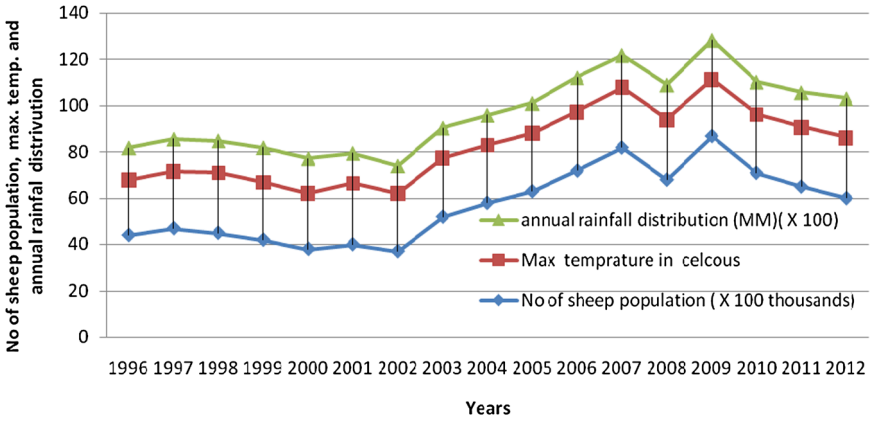

Figure 4. Sheep population, max temperature and annual rainfall distribution in Amhara region.

The farmers also stated that direct effects of climate change on feed resources can have a significant impact on livestock productivity, the carrying capacity of rangelands, the buffering ability of ecosystems and their sustainability, prices of stoves and grains, trade in feeds, changes in feeding options, greenhouse gas emissions and grazing management. The main pathways in which climate change can affect the availability of feed resources for livestock are temperature increases and rainfall increases or decreases or land use and systems changes, changes in the primary productivity of crops, forages and rangelands, changes in species composition and quality of plant material.

Impact of climate variability livestock population dynamics

The improved cattle population trend was increasing from 1997 to 2005 but decreased in the year 1998, 2002 and 2008. The reason may be shortage of rainfall, increments of temperature and feed shortage (Figure 3).

The result also showed that climate change has a negative impact on improved cattle population than indigenous cattle and in sheep (Figure 4).

Trends of livestock and climate variability

This study revealed that climate affected cattle and sheep in 1985, 1993 and 1998 and cattle numbers dropped by 37 percent after the drought of 1983–1985. The herd then quickly grew to about 85 percent of the previous peak size by 1990. Another crash occurred in the early 1990s with a 42 percent reduction in cattle numbers, but interestingly the corresponding change in annual rainfall was less apparent in the early 1990s compared with that observed in 1983–1985 (Solomon *et al.,* 2003). The cattle population dynamics resembled a "boom and bust" pattern where longer periods of gradual herd growth were punctuated by sharp crashes in 1983–1985, 1991–1992 and 1998–1999 when 37–62 percent of the cattle population perished (Solomon *et al.,* 2003). Rainfall variability greatly influenced herd dynamics under the communal and ranch management in terms of herd die-offs and lower birth rates, which also considerably affected milk production for household consumption (FAO, 2009). Droughts of the 1980s and 1990s caused 49 percent herd losses under the communal land use, while 57 percent of the cattle mortality under ranch management was attributed to droughts of the 1990s (Table 2).

Table 2. Relationship between ruminant livestock dynamics and climate change.

Species	Unstandardized coefficients		Standard. coefficient	*t*-value	Sig.	95% Confidence interval		Correlations
	B	SE	Beta			Lower bound	Upper bound	Partial
Cattle								
(Constant)	95.870	22.70		4.22	0.000	49.28	142.45	
Temperature	−2.317	0.80	−0.475	−2.86	0.008	−3.975	−0.66	−0.48
Rainfall	−0.078	0.05	−0.219	−1.32	0.196	−0.199	0.04	−0.24
Sheep								
(Constant)	103.91	51.61		2.01	0.055	−2.616	210.45	
Temperature	−2.31	1.97	−0.208	−1.17	0.252	−6.395	1.76	−0.23
Rainfall	−0.24	0.09	−0.448	−2.53	0.018	−0.436	−0.04	−0.45
Goats								
(Constant)	−149.82	46.7		−3.20	0.003	−245.81	−53.83	
Temperature	4.96	1.6	0.414	2.98	0.006	1.548	8.379	0.49
Rainfall	0.509	0.12	0.581	4.18	0.000	0.259	0.75	0.62

Table 3. Correlations among dependent variables (cattle, sheep and goat) and the predictors (rainfall and temperature).

Model	*R*	R^2	Adjusted R^2	SE of the estimate	Change statistics				
					R^2 change	*F* change	df1	df2	Sig. *F*
Cattle	0.512[a]	0.262	0.207	3.239	0.262	4.792	2	27	0.017
Sheep	0.535[a]	0.286	0.226	4.825	0.286	4.807	2	24	0.018
Goats	0.345[a]	0.235	0.189	6.674550	0.201	12.548	2	27	0.000

[a]Correlation of predictors – rainfall and temperature and dependent variable – cattle, sheep and goats.

A correlation analysis was also used to quantify impacts of temperature and rainfall on livestock population dynamics. The analyses revealed that sheep ($r = 0.535$, $P < 0.05$) and cattle ($r = 0.512$, $P < 0.05$) were negatively affected by climate change. Whereas goats were having positive relationship ($r = 0.345$, $P < 0.001$) (Tables 2 and 3). As the average maximum temperature steadily increases, the population dynamics ruminant livestock fluctuated after the year 1996. The population of goat have shown boom after that year and the population of sheep and cattle have shown dramatic increase and revived again.

Farmers' perception towards climate change

About 92.2, 78 and 83.3 percent respondents in Farta, Gondar Zuria and Bahir Dar Zuria districts, respectively said that there is a change in amount of rainfall distribution during the main rainy seasons due to climate change. About 84.5 percent of respondent in the three districts believe that climate change made variation in rainfall distribution (Table 4).

Coping strategies from community-based knowledge

About 52 percent of the respondents suggested that if livestock is to be protected from climate change and related effects, changing the farming system with appropriate breed (productive) are important. This can be achieved with zero-grazing system. The second preference of the farmers to adapt climate change was stocking climate change adapting and productive breeds (Table 5).

Conclusion

From this study, it was possible to see that climate change affected livestock negatively. The livestock responds to these changes by walking here and there for searching of pasture and water as the temperature increases steadily. The trends of livestock breed distribution pattern varied from year to year and from agro-ecology to agro-ecology (move towards the highland). It was noted especially that the number of cattle and equines are decreasing as the number of goats population responds positively to the change. The direct effects of climate change on feed resources significantly affected livestock productivity, the carrying capacity of rangelands, the buffering ability of ecosystems and their sustainability and grazing management. From the cattle population, exotic cattle were highly affected. Farmers also believed that climate change made variation in rainfall distribution. According to the farmers' perception, if livestock is to be protected from climate change and related effects, changing the farming system (intensification with appropriate management) with appropriate breed, zero-grazing system and reforestation is critical.

Table 4. Farmers' perception of climate change and its effect on livestock based on the last 20–30 years' experience (% of respondents).

Perception indicators	Farta district			Gondar Zuria district			Bahir Dar Zuria district			Total		
	No. of respondents	Yes	No	No. of respondents	Yes	No	No. of respondents	Yes	No	Respondents	Yes	No
Change in amount of rainfall during main rain season?	100	92.2	7.8	100	78	22	100	83.3	16.7	300	84.5	14.5
Is rainfall increasing in amount during main rain seasons?	100	32.5	67.5	100	22	78	100	35	65	300	29.83	71.17
Is the timing of the onset of rain in the main season shifting?	100	23	77	100	27	73	100	32	68	300	27.3	69.7
Is your planting date changing due to change in the onset of rain?	100	85.4	14.6	100	90	10	100	79	21	300	84.8	15.2
Increase problem of livestock health related to climate change?	100	47	53	100	59	41	100	52	48	300	52.7	47.3
Is rain starting late than normal?	100	93	7	100	88	12	100	87.3	12.7	300	89.4	9.6
Is the amount of precipitation sufficient for full cropping and animal feed development during short rainfall	100	44	46	100	33.7	66.3	100	52	48	300	43.3	56.7
Is the breed pattern changing?	100	53	47	100	56	44	100	49	51	300	52.7	47.3
Do you feel temperature of the area is changing?	100	97	3	100	82.1	17.9	100	78	22	300	85.7	14.3
Do you believe that the no. of cattle is decreasing from goats?	100	51	49	100	57	43	100	49	51	300	52.3	47.7
Do you feel that livestock are being affected by the change?	100	92	8	100	95.2	4.8	100	71	29	300	86	14

Table 5. Adaptation mechanisms suggested by the farmers interviewed.

Adaptation mechanisms suggested	Total no. of respondents	Respondents preference	%
Adopting drought resistant improves forages	271	28	11
Stocking climate change adapting breeds like goats	271	48	19
Changing the farming system with appropriate breed (productive)	271	141	52
Stocking selected indigenous cattle	271	19	8
Stocking improved dairy cattle like Holstein Frisian	271	11	1
Conserving natural resources and forestation	271	24	9

Acknowledgements

We would like to thank all staffs of agricultural offices, farmers, development agents of Farta, Gondar Zuria and Bahir Dar Zuria districts for their cooperation in facilitation, data collection, providing secondary data sources. I would like to thank also Bahir Dar University for funding this research.

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Animal Genetic Resources, 2016, 59, 123. © Food and Agriculture Organization of the United Nations, 2016
doi:10.1017/S2078633616000291

Recent Publications

25 YEARS WITH DAGENE - The Jubilee Proceedings, which comprise the history of this international NGO in field of preservation of rare domestic animal breeds of countries in the Danube basin

Edited by Pál Hajas and András Gáspárdy
DAGENE- International Association for the Conservation of Animal Breeds in the Danube Region Printed by Palatia Printing and Publishing Ltd., Győr, Hungary Published in 2015, pp. 196
ISBN 978-963-12-3101-4

doi:10.1017/S2078633616000291

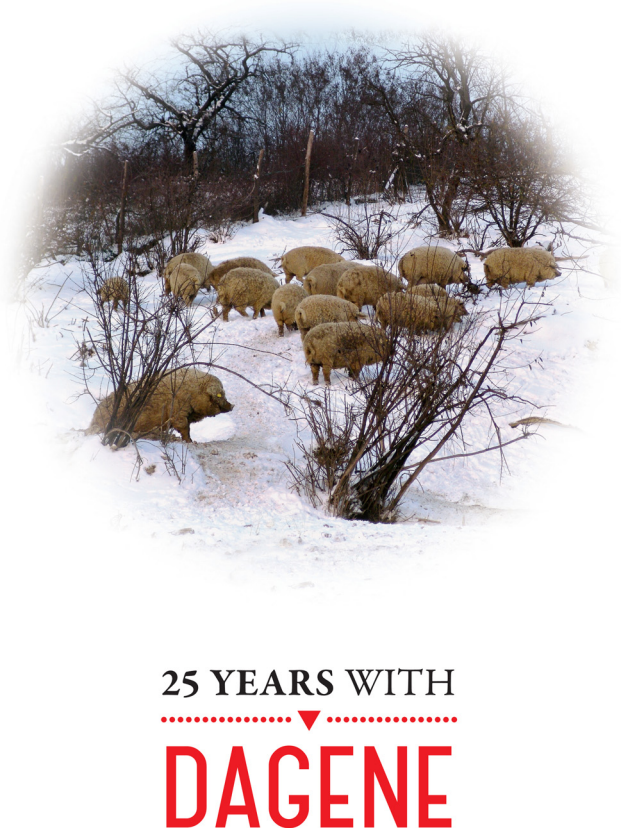

Fig. - Colour online

This publication provides a review of the 25-year-long work of an international association known as DAGENE (International Association for the Conservation of Animal Breeds in the Danube Region, headquarter 1078 Budapest, István street 2., Hungary).

The organisation which originated initially from two founder countries' (Austria and Hungary) aims to promote professional and scientific cooperation of Danube countries in relation to animal husbandry.

This special jubilee book contains contributions from the scientific community of the currently ten member countries. The book guides the reader through the professional scientific-educational process which is the hallmark of the first 25 years.

DAGENE association was established in 1989 by researchers and breeders with the aim of protecting endangered autochthonous animal breeds in the Danube-valley. The book is written in the two official languages of the association, English and German. DAGENE is actively involved in the discussion of scientific and economic issues related to the conservation of animal genetic resources. DAGENE's Annual Conference gives members the opportunity to meet and discuss current issues as well as the implementation of various methods. In the period between meetings, the association's well-established network connects and supports the work and advocacy of the members.

Contributions are made with joint transnational researchers in relation to the maintenance of breeds native in many countries. Some of the most important tasks DAGENE is involved with are to find varieties not yet registered; the replacement of breeding animals towards blood refreshment, the organization and participation in tenders and projects as vocational training as well as preparing information materials.

Following today's international networking, DAGENE cooperates with similar organizations in the Baltics (Baltic Genofond), the SAVE Foundation (a European foundation bringing 26 countries together), as well as the ERFP (the European organization of animal genetic coordinators).

Readers can get acquainted with the people who have played prominent roles in the history of the organisation, those involved with international scientific responsibility as well as those in the regional networking. The book updates the reader on DAGENE's activities contributing to the maintenance of several native animal (e.g. Murinsulaner Horse, repatriation of Carpathian Brown Cattle, ecotypes of Tsigai Sheep).

This publication contains papers and posters presented on the 25th annual scientific symposium, these serve as a small example of the great biological diversity of our living environment which is of supreme importance to maintain.

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I6686T/1/12.16