Chapter XI

BUFFALO MEAT AND MEAT INDUSTRY

Antonio Borghese

Istituto Sperimentale per la Zootecnia
(Animal Production Research Institute)
Via Salaria 31, 00016 Monterotondo (Rome), Italy

In the 1990s the average consumption of meat was 12 kg/ head per year for sub-Saharan Africa, 18 kg/head per year for Asia and 45 kg/head per year for Latin America (FAO, 1998) compared to an average of 76 kg/head per year in developed countries. Although a number of factors affect the long-term estimates for per capita demand for livestock products, the scenario predicted for changes in consumption patterns based on economic development has been considered (Bouwman, 1997) and the per capita demand (kg/year) for all the developing countries will increase from 17 kg in 1989/91 to 25 kg in 2010 and to 30 kg in 2025. It is considered that buffalo meat has a strong potential for meeting this requirement for increased per capita consumption. (Kondaiah and Anjaneyulu, 2003).

The production of buffalo meat has high growth possibilities and poses a minimal level of risk from pesticides and veterinary drugs when compared to beef production in developed countries. Buffalo meat is produced primarily in Asia. The contribution of buffalo meat to world total meat production is only 1.3 percent. India produces 1.43 million tonnes of buffalo meat annually and accounts for 36 percent of total meat production contributing significantly to human nutrition. As the meat produced is mainly from spent animals, it is coarse and fibrous. The demand for buffalo meat is high as it is relatively lean with a fat content below 2 percent and it is free from Mad Cow Disease as the animals are only fed grass and farm by-products. The functional proprieties of buffalo meat for product processing could be improved by increasing its popularity on the Indian market. For these reasons the future potential for buffalo meat and meat products is promising for India both on the domestic and international markets (Murty and Prince Devadason, 2003).

The quality and quantity of buffalo meat depend on many factors, the most important of which are the water buffalo type and breed, age, feeding intensity, management system and environmental conditions.

Generally, cattle are superior to buffaloes in their growth rate and there are also differences between the two water buffalo subspecies: River and Swamp.

The possibility of producing buffalo meat in Italy was initially studied by Bartolo Maymone (Maymone, 1945), the first Director of the Istituto Sperimentale per la Zootecnia (Animal Production Research Institute), and later by Beniamino Ferrara at the Veterinary University in Naples (Ferrara et al., 1964).

Studies relating to early weaning and growth rate in water buffalo calves were reported (De Franciscis and Zicarelli, 1974) during the First International Congress on Buffalo Livestock held in Caserta in 1974. The authors found a 154-163 kg live weight at 180 days of age with daily gains between 639 and 707 g without significant differences at different feed levels, with about 2.5-3.0 FU/kg live weight as conversion ratio. However, many animals presented myodistrophic disease and died or did not show acceptable performances. The first problem which was tackled in Italy was the question of early weaning in order to save buffalo milk for the market (the actual price is €1.20/litre) and spend less on milk replacers.

Many studies were carried out on calf early weaning and it was found that calf adaptation to

artificial feeding was easier if the calf never suckled the mother's milk. It was also observed that feeding and health control in individual boxes during the first month of life can reduce calf mortality.

But the main problem affecting the success of early weaning was the chemical composition of milk powders produced at that time for the calve bovine market, which, when reconstituted, had a chemical composition similar to cattle milk: crude protein 19 percent and fat 14 percent on dry matter. So Romita and Dias da Silva (1975) developed a milk powder with 21 percent crude protein and 25 percent fat on DM and obtained a daily weight gain of 919 g in males and 933 g in females up to 220 kg live weight and 59.72 percent and 58.60 percent as dressing percentage respectively. Many studies were carried out in order to ascertain the effects of different milk powders, different concentrations, varying ages at weaning and integrations with pre-starter concentrates or lactobacillus in comparison to bovine or buffalo milk (Palladino et al.,1993, Di Lella et al., 1998, Tripaldi et al., 2001, Roncoroni et al., 2001). Presently, acidified milk, with 25 percent crude protein and 23 percent fat on D.M., with probiotics and without copper because of its toxicity in buffaloes (Zicarelli et al.,1981) are used at normal climatic conditions, without causing problems of preparation (16-18 percent concentration) or mortality in calves that can be weaned within three months of age, with 700-800 g daily gains and with about 100 kg live weight.

Giovanni De Franciscis organized the Second International Congress on Buffalo Livestock at the Royal Palace in Caserta in 1982, where Ferrara (Ferrara et al., 1982) reported on the first review of the results of the different trials to produce buffalo calves and young bulls for meat production in Egypt, Romania, Yugoslavia, India, Indonesia and Italy. These results revealed a high daily gain (904 g) for a low slaughter weight (320 kg), and a low daily gain (200 g) for a high slaughter weight (683 kg); and the dressing percentage was increased with increasing live weight changing from 52.7 to 60 percent (Di Lella et al., 1975). These authors suggested that it was not economical to produce animals heavier than 450 kg because of the reduction in growth and efficiency in both males and castrated animals, and particularly because of the organoleptic characteristics of the meat, which smelt badly of musk. In Italy many trials were carried out by the Istituto Sperimentale per la Zootecnia on water buffaloes and Friesian bovine males, under the same feeding and environmental conditions, and at different slaughter ages in order to compare the meat performance and these were also referred to at the Second International Congress on Buffalo Livestock. Six trials were carried out on 116 males: 58 Mediterranean Italian buffaloes and 58 Friesian bovines which were fed milk substitutes and concentrates and slaughtered at 20, 28 and 36 weeks, or fed milk substitutes, hay and concentrates and slaughtered at 36, 52 and 64 weeks of age. The Friesians showed better performances than the buffaloes, the latter realized the following daily gains at 20, 28, 36(1), 36(2), 52, and 64 weeks of age: 795, 807, 746, 963, 930, 949 g/d (Romita et al., 1982; Table 1), and the following respective net dressing (the net dressing is about 4.5 percent more than the normal dressing because it is the ratio carcass/net live weight, without stomach and intestine contents) percentages: 59.6, 58.2, 57.6, 55.3, 57.0, 56.6 percent (Romita et al., 1982, Table 2). These trials proved that buffaloes could be bred until 15 months of age following an early weaning with good performances and a similar body growth trend in comparison to Friesians. The head and skin of the buffaloes were always heavier than that of the bovines, which showed higher intestine length and circumference; the carcasses were heavier in the bovines at all ages, but buffaloes showed a significantly higher percentage of meat on the carcasses at 20, 28, and 36(1) weeks of age. However at later ages these differences disappeared with the fattening increasing in buffaloes. In particular, buffaloes showed significantly more subcutaneous fat and less intermuscular and intramuscular fat at 36(2), 52, and 64 weeks of age, so the meat percentage on the carcass transpired as similar (62-64 percent) in both species (Gigli et al.,1982, Table 3).

As in the previous trials the buffaloes were fed hay and concentrates. In the 1990s the buffalo fattening was effected by using maize silage because of the lower cost/UFC (Meat Feed Unit). Gigli et al., (1994) fed 24 male Mediterranean Italian buffaloes with maize silage ad libitum,

0.9 kg/d soya bean meal 50 percent CP, 0.1 kg/d vitamin mineral nucleus (group A) in comparison to 24 animals fed the same diet (group B) with 50 percent maize silage of the quantity fed to group A. Eight animals from each group were slaughtered at 10, 14 and 18 months respectively: the buffaloes fed ad libitum realized a similar growth (Table 4) to the previous trials with about 970 g/d gain up until 14 months, reduced to 798 g/d at 18 months; the lower feeding level strongly affected the animal growth rate; the conversion ratio (feed intake/gain) increased with increasing age as did the net dressing percentage with values of more than 60 percent. Conformation and fatness scores also increased with the age of the animal as did the subcutaneous and intermuscular fat percentage on the carcass (Table 5), and consequently the meat percentage on the carcass decreased. The carcasses of the animals of group B registered lower fat and more meat than the group A buffaloes. Di Lella et al., (1998) using three different isoenergetic-isoproteic diets, obtained significantly higher daily gains (977 g) with 50 percent maize silage, 15 percent alfalfa hay and 35 percent concentrates on DM, 0.84 UFC/kg DM, 14.2 percent CP, between 6 and 12 months, in comparison with a diet with 25 percent corn silage, 50 percent concentrates and 25 percent NH3 treated straw. In the period between 360 and 480 days with 0.80 UFC/kg DM, 12 percent CP again the best daily gain (887 g) was obtained in the first group in comparison with the second one (818 g).

Silva et al., (1997), in a study undertaken at the Instituto Agronomico do Paraná (IAPAR) (Pinhais/PR), involving 18 Murrah animals with isoproteic and isocaloric feeds, in three proportions of volume: concentrate (75:25, 65:35 or 55:45) achieved DWG (Daily Weight Gain) of 1.23, 1.23 and 1.21 kg/day, respectively. Restle et al., (1990) established DWG of 1.032 and 1.345 kg/day in buffaloes and cattle, respectively. In a study with calves of different genetic groups, Jorge et al., (1997a) observed similar results, with buffaloes, the DWG was smaller than two cattle breeds and greater than another one. However, these results differed from those of Moletta and Restle (1992), who evaluated the performance of calves of different genetic groups, finding an average DWG for buffaloes of 1.032 kg/day and for cattle of 1.029 kg/day, with all the animals being subjected to similar treatments under confinement. Villares et al., (1979a) observed an average daily weight gain of 0.991 kg, with 18 month old animals from three different buffalo breeds (Jafarabadi, Mediterranean and Murrah), when all of them were subjected to the same nutritional management. In weight gain tests of young Mediterranean buffaloes, Nascimento and Veiga (1973) demonstrated the great meat potential of this breed, which demonstrated an average daily gain of 0.857 kg. Velloso et al., (1994), comparing the DWG of buffaloes and zebu cattle, obtained values of 1.027 and 0.808 kg/day, respectively, which represents a difference of 20 percent. In a test involving buffaloes fed with different diets from 26 months (330 kg) and slaughtered at about 30 months of age (408 kg), Tonhati et al., (2001a), working with Murrah buffaloes, fed with sugarcane as volume and three protein sources (amiferm, poultry litter and wastes, and soya bean bran), observed a DWG of 0.39, 0.88 and 0.78 kg, respectively, with an overall average of 0.68 kg.

Johnson and Charles (1975), in a comparative study involving confined (132 days) buffaloes and Holstein, Angus and Hereford cattle, matured to ages between 20 and 30 months with a diet rich in concentrate, concluded that the buffaloes had a lower yield (53.3 percent) than the cattle (58.4 percent; 63.3 percent and 62.1 percent in the three genetic groups). Felicio et al., (1979) evaluated the carcass composition and meat quality of eight whole male buffaloes, of Jafarabadi breed, matured to approximately 24 months, with a slaughter weight of 400.6 kg and an empty weight of 349.5 kg. The results indicated a carcass yield of 48.7 percent in relation to slaughter weight and 55.8 percent in relation to the empty weight. Villares et al., (1979b), in Botucatu (SP), analysed the meat production of 15 Mediterranean buffaloes, 10 males and 5 females, matured to about 24 months, in a free stall regimen and with an average weight before slaughter of 364.07±46.10 kg. The weight of the warm carcass was 183.36±13.65 kg, representing a 50.36 percent yield in relation to the live weight before slaughter. Cockrill (1974) reported a yield of 49 percent for Italian female buffaloes at eighteen months of age, and also reported that in the former USSR, female buffaloes had greater yields than the males, 48.70 percent and 47.80 percent, respectively. Mattos et al., (1998) obtained an average warm carcass yield (WCY) of 52.20 percent, 52.05 percent and 53.10 percent, for females, non

castrated and castrated males, respectively. The lower carcass yield of buffaloes in relation to cattle was observed by several authors and can be attributed to the fact that these animals have a thicker skin and a larger percentage of head, horns, hoofs and guts. Jorge et al., (1997b) observed an average carcass yield of 49.44 percent in buffaloes slaughtered at different stages of maturity, fed with a diet containing 2.4 Mcal metabolizable energy/kg DM. Oliveira et al., (1991) observed an average carcass yield of 49.30 percent in confined buffaloes , in addition to differences in body composition between buffaloes and zebus. Afif et al., (1974) observed an average carcass yield between 50 and 55 percent for male buffaloes at different ages and weights at slaughter. Pillai et al., (1988) studied the carcass yield of 15 male whole buffaloes and 30 females, of different breeds, originating from two different regions of India. In one of the regions, the male and female buffaloes produced, respectively, 410.57±104.92 kg and 470.18±72.74 kg of slaughter weight; 41.58±1.83 percent and 43.06±1.01 percent of carcass yield in relation to the slaughter weight. In similar studies, Lorenzoni et al., (1986) found a carcass yield of 53.2 percent for buffaloes and 58.7 percent for Nelore. Mattos et al., (1997) evaluated carcass characteristics for eight Mediterranean buffaloes and six Nelore cattle, confined for 120 days, obtaining a smaller carcass yield for buffaloes (52.09 percent) and greater for Nelore (56.28 percent). In a study undertaken by Franzolin et al., (1998), 15 male Mediterranean buffaloes, fed with three different energy levels, produced an average carcass yield varying from 49.66 percent to 50.76 percent, with the first average as the least energetic and the last one as the most energetic. Lourenço Jùnior et al., (1987), comparing carcass yield in relation to empty body weight (EBW), found Mediterranean, Carabao and Jafarabadi buffaloes, respectively, 54.08, 53.76 and 53.32 percent, which showed little difference between them. Gazzeta et al., (1995), evaluating 12 Jafarabadi buffaloes, 12 Mediterranean and 6 Nelore cattle, obtained carcass yields of 51.45 percent, 51.44 percent and 57.18 percent, respectively. Tonhati et al., (2001b) showed an average carcass yield of 48.65 percent for Murrah buffaloes slaughtered at 30 months. Preston and Willis (1974) demonstrated that several factors can affect carcass yield values, such as the assessment basis (in relation to live weight or in relation to empty body weight). When live weight is used, the yield is affected by the diet type and fasting period that the animals have been subjected to prior to slaughter. Jorge (1993), considered that carcass yield, often does not provide a good estimation of quality meat yield, especially when considering animals that have been excessively finished, due to the dilution effect of the fat tissue on other carcass components: i.e. muscles and bones (Jorge and Fontes, 1997). In a study covering Mediterranean animals, Jorge et al., (1997) showed values of 55.86, 27.64 and 16.51 percent for muscles, fat and bones, respectively. In the same presentation order, Tonhati et al., (2001c) found averages of 52.01, 30.50 and 17.24 percent for Murrah 30 months old. In Egypt, Abdallah et al., (1981) investigated the comparative performance of buffaloes and cattle weighing 167 kg of warm carcass, and found values of 65.6 percent and 60.0 percent for muscles, 10.1 percent and 8.1 percent for fat, and 17.1 percent and 15.9 percent for bones, respectively (Tonhati and Ferreira Lima, 2003).

In South American and Asian countries buffalo meat production is generally undertaken utilizing extensive systems, using pasture or poor crops, with no incentive to use high energy diets, realizing low daily gains (500 g) and producing bulls weighing 400 kg at about two years. In Italy the aim is to obtain a minimum daily gain of 800 g and up to 900 g, and to produce young bulls weighing 400 kg within 15 months. Therefore, specific diets are used, as shown in Tables 6, 7 and 8 with an energetic concentration varying from 0.86 to 0.80 UFC (Meat Feed Unit)/kg DM (Dry Matter) and with about 10 percent of DCP (digestive crude protein). 40 kg prior to slaughter a fattening period is effected adding 1 kg cereal meals/pro die/pro capite.

The buffalo performances for meat production i.e. growth, feed efficiency, conversion ratio, dressing percentage, carcass evaluation and composition and meat quality cuts, are very important in economic terms but the priority focus for expanding the buffalo meat market is meat quality, which means chemical, physical, organoleptical and hygienic characteristics and a good presentation to the consumer.

Many years ago a study was undertaken on meat quality in buffalo males slaughtered at different ages. This trial covered 30 Friesian male calves and 30 Mediterranean Italian buffaloes reared under identical feeding and environmental conditions and slaughtered at 20, 28 and 36 weeks of age (Borghese et al.,1978b). The water buffalo meat, upon the visual inspection of the judges, was lighter than the bovine meat and a colorimeter confirmed this fact; it became darker with the increasing age of the animals. Cooking losses also decreased with the age of the animal. The meat tenderness using the Warner Bratzler Shear machine and according to a panel taste decreased as the age increased, as did flavour scores, while juiciness scored better after 36 weeks of age.

Many studies have been undertaken in this field comparing buffaloes to Friesian bovines up to 52 and 64 weeks of age, including analysis of the fatty acid composition of subcutaneous, intermuscular, intramuscular, perivisceral and perinephric lipids at different ages (Borghese et al., 1978a) but only a few of these results are reported here covering the meat quality of Italian buffaloes fed with hay and concentrates and slaughtered at 52 weeks of age compared with Friesian bovines reared under the same conditions (Borghese et al.,1996).

Muscle pH was approximately 6.2 at slaughter, 5.7 after two hours, and 5.5 after six hours. Only the *longissimus dorsi* in bovines showed a significantly ($P<0.05$) higher value (6.2 after two hours and 5.8 after six hours) than other muscles (*semimembranosus* and *semitendinosus*) and than in buffalo *longissimus dorsi*. After 24 hours, the pH was about the same (5.5-5.6) for all the muscles in both species, with a slight increase (5.7) from the sixth day on. Therefore, after ageing, pH characteristics are practically the same in both species.

The trial demonstrated that normally there were no significant differences between species in the nine studied muscles for all the physical parameters, using the Instron machine (Table 9). The hardness of the raw meat tested by the Warner Bratzler Shear machine was significantly ($P<0.05$) higher in buffalo only in the *iliopsoas* muscle, while in bovine bulls it was higher than in buffaloes only in the *semitendinosus* (Fig. 1), while significant differences were found in the force used only in the *caput longum tricipitis brachii* (Fig. 2). With the compression test only gumminess in the *supraspinatus* was significantly higher in the bovine (Table 9).

The chewiness, that is the synthesis of physical parameters, shows a tendency to be higher in bovine bulls. This could explain why generally people say that buffalo meat is more tender.

The results of the Warner Bratzler Shear tests on cooked meat are reported in Table 10: *longissimus dorsi* (Fig. 3) was more tender than in the raw meat, particularly if baked; after baking, both species showed the same values, while buffalo meat appeared more tender after being cooked in boiling oil ($P<0.09$).

Figure 1. *Semitendinosus* muscle in buffalo young bull
(Borghese photo, 2004)

Figure 2. *Caput longum tricipitis brachii* in buffalo young bull
(Borghese photo 2004)

Figure 3. *Longissimus dorsi*
(Borghese photo, 2004)

Figure 4. *Gluteobiceps*
(Borghese photo 2004)

The cooking losses, when the meat temperature reached 70°C, were about 21 percent after boiling in oil, and about 12 percent after baking, with no differences between species (Table 10).

Further quantity was lost, ten minutes after cooking with liquids: seven percent after cooking in boiling oil, and approximately six percent after baking, with the same trend for both bovine and buffalo meat.

The percentage of judges that identified the species was 22.9 percent for meat slices cooked in the open pan and only 7.5 percent for meat cooked by pressure cooking, less than casual probability. The percentage was significantly ($P<0.01$) higher for slices cooked in the open pan, where the meat was less cooked and quite natural. A large number of judges declared it impossible to identify the meat (52.0 to 74.5 percent for the open pan and pressure cooker respectively), while 25.1 and 18.0 percent respectively mistook the identification. No judge identified the species in all the tests. No difference was found with regard to the evaluation of tenderness, flavour and juiciness (Table 11).

Only in two taste tests was buffalo meat significantly overscored by one judge on the panel. The judges always gave better scores ($P<0.05$) to the meat cooked in the pressure cooker than that cooked in the open pan (Borghese et al., 1996).

Regarding the tests on tenderness values undertaken with the Instron machine on several muscles of Italian buffaloes slaughtered at 190 days, Failla et al. (2001) found values in raw meat varying from 2.98 to 4.87 kg/cm, while Tonhati et al. (2001d) found a mean of 4.52 kg/cm in Murrah 30 months old.

With respect to the nutritional characteristics of buffalo meat new data (Infascelli et al., 2003) confirmed the very low lipid content (1.36 ± 0.1 percent), correlated to the low energy value of the diet (0.84 UFC/kg DM) fed to the animals. The cholesterol content (48.8 ± 2.9 mg/100 g) was lower than that reported for Italian bovine genotypes with an aptitude for meat production. The content of myristic, palmitic and stearic acids, the first two with both atherogenic and thrombogenic activity, was also very low. Thus, despite the low values of oleic acid and polyunsaturated acid of the ω-6 and ω-3 series, both the atherogenic and thrombogenic indexes were very low (0.53 and 1.48, respectively). Based on these results, the nutritional quality of buffalo meat can be considered of great interest. Many researches are being carried out in Italy regarding the physical, chemical and organoleptic characteristics of industrial products derived from buffalo meat, due to the marketing and managerial interest in this field. Buffalo Beef is a company in Capua (Campania region) that produces a lot of typically Italian buffalo meat products: bresaola (salted rump lean muscle, particularly *Semitendinosus*, Fig. 1), salami, sausages, cacciatorini (very little salami), buffalo cheese rolls containing salami, ham or dry meat etc. (Fig. 5).

Figure 5. Bresaola and typical salami produced by Buffalo Beef

In Milan also, Paleari et al. (2000) studied characteristics of buffalo bresaola as compared with the typical IGP (Indication of Protected Geographic Origin) bovine bresaola of Valtellina (Prealpine Lombard region), which is just entering several international markets. There were no significant differences in the percentage weight loss (32.3 percent) and pH after thawing and curing (5.67) for the buffalo and beef samples. Significant differences were found for all the chemical composition parameters (moisture % 62.94 *vs* 60.95, protein % 29.79 *vs* 31.96, collagen % 0.9 *vs* 0.78 in buffalo and in bovine respectively) except the fat (1.75 percent) and ash (5.43 percent). In terms of brightness and hue there were no significant differences, either inside or outside; however, the saturation value revealed the buffalo bresaola to be darker. Furthermore, the buffalo bresaola was less tender than the beef one, tenderness being expressed as shear force (0.189 *vs* 0.157 kg/mm^{-2}). The investigation demonstrated the possibility of transforming cuts of buffalo rump into a product similar to that of beef. Thus buffalo beef can be transformed into various cured products, especially that of the male animal, which would be of increased value, with clear advantages to the breeder. In this way a range of typical products with their own niche in the market place could be created. This could also satisfy consumer demand for lean products with a low energy content which could be well integrated into the modern diet.

At present Italian breeders (particularly three cooperatives are of particular interest: Consorzio Alba, Consorzio La Baronia and Buffalo Beef) are trying to produce high quality meat for the luxury market (restaurants and gourmet food) adopting a production protocol in accordance with the IGP symbol (Indication of Protected Geographic Origin) "Carne di bufalo mediterraneo" (Mediterranean buffalo meat). The geographic territory where the buffaloes are reared is the same as the D.O.P. "Mozzarella di bufala Campana": Campania and the south Lazio regions and some parts of the Puglia and Molise regions.

The calves can be weaned with milk substitutes or by nursing bovine cows. Following this they can be fed fresh or conserved (silages or hay) forages and meals or concentrates until they reach no more than 450 kg live weight in order to avoid carcasses which are too fat or bad smelling. The daily gain must be between 800 and 950 g for young bulls in order to avoid sick animals or hormone treatments. Four months prior to slaughter the use of maize silage and of particular feeding stuffs is prohibited in order to avoid bad flavour in the meat and the animals must be reared on slattered floors or on floors where the straw is changed each week to avoid the smell

of urine and faeces. Stress before and during slaughter must be avoided for the quality of meat. The carcasses must be included in the medium and abundant classes for fatness and in the good and optimum classes for conformation. Ageing must be effected for nine days at least and the quality characteristics of the *longissimus dorsi* muscle must be: PH 5.5 5.9, intramuscular fat <3 percent, protein >20 percent, cholesterol <50 mg/100 g, iron > 1.5 mg/100 g, and total mesophil bacteria< 10^5 Units/cm^2. These regulatory measures guarantee the high quality of the meat and of the products which are produced in the different industries such as: bresaola, salami, cacciatorini, and buffalo cheese rolls with salami or ham.

Therefore, the aim of the Italian market is to develop products of good nutritional and organoleptic quality. In South America also, particularly in Brazil where most of the South American buffalo population can be found (3 000 000 head), buffalo meat is recognized as a differentiated product as compared to bovine meat. Buffalo meat is also leaner and presents 40 percent less cholesterol and 55 percent less calories, 11 percent more protein and 10 percent more minerals (Table 12). Buffalo meat, which is to be placed on the market, should derive from young animals, preferably from 18 to 24 months of age, since older animals have fibrous meat and meat with lower organoleptic qualities. Therefore, due to these advantages, there is an open and guaranteed market for buffalo meat, not only in Brazil, but all over the world. In Brazil buffalo meat is already sold in special kits, with cuts ready to be used. At present, the demand for this product comes mainly from specialized restaurants (Rocha Loures, 2001).

Clearly the priority in Asia is for food production to satisfy human needs, particularly for animal proteins, and the possibility to produce buffalo meat could strongly assist in finding a solution to this planetary problem. India is the highest producer of buffalo meat in Asia.

Meat production in India is estimated at 4.7 million tons, taking eighth place in the ranking of the world's meat production. Buffalo in India contribute about 30 percent of the total Indian meat production with 1.43 million tons annually. The contribution from cattle, sheep, goats, pigs and poultry is 31 percent, 5 percent, 10 percent, 10 percent and 13 percent, respectively (FAO, 2003). The trends in livestock population, slaughter rate (number slaughtered as percentage of population), carcass weight and meat production in India in 2003 are shown in Table 13. The major export of meat is from buffaloes, as is shown in Table 14 (Ranjhan, 2004).

It can be seen that exports of buffalo meat have increased significantly over the last three years. The export of buffalo meat in 1997-98 was only 176 328 MT, and this increased to 243 355 MT in 2001-2002 accounting for an increase of 43 percent. In 2002-2003, exports were almost 300 000 MT valued at 13,054 million Indian rupees equivalent to about US$300 million. In addition, the export of meat from small ruminants (sheep and goats) has increased over the last three years. India also has a large number of dogs and cats, which are kept as pets but there is no scientific production of pet food. The international market is vast and the demand for pet food runs into billions of dollars. Slaughterhouses produce large quantities of raw material for pet food and the technology for its commercial utilization is evolving in India. In the next five years this sector is poised for a quantum leap with many world leaders in this field working towards joint ventures with Indian companies (Ranjhan, 1996, 2004).

In India the intensive feeding of male buffalo calves for meat production was never visualized and was considered a taboo. However, intensive feeding has been implemented over the last five years (1999-2004) for the first time in a commercial feedlot for the production of quality meat. In a village demonstration farm with HAIL, a commercial feedlot housing 5 000 male calves has been established. The facilities include environmentally controlled animal houses with slatted floors where urine and dung are collected in the Keller and are regularly pumped out for spraying on the forage field. The male calves are purchased from the farmers at the age of eight to ten months and are then quarantined for 15 days during which time vaccinations and de-worming are provided. Thereafter, they are brought to the main farm and are fed on a high protein/high-energy diet in order to gain an additional weight of 120 kg in four months and produce quality meat. The composition of the feed is as follows:

Composition of the feed (pelleted)

Straw	50%
Rape Seed	30%
Bran	5%
Urea	2%
Molasses	10%
Mineral Mix	1%
Salt	2%

The above mixture is pelleted and contains above 18 percent crude protein equivalent and 60 percent TDN. Each animal is fed 2.5 - 3.0 kg of pellets; 3 kg of spent brewer's grains and 2 kg whole sugar cane/sorghum per day. This above ration provides 20 percent crude protein and 65 percent TDN with a dry matter intake of 4.0 to 5.0 kg per animal per day. The above composition of the feed can be changed according to the local availability of feed resources (Ranjhan, 2004).

The Murrah calves grow at the rate of 900 to 1 000 g per head per day with a feed conversion ratio of 5.0:1. The cost of 1 kg live weight gain comes to around Rs.25.00 (US$0.5) per kg live weight gain (feed cost Rs.20.00+overhead cost Rs.5.00 per day). The dressing percentage of such animals is around 65 percent. A 250 kg live animal produces a carcass of about 150 kg fetching a price of Rs.65.00 to 90.00 (US$1.5 to 2.0) per kg on the international market. The quality of the buffalo meat is excellent, since it is lean, tender and juicy. It is no exaggeration that buffalo is black gold. The other positive aspects are that there is identification and certification of origin and traceability which are essential tools for accomplishing control of diseases, for improving production standards and providing the consumer with a more transparent and reliable market. These are the hallmarks of the Codex Alimentarius. Buffalo are also never fed on antibiotics, hormones and growth promoters. It should not be forgotten that with the globalization of the meat trade, the expansion of animal production will lead to global warming and an indiscriminate use of medication, increasing the risks for human beings. These factors could also disrupt the existing eco balance.

In India about 40 million people are engaged in the meat sector in the trade of live animals, hides, bones, horns, hooves etc. (Ranjhan, 2004).

Table 1. Weight, gain and feed intake at different ages (Romita et al., 1982)

Age (weeks)	20		28		36 (1)		36 (2)		52		64	
	Bovines	Buffaloes	Bovines	Buffaloes	Bovines	Buffaloes	Bovines	Buffaloes	Bovines	Buffaloes	Bovines	Buffaloes
Starting weight (kg)	48.4	46.1	39.6	45.6	48.5	48.1	48.1	50.7	48.6	50.8	49.1	50.0
Final weight (kg)	161.5*	151.8	229.3***	203.7	291.0***	231.1	285.7	273.7	402.8	385.0	481.1	466.4
Total gain (kg)	113.1*	105.7	189.7***	158.1	242.5***	183.0	237.6	224.7	354.2	334.2	432.0	416.4
Daily gain (kg)	0.850*	0.795	0.968***	0.807	0.988***	0.746	1.007	0.963	0.980	0.930	0.987	0.949
Milk (kg)	199.6	199.6	292.3	292.3	456.2	456.2	39.4	41.8	43.7	43.7	43.7	43.7
Concentrate (UF)			170.8***	142.3	274.3***	188.7	772.0	733.5	1448.6	1381.3	1970.7	1885.0
Hay (UF)							260.0	243.0	431.7	395.1	653.1**	552.1

(* p≤5%, ** p≤1%, *** p≤0.1% between species, within ages)

Table 2. Fast live weight, net live weight, carcass and dressing at different ages (Romita et al., 1982)

Age (weeks)	20		28		36 (1)		36 (2)		52		64	
	Bovines	Buffaloes	Bovines	Buffaloes	Bovines	Buffaloes	Bovines	Buffaloes	Bovines	Buffaloes	Bovines	Buffaloes
Fast live weight (kg)	161.5*	151.8	228.3***	203.4	291.4***	230.2	285.7	273.7	402.2	384.5	479.2	465.7
Net live weight (kg)	145.8	141.0	201.7**	185.3	263.0***	210.1	257.5	251.1	360.6	353.9	433.2	428.4
Carcass weight (kg)	92.9**	84.0	125.5***	108.0	159.2***	121.0	152.6**	138.9	225.0**	201.8	262.3*	242.7
Dressing (%)	57.52	55.33	54.97	53.09	54.63	52.56	53.41	50.74	55.94	52.48	54.73	52.11
Net dressing (%)	63.70**	59.60	62.40***	58.25	61.24***	57.65	59.28***	55.33	62.31***	57.03	60.56***	56.64

(* p≤5%, ** p≤1%, *** p≤0.1% between species, within ages)

Table 3. Composition of the carcass (Gigli et al., 1982)

Age (weeks)	**20**		**28**		**36 (1)**		**36 (2)**		**52**		**64**	
	Bovines	Buffaloes	Bovines	Buffaloes	Bovines	Buffaloes	Bovines	Buffaloes	Bovines	Buffaloes	Bovines	Buffaloes
Half carcass weight (kg)	43.7**	39.0	59.2***	50.7	75.6***	57.3	71.7*	65.3	108.5**	97.5	125.0	117.3
Meat (%)	65.0**	67.6A	64.16**	66.36AB	63.26*	65.40B	64.35	63.80C	62.34	61.41D	61.67	62.00D
Bone (%)	26.4**	24.9A	23.16	22.09B	22.65	21.96B	21.38*	19.5C	19.48***	17.57D	20.20***	16.87D
Total Fat (%)	8.6	7.51D	12.68	11.55C	14.04	12.64C	14.37*	16.70B	18.19*	21.02A	18.12**	21.13A
Other tissues (%)							0.91	0.99	0.86	0.92	1.17	1.22
Subcutaneous fat (%)							4.56***	7.28	5.20***	8.86	5.25***	9.41
Intermuscular fat (%)							6.48	6.78	9.52	9.13	8.57	8.26
Perineal fat (%)	1.9**	0.7	2.23***	0.87	2.30***	0.97	2.42*	1.65	2.61	2.11	3.13**	2.23

(* p≤5%, ** p≤1%, *** p≤0.1% between species, within ages)
Different letters mean significant differences ($p<0.05$) between ages

Table 4. Performances in vivo and at slaughtering (Gigli et al., 1994)

	n	Daily gain (kg)	ME/d (MJ)	Conversion ratio (MJ/kg)	PDI (g/d)	n	Net live weight (kg)	Net dressing (%)
6 months						10	128.7F	56.23C
10 months								
A	24	0.962A	53.34C	55.79C	577.54C	8	212.8D	59.63B
B	24	0.594CD	31.66E	54.07C	439.96E	8	178.0E	59.48B
14 months								
A	16	0.979A	64.46B	66.36B	649.86B	8	326.3B	60.58AB
B	16	0.647C	35.27DC	54.88C	463.87C	8	251.5C	59.73B
18 months								
A	8	0.798B	72.06A	87.42A	696.45A	8	409.1A	61.68A
B	8	0.550D	37.21D	65.35B	473.01D	8	316.6B	60.22AB
Mean	96	0.772	46.98	60.40	537.45	58	255.9	59.53
Error variance		0.009	34.763	55.380	1645.676		77537	2.194

Different letters mean significant differences ($p<0.05$)

Table 5. Composition of the carcass (%*) (Gigli et al., 1994)

	Half carcass (kg)	Total meat	Subcutaneous fat	Inter-muscular fat	Total fat	Bone
6 months	33.9E	62.06AB	3.98E	5.91D	9.89D	24.93A
10 months						
A	60.2C	63.79A	5.37D	8.01BC	13.38BC	20.20C
B	50.2D	63.86A	4.74DC	6.40CD	11.15ED	22.14B
14 months						
A	93.2B	60.37C	9.00B	11.02A	20.02A	17.47D
B	70.5C	62.76A	6.48C	7.68BC	14.16BC	20.33C
18 months						
A	118.4A	59.16C	10.69A	10.93A	21.62A	17.17D
B	91.1B	62.37AB	6.93C	8.31B	15.25B	19.98C
Mean	72.5	62.04	6.65	8.24	14.89	20.48
Error variance	76.81	4.826	1.208	2.636	5.674	1.436

* Without other tissues
Different letters mean significant differences ($p<0.05$) between ages

Table 6. Diet for weaned calves from 150 kg

	kg t.q.	kg DM	UFC	g DCP	g CF	Cost
Maize silage	2	0,62	0,56	29	100	€ 0,10
Italian Ryegrass hay	2	1,76	1,1	146	644	€ 0,28
Barley grain	1	0,9	1	63	51	€ 0,20
Leguminous seed	1	0,9	0,94	220	78	€ 0,20
Total	**6**	**4,18**	**3,6**	**458**	**873**	**€ 0,78**

Table 7. Diet for weaned calves from 200 kg

	kg t.q.	kg DM	UFC	g DCP	g CF	Cost
Maize silage	3	0,93	0,84	43	150	€ 0,15
Italian Ryegrass hay	3	2,64	1,65	219	966	€ 0,42
Barley grain	1	0,09	1	63	51	€ 0,20
Leguminous seed	1	0,9	0,94	220	78	€ 0,20
Total	**8**	**5,37**	**4,43**	**545**	**1245**	**€ 0,97**

Table 8. Diet for weaned calves from 300 kg

	kg t.q.	kg DM	UFC	g DCP	g CF	Cost
Maize silage	4	1,24	1,12	58	200	€ 0,20
Italian Ryegrass hay	4	3,52	2,20	292	1288	€ 0,56
Barley grain	1	0,9	1	63	51	€ 0,20
Leguminous seed	1	0,9	0,94	220	78	€ 0,20
Total	**10**	**6,56**	**5,26**	**633**	**1617**	**€ 1,16**

Table 9. Myorheological parameters determined with the Instron instrument on raw meat (Borghese et al., 1996)

Muscle	Species	n	Warner Bratzler Shear		Compression test				
			Hardness (kg)	Work (kgm)	Hardness (kg)	Cohesiveness (max=1)	Springiness (cm)	Gumminess (Hard.XCohe.)	Chewiness (Gum.XSpring.)
C.l.tricipitis brachii	Bovine	9	11.53 c	0.33 Bbcde	31.62 ab	0.35 b	0.19 ab	11.47 a	2.27 a
	Buffalo	9	13.36 ab	0.42 Aab	26.85 ab	0.35 a	0.22 ab	8.80 c	2.00 bc
Supraspinatus	Bovine	9	16.12 b	0.45 bc	24.05 b	0.46 a	0.19 ab	11.07 Aa	2.21 a
	Buffalo	9	14.15 ab	0.40 ab	18.72 c	0.39 a	0.22 ab	7.45 Bc	1.73 bc
Rectus femoris	Bovine	9	10.66 c	0.32 bcde	26.87 b	0.39 ab	0.19 ab	10.93 a	2.17 a
	Buffalo	9	10.20 b	0.30b	24.70 bc	0.35 a	0.20 b	8.86 c	1.83 bc
Guteus medius	Bovine	9	10.55 c	0.33 bc	29.37 ab	0.40 ab	0.20 ab	11.68a	2.47 a
	Buffalo	9	10.27 b	0.30 b	22.26 c	0.38 a	0.21 ab	8.35 c	1.79 bc
Gluteobiceps	Bovine	9	16.15 b	0.42 bcd	39.90 a	0.35 b	0.23 a	13.88 a	3.04 a
	Buffalo	9	13.11 ab	0.36 ab	35.43 ab	0.37 a	0.22 ab	12.86 b	2.88 b
Semitendinosus	Bovine	9	21.34 Aa	0.66 a	35.36 ab	0.42 ab	0.23 a	14.47 a	3.33 a
	Buffalo	9	16.36 Ba	0.54 a	37.55 a	0.43 a	0.26 a	16.97 a	4.31 a
Semimembranosus	Bovine	9	13.56 bc	0.45 bcd	32.39 ab	0.34 b	0.22 ab	10.46 a	2.33 a
	Buffalo	9	13.07 ab	0.46 ab	28.51 ab	0.34 a	0.20 b	9.42 c	1.99 bc
Longissimus dorsi	Bovine	9	9.74 c	0.29 dce	15.31 c	0.33 b	0.18 b	5.14 b	0.94 b
	Buffalo	9	10.69 b	0.32 bc	18.30 c	0.37 a	0.20 b	6.81 c	1.44 c
Iliopsoas	Bovine	9	7.26 Bc	0.24 e	9.24 c	0.26 c	0.22 ab	2.48 b	0.55 b
	Buffalo	9	9.59 Ac	0.28 c	7.83 c	0.23 b	0.20 b	1.75 d	0.36 d

Different letters mean significant differences ($p<0.05$) between muscles if small; between species if capital

Table 10. Cooking losses and hardness (Warner Bratzler) of cooked meat. (*longissimus dorsi* muscle) (Borghese et al., 1996)

	N	BOILING OIL			BAKING		
		at 70°C%	after 10'%	Hardness kg	at 70°C%	after 10'%	Hardness kg
Bovine	10	19.2	26.5	7.0	11.1	17.3	5.9
Buffalo	10	22.2	29.7	6.1	12.6	18.5	5.7
ST. E.		2.41	1.97	0.37	0.91	0.72	0.37

Table 11. Taste panel mean scores (Borghese et al., 1996)

		Open pan				Pressure cooker			
	n	Bovine		Buffalo		Bovine		Buffalo	
		x	cv%	x	cv%	x	cv%	x	cv%
Tenderness	882	6.00b	22.0	6.14b	21.7	7.29a	15.2	7.40a	13.6
Flavour	878	6.28b	17.6	6.18b	18.5	7.25a	11.8	7.35a	11.2
Juiciness	880	4.88b	29.3	4.85b	28.6	5.55a	27.1	5.75a	26.4

Different letters mean significant differences for $P<0.05$.

Table 12. Comparison of some characteristics of bovine and buffalo meat (Rocha Loures, 2001)

Characteristics	Buffalo	Bovine
- Calories (Kcal)	131.00	289.00
- Protein (N x 6.25)	26.83	24.07
- Total fat (g)	1.80	20.69
- Fatty acid:		
- Saturated (g)	0.60	8.13
- Monosaturated (g)	0.53	9.06
- Polysaturated (g)	0.36	0.77
- Cholesterol (mg)	61.00	90.00
- Minerals		
Calcium, Iron, Magnesium, Phosphorus, Potassium, Sodium, Zinc, Copper and Manganese (total mg)	641.80	583.70
- Vitamins		
Ascorbic acid, Thiamine, Riboflavin, Niacin, Pantotenic acid, Vit.B6, Folacin and Vit. B12 (total mg)	20.95	18.52

Table 13. Trends in Livestock Production and Meat Production in India (FAO, 2003)

Livestock Species	Population in Millions	Animals slaughtered (millions)	Percent slaughtered (%)	Carcass weight (kg)	Meat production (million tons)	Share in total meat production (%)
Cattle	180.1	14.2	7.9	103	1.46	31.1
Buffalo	103.0	10.3	10.0	138	1.43	30.5
Sheep	40.1	19.2	47.9	12	0.23	4.9
Goats	124.0	47.0	37.9	10	0.47	10.0
Pigs	18.0	16.0	88.9	31	0.47	10.0
Poultry	820.0	604.0	73.6	0.8	0.63	13.4
Total					**4.69**	**100**

Table 14. Export of Buffalo Meat (MT) from India to some major countries (Ranjhan, 2004)

Countries	2000-2001	2001-2002	2002-2003
Malaysia	77 135	67 251	79 421
Philippines	47 447	50 356	46 971
UAE	41 516	19 988	27 635
Yemen	3 738	3 938	5 604
Qatar	617	8 520	1 334
Mauritius	3 192	3 004	3 382
Oman	7 631	8 101	10 106
Lebanon	4 130	2 980	4 518
Jordan	12 442	15 327	16 212
Iran	12 576	10 741	7 617
Egypt	48 716	17 808	19 524
Total	**268 027**	**243 355**	**297 897**

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Chapter XII

METABOLIC AND HORMONAL PARAMETERS IN BUFFALOES

Giuseppina Maria Terzano [1], Simona Allegrini [1], Antonio Borghese [1], Cristina Roncoroni [2] and Lavinia Alfieri [2]

[1] Istituto Sperimentale per la Zootecnia
(Animal Production Research Institute)
Via Salaria 31, 00016 Monterotondo (Rome), Italy

[2] Istituto Zooprofilattico Sperimentale Lazio e Toscana
(Animal Prophylaxis Research Institute for Lazio and Toscana regions)
Via Appia Nuova 1411, 00178 Capannelle (Rome), Italy

The biochemical characteristics in livestock were initially investigated in order to establish their physiological ranges. In 1970 Payne et al., suggested the possibility of ascertaining nutritional disorders in livestock through blood analysis. Since then many studies have been undertaken in order to set reference values for haematochemical parameters of animals and to discover relationships between blood parameters and the pathological phenomena related to nutrition.

Many studies have also been undertaken in buffalo by several authors, demonstrating the differences with other species (Table 1) and contributing to the advancement of knowledge regarding:

1) the pattern of changes in different physiological states (i.e. growth, dry milk period, pregnancy, lactation);
2) the pattern of changes before and after meals;
3) the pattern of changes due to diets which have a different energy and protein content;
4) the pattern of changes due to transport, handling and the stress connected to slaughter;
5) the pattern of changes due to different environmental factors (housing systems, season, management).

The comparison between species (Table 1) shows that buffaloes are different from cattle and sheep, particularly with regard to:

a. Hematocrit, glucose, creatinine, calcium, phosphorus, magnesium, AST and bilirubin. These are all higher in buffalo than in cattle and sheep.
b. Cholesterol, zinc, total protein and albumen, which are lower in buffalo than in cattle but higher in buffalo than in sheep;
c. Urea, GGT and ceruloplasmin which are higher in sheep, while in buffalo the values are between cattle and sheep;
d. T3 values are similar in the three species, while insulin and cortisol are higher in buf falo and sheep than in cattle.

With regard to reproductive hormonal parameters, it is generally considered that the changes in the ovarian steroids and gonadotrophins blood concentrations during the cyclic ovarian activity in buffalo cows are comparable to those of bovine cows (Jainudeen and Hafez, 2000). Also Seren et al., (1994) when monitoring dynamic ovarian changes by transrectal palpation and ultrasound, found that the observed perioestrus endocrine changes did not indicate clear differences throughout the year and were essentially similar to those recorded in the cow. On the other hand, in the past decade, lower levels of several sexual hormones and some differences in the oestrus behaviour and in other reproductive aspects in buffalo cows and bulls in comparison to that of bovines, have been reported.

Table 1. Mean values of some endocrine-metabolic parameters in buffaloes *vs* cattle and sheep (Bertoni et al.,1994b, modified)

	Variations in		
Parameters	**Buffaloes**	**Cattle**	**Sheep**
Hematocrit (l/l)	0.41	⇓	↓
Glucose (mmol/l)	4.43	⇓	⇓
Cholesterol (mmol/l)	2.43	⇑	⇓
Urea (mmol/l)	5.52	⇓	⇑
Calcium (mmol/l)	2.69	↓	↓
Phosphorus (mmol/l)	2.42	↓	⇓⇓
Magnesium (mmol/l)	1.16	⇓	↓
Total protein (g/l)	76.3	↑	↑
Globulin (g/l)	42.1	↑	⇑
Albumin (g/l)	34.2	⇑	⇑
AST (U/l)	101.2	⇓	⇓
GGT (U/l)	21.2	↑	⇑
Bilirubin (mcmol/l)	5.35	↓	⇓
Zinc (mcmol/l)	10.9	⇑	↓
Ceruloplasmin (mcmol/l)	3.74	↓	↑
Insulin (mcU/l)	11.94	↓	⇑
T3 (ng/ml)	1.33	⇓	↓
Cortisol (ng/ml)	10.47	⇓	⇑

If ↑↓ the variations are small
If ⇑⇓ the variations are great

Parameters of energetic metabolism

Glucose - In all species glucose is used by various tissues and organs for free energy (i.e. ATP) production. In addition, glucose may be converted either into glycogen or triacylglycerols which are subsequently stored within tissues (liver, adipose tissues, muscles) or into lactose which is subsequently incorporated into milk in the case of lactating females.

The destination of glucose is regulated by various hormones such as insulin, cortisol, glucagone, somatotropin and adrenalin, and consequently blood glucose levels depend on the nutritive values of the diets, on social or environmental stress conditions as well as on physiological phases.

In previous studies Campanile et al., (1991) and Borghese (1994) established glucose reference values for productive buffaloes (4.00-4.16 mmol/l) and buffalo heifers (4.20 mmol/l). A number of authors have studied the effect of calving distance on metabolic profile (Campanile et al., 1997) and, according to Bertoni et al., (1994a), the buffalo metabolic response to lactation shows a different pattern compared to other ruminants, as also demonstrated by the low incidence of metabolic disorders. Several researches on buffaloes during the dry milk period and lactation indicated that, among energetic metabolism indicators, serum glucose levels were very constant (Bertoni et al.,1994a; Satriani et al., 2001). Several authors (Setia et al., 1992; Campanile et al., 1997; Montemurro et al., 1997), subsequently, reported increased evidence that nutritional status plays a major role in determining variations of the circulating glucose concentration levels. Low serum glucose levels have been found in buffaloes intaking less than

1 020 Kcals/l of milk of NE/l (net energy/lactation), inversely correlated with the quantity of milk produced, but positively correlated with the distance from calving (Zicarelli et al., 1982; Elthohamy et al., 1994). Other studies have shown an increase of glucose following a rise of T3 and T4 values in cold climates (Campanile et al., 1994). It is well known that early lactation is characterized by a negative energetic balance, less intense in buffalo than in bovines: plasma glucose concentrations are lower during the catabolic phase of lactation, and are higher during the anabolic phase of lactation when energy intake is equal or superior to the energy release (De Rosa et al., 2001). Terzano et al. (1997), studying the effect of feeding systems and puberty onset on blood metabolites in buffalo heifers, found that blood glucose levels remained within physiological ranges with a mean value (4.08 mmol/l) similar to that reported by Campanile et al. (1991) and Bertoni et al. (1994a) in lactating buffalo cows or by Borghese (1994) in buffalo heifers, while Montemurro et al. (1995a) reported lower glucose values in heifers bred in two different farms (2.80-3.44 mmol/l). In the same study the feeding system significantly affected the glucose level as found by other authors (Montemurro et al.,1995a; Zicarelli et al., 1982), according to the energy level of diet: heifers bred in a feedlot and fed maize silage ad libitum (DM 33 percent, crude protein 8 percent, crude fibre 21 percent, 0.85 MFU/kg DM) plus hay (about 20 percent on fed maize silage) and protein-mineral-vitamin supplement, showed higher daily gains and higher glucose concentration than heifers fed natural pasture (50 percent graminaceae, 40 percent leguminosae and 10 percent other species; DM 20-70 percent, crude protein 10-21 percent, crude fibre 18-35 percent, 0.50-0.85 MFU/kg DM). The same authors reported that glucose levels were significantly affected by the onset of puberty since this probably involves a more intensive energy metabolism. Zia-Ur-Rahman et al. (1997), studying the changes in hormones and haematochemical parameters in buffaloes undergoing transport (150-320 km) handling and slaughter stress, found a strong positive correlation between glucose concentration and struggling time and, specifically, glucose levels rose from a basal value of 3.20 mmol/l to 3.80 mmol/l (after transport), to 3.40 mmol/l (after handling) and to 3.80 mmol/l (after slaughter), due to an increase in cortisol levels.

Triglycerides - The values of serum triglycerides are usually considered as indicators of good nutrition, they increase with high-fat diets (Bertoni, 1989), in the presence of an altered regulatory mechanism of the lipid metabolism or due to degenerated hepatic function. Buffalo cows show higher triglycerides values during pregnancy (0.275 mmol/l) (Zicarelli et al., 1986) and in the dry period (0.29 mmol/l) (Bertoni et al.,1994b) than during lactation (0.17 mmol/l); Piccioli Cappelli et al., (2001) reported plasma triglycerides values of 0.19-0.25 mmol/l during the dry period and 0.10 - 0.15 mmol/l during lactation. Serum triglycerides concentrations increase during lactation and show a positive correlation with milk fat levels (Zicarelli, 1988). One study carried out on 306 buffaloes with different days in milk and fed 17 different diets evidenced that triglycerides represent a good index of meeting energy requirements in the early stage of lactation (1-31 days) while total cholesterol and HDL in the intermediate phase (31-110 days) and glycaemia after 110 days of lactation, are also positively correlated with the energy supplied by the diet (Di Palo et al., 1990). It is well known, in fact, that the NEFA which are released at early lactation following intense fat mobilization, are used by the liver for the synthesis of the triglycerides only if the balance between energy absorbed in the diet and that emitted due to production is not especially deficit. Bertoni et al. (1997), clarified the pattern of changes of blood metabolites and hormones, in relation to the energy content and protein degradability of diets (0.17-0.20 mmol/l) for lactating dairy buffaloes showing that plasma triglycerides levels were dependent on the energy level (ranging between 0.10 mmol/l and 0.12 mmol/l) and varied before and after meals (ranging between 0.07 and 0.17 mmol/l). However, serum triglycerides increase in buffaloes under different environmental conditions (low temperatures and season) (Satriani et al., 2001).

Not esterified fatty acids (NEFA) - The concentration of NEFA in blood reflects the degree of adipose tissue mobilization, therefore, the greater the extent of negative energy balance, the more NEFA are released from body fat and the higher the concentration of NEFA in the blood. Animals adapt to negative energy balance by mobilizing energy from adipose tissue in the form of NEFA: metabolic and endocrine factors regulate the rate of NEFA release but with a low

degree of sensitivity. The energy balance is the main determinant of plasma NEFA concentration but other factors have an important influence on plasma NEFA concentration (impending parturition, stress, previous nutritional history, etc.). Buffalo cows in early lactation have higher energy requirements that cannot be supported by dietary intake. As a result, the cow must utilize body fat as a source of energy. Buffalo fat mobilization begins towards the end of gestation (Campanile et al., 1997; Grasso et al., 2004) and in early lactation plasma concentrations of NEFA are high, but never reach the levels found in the cow. In postpartum buffalo cows the plasma NEFA concentration was reported to be highest at d 20, (0.48 mmol/l) then decreasing and returning at about d 110 to the plasmatic levels of the dry milk period (0.17 mmol/l). Bertoni et al. (1997), clarified the pattern of changes of blood metabolites and hormones, in relation with the energy content and protein degradability of diets (0.17-0.20 mmol/l) for lactating dairy buffaloes showing that plasma NEFA levels were dependent on the energy level and varied before and after meals (ranging between 0.07 and 0.17 mmol/l).

Cholesterol - The values of serum cholesterol are usually considered as an indicator of good hepatic lipoproteins production used as carriers of triglycerides, synthesized from NEFA (NEFA-AcilCoA-glicerphosphate---trygliceride s). Total cholesterol and HDL-fractions indirectly reflect the degree of exogenous energy availability and the hepatic functionality: its levels rise owing to a moderate negative energetic balance: lactation, low temperatures and high thermal ranges (Campanile et al., 1994). In a recent study Grasso et al. (2004), observed a marked effect of calving distance on this parameter (ranging between 2.05 and 3.01 mmol/l); in the same study the authors showed that housing systems (intensive vs traditional system) did not markedly affect the plasma cortisol level of buffalo cows. Terzano et al. (1997), studying the effect of feeding systems and puberty onset on blood metabolites in buffalo heifers, reported that serum cholesterol level was not affected by the feeding system (1.93 mmol/l) but it was significantly affected by puberty, showing an increasing trend with a significant difference after puberty (1.83 vs 2.03 mmol/l). The higher concentration of cholesterol with the advancement of age is probably a physiological adjustment to meet growth requirements.

Beta-hydroxybutyrate - Acetoacetate, beta-hydroxybutyrate (BOHB), and acetone are collectively called ketone bodies. Acetone is formed by spontaneous decarboxylation of acetoacetate while the first two are synthesized from acetyl CoA, in the mitochondria of liver cells. Acetyl-CoA can be oxidized to carbon dioxide in the citric acid cycle for the production of energy but during periods of increased fat metabolism and decreased carbohydrate metabolism (fasting, early lactation, diabetics), oxaloacetate may not be available for use in the Tricarboxylic acid cycle (TCA) as it is consumed by gluconeogenesis. The liver mitochondria can convert acetyl-CoA to ketone bodies that, unlike fatty acids and triglycerides, are water-soluble and after having been exported from the liver, are taken up by other tissues, notably the brain, skeletal and cardiac muscles; they are the preferred energy source for the heart muscle and the kidney cortex. There, they are broken down to acetyl-CoA which is oxidized via the TCA cycle to yield energy. Under normal physiological conditions, the production of ketone bodies occurs at a relatively low rate. If the production of ketone bodies exceeds the ability of peripheral tissues to oxidize them, the result is a lowering of the pH of the blood, and a high anion gap metabolic acidosis due to an excessive blood concentration of keto-anions. Blood acidification is dangerous, chiefly as it impairs the ability of hemoglobin to bind oxygen. Beta-hydroxybutyrate is stable, and it is usually present in higher concentrations than the other ketones, it is a more sensitive test to use when monitoring for ketoacidosis and it is stable in whole blood for up to 48 hours at room temperature.

Lean et al. (1992) found that in multiparous cows, milk yield was positively associated with BOHB while dry matter intake was negatively correlated with it. Free fatty acids (FFA) are positively associated with BOHB production, glucose concentrations are negatively cross-correlated with BOHB concentrations and the estimated net energy balance is negatively cross-correlated with beta hydroxybutyrate. In buffaloes, Campanile et al. (1997) found an increase in BOHB blood levels when a greater quantity of protein was fed in the diet, because

of a "relative" energy deficiency. BOHB blood levels found in buffaloes during early lactation period have been 2.69 mg/dl in summer and 4.40 mg/dl in winter (Fagiolo et al., 2004). In buffalo calves it is lower until weaning (1.3-2.4 mg/dl) (Cavallina et al., 2003).

Free radicals and antioxidants

Free radicals - Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Free radicals are very unstable and react quickly with other compounds to capture the electron needed for stability. Generally, free radicals attack the nearest stable molecule making it lose its electron and become a free radical itself. A chain reaction begins. Once the process is started, as a cascade, it can finally result in the disruption of a living cell. Their chief danger comes from the possibility of reaction with important cellular components such as DNA or cell membranes. Cells may function poorly or die if this occurs.

Free radicals arise normally during metabolism and sometimes are purposely created by the body's immune system's cells to neutralize viruses, bacteria and parasites. This has been detected in the buffalo species too: after activation with opsonized zymozan or lipopolysaccharide, buffalo polymorphonuclear cells increase H_2O_2/O_2-production (Singh et al., 1997). Some free radical blood levels found in buffaloes are 94.62 U car during the dry milk period and 80-69 U car in lactation during the catabolic and anabolic phase (Fagiolo et al., 2004; unpublished data).

However, environmental factors such as pollution, radiation and herbicides can also spawn free radicals. To prevent free radical damage the body has a defence system of antioxidants.

Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged.
Antioxidative status consists of two mechanisms: nonenzymatic and enzymatic mechanisms. Nonenzymatic mechanisms are composed of antioxidants, scavengers of free radicals, transition metal ions, sequester transition metal ions, albumins, ceruloplasmin, and metallothioneins. The principal micronutrient antioxidants are vitamin E, beta-carotene, and vitamin C. Additionally, these mechanisms depend on the nutritional status of antioxidant minerals, especially copper, zinc, iron, selenium, silicon, and manganese. The nutritional status of cattle in different regions of the world is often characterized by a lack of these minerals; therefore, there is a great potential for changes in the activity of defence mechanisms against free radicals (Kleczkowski et al., 2003). These micronutrients must be supplied in the diet as in buffaloes, vitamin A, beta-carotene and selenium proved to enhance in vitro phagocytic and kill activities of polymorphonuclear leukocytes isolated around parturition (Ramadan et al., 2001). Moreover immunopotentiation in late gestation with vitamin E and Selenium reduced the calving to first oestrus interval and the length of the postpartum service period, shortening also the uterine involution period (Qureshi et al., 1997). Buffaloes show a deficiency in β–carotene, probably due to rapid transformation into vitamin A. Average values of vitamin A and E are 597 g/l and 175 g/l, respectively. They present the same trend with cholesterol, increasing with the distance from calving and reducing after 120 days of lactation (Campanile et al., 1997).

The most important enzymatic mechanisms which protect an organism against oxidative stress are superoxide dismutase (SOD), peroxidase (Px), e.g. glutathione peroxidase (GSH-Px) and ascorbate peroxidase, catalase and glutathione reductase. Their activity depends on many trace elements. Normally, those mechanisms allow the body to handle free radicals, but if antioxidants are unavailable, or if the free-radical production becomes excessive, damage can occur. New and re-occurring metabolic and infectious diseases of cattle emerge when there is a disproportion in the balance between reactive oxygen species and the antioxidative enzymatic barrier (Kleczkowski et al., 2004). Buffalo polymorphonuclear cells (PMN) present oxygen-dependent and oxygen independent antimicrobial systems (Singh et al., 1997).

Reddy et al. (1988) examined Leucocyte-superoxide dismutase (SOD), GSH peroxidase (GSH-Px), GSH-reductase (GR), GSH-S-transferase (GSH-S-t) and arginase in samples from buffaloes infected with Anaplasma marginale: SH S t, GSH- and glutathione-reductase (GR) levels in leucocytes decreased in infected animals suggesting a decline in the efficiency of the GSH-oxidant defence system. SOD levels increased but there was no change in leucocyte-arginase activity due to infection. Infection caused no significant changes in red cell SOD, GSH-Px, GR and GSH. However, GSH-S-t significantly decreased ($P < 0.05$).
Buffaloes suffering from post-parturient haemoglobinuria showed a drastic reduction in the red cells glutathione content compared to healthy control buffaloes. They also exhibited severe hypophosphataemia (Chugh et al., 1998).
Retinol and alpha-tocopherol levels in milk are correlated to the plasma level of triiodothyronine that enhances the transport of both antioxidants through the blood-mammary barrier (Spagnuolo et al., 2003).

Proteic metabolism

Total protein, albumin, globulin - Unlike lipid metabolism, protein metabolism is not markedly influenced by the energy-protein content in diets or by different environmental conditions. When the protein level of the diets is high, animals enhance gluconeogenesis by amino acids from protein degradation; on the contrary, when the protein level of the diets is low, animals reduce production (meat and milk) and afterwards enhance hepatic protein synthesis and the production of microbial protein which may represent a significant part of total amino acid entering the small intestine of host animals. Thus, microbial protein contributes to satisfying the protein requirement of the animal for tissue maintenance and growth and for milk and wool production.
In a previous study Bertoni et al. (1994b), studying the serum emoprotein levels of lactating buffaloes reported no significant variations during lactation. Campanile et al. (1991), setting the metabolic conditions of healthy and affected buffaloes in farms with a high incidence of endometritis, found that the endometritis affected animals tended to have a significant increase of γ-globulins; the latter, therefore, can represent a useful indicator of this pathological phenomena. Montemurro et al. (1997) reported low serum total protein and globulin levels in buffaloes before calving and after 45 days of lactation. The same authors reported high serum albumin levels at the end of pregnancy, while the serum total protein levels have been found to show very little variation during lactation, as reported by Campanile et al. (1997). Montemurro et al. (1995a) showed that, in heifers, different seasons and feeding did not markedly affect the serum total protein, albumin and globulin levels. On the contrary, Setia et al. (1992) and Campanile et al. (1994) found a significant effect of feeding systems on serum total protein levels. In the study of Terzano et al. (1997), feeding systems affected serum total protein levels as heifers fed natural pasture benefited from more protein than the heifers bred in the feedlot and fed maize silage ad libitum (69.4 g/l vs 73.2 g/l, respectively). The same authors reported serum total protein levels to be significantly affected by puberty, showing an increasing trend with significant differences after puberty (69.0 g/l vs 73.6 g/l). Zia-Ur-Rahman et al. (1997), in a study on hormonal and haematological profiles in buffaloes after transport, handling and slaughter stress, found higher serum total protein and albumin levels after transport, higher serum total protein after handling, higher serum albumin levels and lower serum globulin levels after slaughter.

Urea - The concentration of urea in the blood reflects the degree of protein catabolism and is synthesized in the liver from CO2 and NH3. Serum blood urea levels are influenced not only by renal function but also by external factors. In fact, when the protein level of diets is excessive or when the energy/protein ratio is very low an insufficient ruminal protein synthesis follows; a high quantity of ammonia is formed and absorbed through the ruminal wall into the portal blood and is converted to urea in the liver. This, in the long run, may cause hepatic failure and degeneration causing infertility, mastitis, puerperal collapse, lameness and steatosis. When the scarcity of protein is heavy and lengthened, the protein synthesis is reduced and changes in haematochemical parameters and in productive and/or reproductive functions follow. The

values of serum urea are also considered as indicators of stress conditions as they rise after a too high protein degradation under the stimulus of adrenalin and cortisol hormones (Maianti et al., 1990). In buffaloes serum urea levels rise during lactation, whether by high protein intake or by tissue protein mobilization. In fact, Montemurro et al. (1997) found that serum urea levels in buffaloes rose from a basal value of 5.48 mmol/l (before calving) and of 5.15 mmol/l (at 45 days of lactation) to a peak of 10.29 mmol/l (at 160 days of lactation). Bertoni et al. (1994b) reported urea values of 5.48 mmol/l during the dry milk period and 6.14 mmol/l during lactation. In buffalo cows, as well as in dairy cows, dietary protein characteristics and protein/energy (P/E) ratio influence urea levels in blood serum and milk (Campanile et al., 1996). As the buffalo cow adapts itself better to a lack of protein than the dairy cow, Bertoni et al., 1993 and Campanile et al. (1996), reported low values of serum and milk urea levels, compared to the cow, when the protein content in the diet decreased from 12 percent to 9 percent. The same authors found that longterm diets with protein concentrations of less than 9 percent did not determine a further lowering of serum urea levels, but rather caused an increase in the milk freezing point, especially if the diet showed a high amount of fermentable energy. It would seem relevant, therefore, to evaluate the protein/energy ratio in order to obtain a correct diet. In buffalo such a ratio can be increased: diets with a high protein concentration, in fact, determine less harmful effects in buffalo compared to that in bovine milk cows. Buffalo cows make better use of ingested nitrogen than bovine cows, even if there is a carbohydrate deficit, since buffalo ruminal milieu is more favourable to the growth of microrganisms, using non proteic nitrogen (Langer et al., 1969). Serum and milk urea levels are also affected by the crude protein/non structural carbohydrate (CP/NSC) ratio in buffalo cows. Specifically, Smith (1969) reported that nitrogen deficiency, in ruminants living in tropical areas, reduces renal clearance of urea increasing its return to the rumen and decreasing its haematic levels. This, in turn, would promote a better urea utilization in the digestive tract and a better protein synthesis by ruminal bacteria (Houpt, 1970).

Serum blood urea is influenced by the days in milk (Campanile et al., 1997; Grasso et al., 2004), by the diet (Campanile et al., 1997) and by the season (Satriani et al., 2001). The values of serum blood urea are considered to be an indicator of total protein intake and its determination with creatinine is important in order to exclude renal damage, which is fairly frequent in this species.

In the study of Terzano et al. (1997), the feeding system affected the serum blood urea levels as heifers fed natural pasture benefited by more protein than heifers bred in the feedlot and fed maize silage ad libitum (9.82 mmol/l vs 3.60 mmol/l). The same authors reported that serum blood urea levels were not significantly affected by puberty (mean value: 6.71 mmol/l).

Blood serum enzymes activity

The high variability in the blood activity of serum enzymes suggests considering age, physiological conditions and lactating period in establishing reference values (Pizzuti and Salvatori, 1993).

Asparagine aminotransferase (AST or GOT) - This enzyme transfers the amminic group from aspartate to -ketoglutaric acid, forming glutamate and oxaloacetate. In ruminants, AST is present in greater concentrations in the muscles and heart as opposed to the liver, and increases in serious cases of prolonged fasting and infectious, infestious and nutritional liver disease. In fact it is localized at the mitochondrial level, so that it increases only in the case of extensive hepatic necrosis. It can be referred to muscular cells damage and tissue changes related to the neonatal phase in buffalo calves (Campanile et al., 1997). In buffalo heifers near to puberty, values of 216 U/l have been found (Borghese, 1994). Finally in adult buffaloes AST varied from 120 to 145 U/l in the dry milk and lactating period, respectively; higher levels were measured at 30 and 190 days of lactation (Bertoni et al., 1994a). In the early months of lactating, De Rosa et al. (2001) detected values ranging from 143 to 160 U/l. Decreasing metabolism is linked to lower AST hematic concentrations, while thyroid hormones,

particularly T4, induce a greater emission of the enzyme increasing mitochondria membrane permeability (Campanile et al., 1997).

Alanine aminotransferase (ALT or GPT) - This enzyme transfers the aminic group from alanine acid to ketoglutaric acid, forming glutamate and piruvate. In ruminants, ALT is present in small quantities in the liver and in various tissues, particularly in the muscles. It is in fact referred to, together with AST, as an index of muscular integrity. In buffalo heifers Borghese (1994) found values of 60 U/l, in adult buffaloes at different pre- post- partum time intervals, Pizzuti and Salvatori (1993) found ALT values ranging from 176 to 219 U/l. In the early months of lactation, De Rosa et al. (2001) detected values ranging from 83 to 116 U/l. ALT and AST, together with hemoglobin, increased in autumn, in buffaloes fed at pasture until early autumn. Decreasing metabolism is linked to lower ALT hematic concentrations, while thyroid hormones, particularly T4, induce a greater emission of the enzyme, increasing mitochondria membrane permeability (Campanile et al., 1997).

g-glutamyltransferase (GGT) - GGT is a membrane linked enzyme that transports amino acids inside the cells. It is present in various tissues in the kidney, pancreas, mammary gland, liver and so on. In water buffalo mature females, the enzyme GGT was significantly higher compared to that in immature females (Canfield et al., 1984). This has been verified by Borghese (1994) who found values of 24.7 U/l in buffalo heifers, and by Bertoni et al. (1994a), who recorded, in adult buffaloes, a GGT variation from 29 to 35 and 41 U/l in the dry milk period and 30 and 230 days of lactation, respectively. In the early months of lactation, De Rosa et al. (2001) detected values ranging from 37 to 52 U/l. It is an important index in liver diseases as it is the first serum enzyme that increases even in mild liver disease. GGT decrease during the growth period has been recorded from 35 U/l (at 30 days) to 18 U/l (at 50 days of age). This progressive lowering in GGT and g-globulines values, after the increase due to absorption during the colostral phase, is an index of good liver and kidney function (Campanile et al., 1997).

LDH - This is a blood test that measures the amount of lactate dehydrogenase (LDH). LDH is a cytoplasmic enzyme that converts piruvic acid into lactic acid. The enzyme comes from the myocardium, skeletal muscles, liver, kidneys, pancreas, red blood cells and the lungs. In adult healthy buffaloes, LDH values (U/l) ranging from 1272 to 1741 and from 713 to 1047, have been found in different housing and seasonal conditions (Terzano et al., 2000; Fagiolo et al., 2004). Lower values have been found for calves: 1325 UI/l (Cavallina et al., 2003, unpublished data). Higher-than-normal levels may indicate: intestinal ischemia (blood deficiency) and infarction (tissue death); liver disease (for example, hepatitis); muscle injury; muscular dystrophy; neoplastic (new abnormal tissue formation) states; pancreatitis; pulmonary infarction (tissue death); heart attack; hemolytic anaemia; hypertension. In the neonatal phase, the growth and changes that involve a turbulent synthesis activity, are responsible for the progressive increase of the LDH serum levels in buffalo calves. In adult buffaloes, higher LDH values are a proof of hepatic sufferance induced for example by acidosis, that causes a greater effort to be made by this organ in transforming lactic acid into propionic acid (Campanile et al., 1997).

Alkaline phosphatase - ALP is an enzyme made in the liver, bone, and the placenta and is normally present in high concentrations in growing bone and in bile. The enzyme is termed alkaline phosphatase because it works under alkaline (non-acidic) conditions, as opposed to acid phosphatase. Alkaline phosphatase is released into the blood during injury and during such normal activities as bone growth and pregnancy. Abnormally high blood levels of ALP may indicate disease in the bone or liver, bile duct obstruction, or certain malignancies. However, higher values are found in buffaloes during the first 40 days of life due to the more intense bone remodelling. An increase of ALP during early lactation in buffaloes is proof of speedy parathyroid activation (Campanile et al., 1997). Pizzuti and Salvatori (1993) found differences in the ALP mean values of buffaloes at various distances from partum, increasing in the advanced phases of pregnancy and decreasing before parturition; they ranged from 159 to 228 U/l. Terzano et al. (2000) reported ALP values ranging from 200 to 650 U/l, in adult

buffaloes under different housing conditions.

Micro - and macroelements

The body uses over 80 minerals for its maximum function. Every living cell depends on minerals for its proper structure and function. Nutritionally, minerals are grouped into two categories: bulk or essential minerals, also called macrominerals, and trace minerals or microminerals. Macrominerals such as calcium and magnesium are needed by the body in larger amounts. Although only minute quantities of trace minerals are needed, they are nevertheless important for good health. Microminerals include boron, chromium, iron, zinc, and many others. Essential trace elements range from metals to non-metals. During the buffalo dry period, even minimum but long-term deficiencies can cause damage that will influence the health state of the following lactation (Campanile et al., 1997).

Ca - Most calcium in the body, about 90 percent, is in the bones, where it can be reabsorbed by blood and tissue, but about one percent is used for nerve impulses and muscle contractions (including the heart, kidney, and other organs). Calcium participates in the protein structuring of RNA and DNA, and also contributes to the formation of intracellular cement and cell membranes. It helps in normalizing blood clotting action, to metabolize the body's iron and it is more effective when combined with: vitamins A, C and D. Buffalo calcium blood levels show limited variability during lactation and dry milk period (Bertoni et al., 1994a); higher levels have been found in the last month of pregnancy and lower ones at the end of the lactation period (Montemurro et al., 1997). A seasonal variation has been evidenced with higher values during the winter. Ca/P ratio is maintained at 1.4-1.6 (Montemurro et al., 1995a). Campanile et al. (1997) found constant values of about 10 mg/dl, confirmed by Terzano et al. (2000) who found 8.87-10.63 mg/dl of Ca blood levels in adult buffaloes. In buffalo species calcium excesses could alter the Ca/P ratio during the dry milk period, inducing parathyroid hypoactivity which would cause magnesium to increase and calcium to decrease at the beginning of the lactation due to a non immediate calcium mobilization by the bones. The altered Ca/Mg ratio favours utero-vaginal muscular release, responsible for uterus atony and eventually uterine prolapse (Campanile et al., 1997).

Phosphorus - Phosphorus is the second most plentiful "essential mineral" in the body and is a key component of DNA, RNA, bones, teeth, and many other compounds required for life. It plays an important role in the energy metabolism of cells, affecting carbohydrates, lipids and proteins. Phosphorus also stimulates muscle contraction and contributes to tissue growth and repair, nerve-impulse transmission, central nervous system health, and proper heart and kidney function. Phosphorus deficiency during buffaloes' dry milk period is responsible for the most frequent causes of vaginal and/or uterine prolapse in this species. If the diet is deficient in P before calving, calcium levels decrease upon calving while phosphatemia is normal (Campanile et al., 1997). On the other hand, diets rich in silage and/or concentrates (>59 percent), have been associated with high P and Cu hematic values and subclinical metabolic acidosis, that is frequently connected with uterine prolapses and endometritis (Campanile et al., 1997). Phosphorus levels in buffaloes have been found to be quite stable at six mg/dl (Campanile et al., 1997). An increasing trend has been evidenced starting from the pre-partum period (6.3 mg/dl) to 160 days of lactation (7.9 mg/dl) (Montemurro et al., 1997).
In water buffalo mature females, inorganic phosphate was significantly higher compared to that of immature females (Canfield et al., 1984). Buffalo heifers showed higher P blood levels in winter (Montemurro et al., 1995a). Buffaloes suffering from post-parturient haemoglobinuria showing a decrease in the reduced glutathione content in the red cells, also exhibited severe hypophosphataemia (Chugh et al., 1998).

Potassium - Potassium is the third most abundant mineral in the body, after calcium and phosphorus. Potassium works closely with sodium and chloride to maintain fluid distribution and pH balance and to augment nerve-impulse transmission, muscle contraction, and regulation of heartbeat and blood pressure. Potassium is also required for protein synthesis,

carbohydrate metabolism, and insulin secretion by the pancreas. It works with sodium to regulate the body's water balance. Deficiencies are rare in ruminants while excesses in the diet can reduce Mg, Ca and P digestion. Low blood levels have been observed in cattle fed with high concentrate levels and have also been associated with stress conditions. In buffaloes physiologic values range from 4 to 5 mmol/l (Bertoni et al., 1999).

Sodium - Sodium is one of the three main electrolytes in the body. All body fluids - including blood, tears, and perspiration - contain sodium. Together with potassium and chloride, sodium maintains fluid distribution and pH balance; together with potassium, sodium also helps in the control of muscle contraction and nerve function. Blood levels are strictly controlled as a result of the surrenalic hormone aldosterone, so alterations are usually only possible in cases of homeostasys problems. Buffaloes' hematic values are 144-146 mmol/l during the dry milk period and 143-147 mmol/l in early lactation (Bertoni et al., 1999).

Magnesium - Magnesium is involved in more than 300 enzymatic reactions. Magnesium is essential for the conversion of vitamin D to its biologically active form which helps the body absorb and use calcium. The highest magnesium concentration is found in the tissues that are most metabolically active including the brain, heart, liver and kidney. Magnesium is a key substance in the proper functioning of nerves and muscles. It is also required for the healthy maintenance of bones. Mg deficiency is well known as grass tetany, while the excess during post partum puerpueral collapse seems to be caused by renal failure. Physiologic values in buffaloes are 1.1-1.3 mmol/l in the dry milk period and 1.2-1.4 mmol/l during lactation (Bertoni et al., 1999).

Iron - Iron mainly works in the red blood cells hemoglobin, which transport oxygen from the lungs to the body's tissues, including the muscles and the brain. Iron is also a component of myoglobin, a similar protein in the muscle, that stores and provides oxygen during muscle exertion and is found in the part of the cell involved in energy production and as a co-factor for several enzymes. The diet of ruminants usually provides enough iron and it is well utilized in digestion (Bertoni et al., 1999). Iron deficiency generally occurs during the growth period or when intakes fail to replace the iron loss associated with blood losses. Excessive amounts of phosphates, calcium, zinc and manganese can also inhibit iron absorption. When iron stores are depleted and there is an inadequate production of heme (the portion of hemoglobin associated with iron), the red blood cells become small (microcytic) and have a decreased capacity to carry oxygen. There is also a drop in iron-containing enzymes that are important in cellular metabolism. This results in decreased work capacity, fatigue, paleness, dizziness, sensitivity to cold, irritability, heart palpitations and altered behaviour.
Because iron strengthens the immune function, its deficiency may also increase susceptibility to infection. Iron is also an important nutrient for bacteria, that is why one of the body's natural defence mechanisms during infections is to reduce plasma iron, in order to inhibit bacterial growth. Cavallina et al. (2003) found mean iron blood levels in buffalo calves at one and three months of age of 96 and 143 mmol/l, respectively (unpublished data). In lactating buffaloes Fagiolo et al., (2004) found seasonal differences in iron blood levels: higher in winter than in summer (150.07-61.81 mmol/l) though not significantly. Iron blood level analysis can be altered by different factors, it is sometimes preferable to evaluate it indirectly by means of hemoglobin and packed cell volume.

Selenium - Selenium is an essential non-metallic element. Selenium is important for the function of several proteins. One of these is glutathione peroxidase, an enzyme that prevents cellular oxidative damage from a variety of peroxides. It is said to stimulate the metabolism. Together with vitamin E, it is extremely important in preventing free radical damage to cell membranes.
Selenium also supports the immune function and neutralizes certain poisonous substances such as cadmium, mercury, and arsenic. Selenium proved to enhance phagocytic activity in buffalo polymorphonuclear leukocytes starting from parturition up until three weeks post-partum (Ramadan et al., 2001).

Animals grown for the purposes of meat production, in areas with soil deficient in selenium, develop "white muscle disease." Selenium was not detectable in many pregnant buffalo cows suggesting that the prevention of myodistrophy in buffalo calves must be effected in the prenatal period (Pizzuti and Salvatori, 1993).
Symptoms of selenium deficiency include muscle weakness and pain, inflammation of the muscles, fragile red blood cells and degeneration of the pancreas.
Animals grazing on plants that have accumulated selenium show acute or chronic selenium poisoning. Chronic selenium toxicity (alkaline disease) is characterized by muscle degeneration, rough coat, laboured breathing and cardiovascular failure. Selenosis induced in male buffalo calves was associated with an increased activity of the erythrocyte glutathione peroxidase (GSH-Px) and signs of selenium toxicity at Se levels of about 2.0 µg/ml (Deore et al., 2002).

Zinc - Zinc is a part of every cell in the body and forms part of over 200 enzymes that have functions ranging from proper action of body hormones to cell growth.
Zinc is an extremely important mineral for many body functions, down to the very core structure of cells. Zinc is integral to the synthesis of RNA and DNA, the genetic material that controls cell growth, division and function. In various proteins, enzymes, hormones, and hormone-like substances called prostaglandins, zinc contributes to many body processes such as: bone development and growth, development of the testicles, skin integrity, appetite, aiding enzymes in digestion and energy metabolism, cell respiration; wound healing; the liver's ability to remove toxic substances from the body; immune function and the regulation of heart rate and blood pressure. Zinc is a co-factor for many enzymes, which means that it is necessary for the proper functioning of these enzymes. ALP is rapidly inhibited by Zn deficiencies.
Zinc is a critical nutrient of immunity, being involved in so many immune mechanisms including cell-mediated and antibody-mediated immunity, thymus gland function and thymus hormone action. When zinc levels are low, the number of T cells is reduced and many white blood functions critical to the immune response are severely lacking. Like vitamin C, zinc also possesses direct antiviral activity, including activity against several viruses. It is also present in members of a class of proteins called the metallothioneins that are believed to provide antioxidant protection by scavenging free radicals. In buffaloes it normally ranges from 10 to 12 µmol/l in the dry period and from 9 to 12 µmol/l during lactation (Bertoni et al., 1999).
Zinc deficiency may be associated with long-term hypo-nutrition, higher requirements during pregnancy or diseases of the intestine such as paratuberculosis. Excessive zinc interferes with the function of copper and iron.

Hemogram (CBC)

This test evaluates the number and status of red blood cells, white blood cells, and platelets. It screens for anaemia, leukemia, polycythemia, and other disorders that affect blood cells. Buffalo haematological values are comparable with those found in adult cattle (Ciaramella et al., 2005).

Hematocrit (HCT) - This test, also called packed cell volume (PCV), measures the amount of space (volume) red blood cells occupy in the blood. The value is given as a percentage (%, vol/vol) of red blood cells in a volume of blood. For example, a hematocrit of 38 means that 38 percent of the blood's volume is composed of red cells. It is the quickest and most accurate measure of the red cell component of blood. PCV is higher in heifers than in adult buffaloes (Ciaramella et al., 2005). It has shown seasonal differences in lactating buffaloes, proving to be higher in the summer (40.75 percent) than in the winter (32.63 percent) (Fagiolo et al., 2004).

Hemoglobin - Hemoglobin is the major substance in red blood cells. It carries oxygen and gives the red colour to blood cells. The hemoglobin test measures the amount of hemoglobin in blood and is a good indication of the blood's ability to carry oxygen throughout the body. Hemoglobin concentration (Hb) is reported as grams of hemoglobin per decilitre of blood (g/dl). In lactating buffaloes, Hb was found to be higher in the summer (13.62 g/dl) than in the winter (11.37 g/dl)

(Fagiolo et al., 2004).

Mean Corpuscular Volume (MCV) - MCV measures the average size of red blood cells. The mean cell volume indicates the volume of the "average" red cell in a sample. It is expressed in femtolitres (fl; 10-15 litres). In lactating buffaloes mean values in different seasons were about 53 and 56 fl, without significant differences (Fagiolo et al., 2004).
Red cell populations with an MCV above reference range are termed macrocytic; if the volume is normal: normocytic. Common causes of macrocytosis are reticulocytosis and myelodysplastic syndrome (MDS). Common causes of microcytosis are iron deficiency anaemia and chronic liver disease.
A regenerative response can be seen as a population of macrocytic hypochromic cells extending off the normal red cell population. Agglutination can be seen as a macrocytic normochromic cluster of cells that is discrete from the normal red cell population.

Mean Corpuscular Hemoglobin (MCH) - The MCH value is the amount of hemoglobin in an average red blood cell. MCH is the mean cell hemoglobin. This represents the absolute amount of hemoglobin in the average red cell in a sample. Its units are picograms (pg; 10-12 liters) per cell. In lactating buffaloes mean values in different seasons were about 17.8 and 19.5 pg, without significant differences (Fagiolo et al., 2004). It is normally lower in heifers (Ciaramella et al., 2005); in adults a low MCH could be due to smaller than normal cells with normal Hb concentration or normal sized cells with lower than normal Hb concentration. It is preferable to have exact information regarding cell volume and Hb concentration directly.

Mean Corpuscular Hemoglobin Concentration (MCHC) - The MCHC measures the concentration of hemoglobin in an average red blood cell. MCHC is the mean cell hemoglobin concentration, expressed in g/dl. In lactating buffaloes mean values in different seasons were about 33.5 and 34.8 g/dl, without significant differences (Fagiolo et al., 2004). It is lower in heifer buffaloes (Ciaramella et al., 2005). Red cell populations with values below the reference range can be termed "hypochromic". This can occur in strongly regenerative anaemia, where an increased population of reticulocytes with low Hb content "pull" the average value down. Low MCHC can also occur in iron deficiency anaemia, where microcytic, hypochromic red cells are produced as a result of the lack of iron to support hemoglobin synthesis. Values for MCHC significantly above the reference range are not physiologically possible due to limitations on the solubility of Hb. Lipemia or other causes of turbidity in the lysate can cause falsely high values, which raises the apparent MCHC. Furthermore, hemolysis (in vitro or in vivo) will cause a lowering of the HCT and increase the MCHC.

Red Cell Distribution Width (RDW) - The RDW is an index of the variation in cell volume within the red cell population. The RDW indicates whether all the red cells are about the same width, size, and shape. This helps further classify the types of anaemia.
Red cell populations with higher than normal RDW are termed heterogenous; those with normal RDW are homogeneous. For example, increased numbers of reticulocytes will cause an increased RDW. In some instances, the RDW is the first test result to increase with changes in red cell population sizes. For example, in early iron deficiency, there are only low numbers of microcytic red blood cells. This will increase the standard deviation and the RDW, but the mean cell volume is unchanged because there are insufficient numbers of microcytic cells to change the mean volume. In lactating buffaloes mean values in different seasons were about 17.5 and 16.2 percent, without significant differences (Fagiolo et al., 2004).

Red Blood Count - The red blood cell count on the routine CBC is the concentration of red cells, expressed in millions/µL of whole blood (106/µl). In lactating buffaloes this parameter showed seasonal variations from $7.7\text{-}10^6$/µl in summer to $5.8\text{-}10^6$/µl in winter (Fagiolo et al., 2004).

White Blood Count - White blood cells protect the body against infection and are bigger than red blood cells and normally fewer in number. In case of bacterial infection, the number of

white cells can increase dramatically, so the number of white blood cells is sometimes used to identify an infection White cell count (WBC), the total number of leukocytes in a volume of blood, is expressed as thousands/µl (106/µl). In buffaloes above eight years of age it is lower (Ciaramella et al., 2005).

Neutrophils - Cells of the neutrophil line are classified by the shape of their nuclei. Cells with nuclei whose sides are parallel, or nearly so, or that have smooth nuclear outlines are classified as band neutrophils. Band neutrophils and metamyelocytes are blood neutrophils less mature than segmented neutrophils. Segmented neutrophils have nuclei with focal areas that are distinctly narrower than the width of the widest points and usually have irregular nuclear outlines. Fagiolo et al. (2004) found seasonal changes in early lactating buffaloes neutrophil percentage: from 64 percent in summer to 7 percent in winter.

Lymphocytes - Lymphocytes are the most numerous cell type in the buffalo species. Characteristic features include a dense, round nucleus and a scant rim of pale blue cytoplasm. Healthy ruminants have a wide range of lymphocytes in peripheral blood; many are quite large. In addition, in all species, there are low numbers of lymphocytes with small red cytoplasmic granules, so-called granular lymphocytes. These are either natural killer cells or cytotoxic T-lymphocytes and are involved in cell-mediated immunity. Lymphocytes, unlike the other leukocytes, are produced in lymphoid tissue and in bone marrow. Most of the lymphocytes in blood are long-lived cells that recirculate between blood and tissue. Changes in blood lymphocyte number usually reflects changes in distribution rather than changes in production or loss. Early lactating buffaloes showed a decrease in lymphocytes during the summer (41 percent) with respect to winter values (77 percent) (Fagiolo et al., 2004). In buffaloes above eight years of age there is a significant reduction in the absolute values of lymphocytes (Ciaramella et al., 2005).

Monocytes - Monocytes share a common committed stem cell with neutrophils. They are produced in the marrow, circulate briefly in the blood, and migrate into the tissues where they differentiate further to become macrophages.
There is no storage pool of monocytes in the marrow; their numbers in the marrow at a given time are very small. Monocytes in blood are distributed between a marginated and circulating pool. Cytokines (e.g. M-CSF, GM-CSF) produced at sites of inflammation can increase monocyte production. In buffaloes mean values are usually near to 0 percent (Fagiolo et al., 2004).

Eosinophils - Eosinophils are produced in the marrow, circulate in the blood for a few hours, and migrate into the tissues where they survive for several days. Increased production of eosinophils is mediated by interleukin-5 and interleukin-3, which are produced by several cell types, but especially T lymphocytes and mast cells. Corticosteroids decrease the blood eosinophil number but increase the marrow pool of eosinophils.
Increased numbers of circulating eosinophils may be seen in hypersensitivity reactions, as with certain forms of parasitism and allergic conditions. The presence of mast cell tumours in an animal may also be associated with eosinophilia. Increased numbers of basophils (basophilia) sometimes occurs concurrent with eosinophilia. Fagiolo et al. (2004) found the following mean percentage values for early lactating buffaloes in different seasons: 1.64 - 0.16 percent. In water buffalo mature females, eosinophils were significantly higher compared to those in immature females (Canfield et al., 1984) and buffaloes over ten years of age show higher absolute values of eosinophil levels (Ciaramella et al., 2005).

Basophils - Basophils, in general, contain dark purple granules in the cytoplasm. Basophils are produced in the marrow. The number in the blood is very small in all species as in the buffalo species (Fagiolo et al., 2004).

Platelet Count - Platelets (thrombocytes) are the smallest type of blood cell. They play a major role in blood clotting. When bleeding occurs, the platelets swell, clump together, and form a sticky plug that helps stop the bleeding. If there are too few platelets, uncontrolled bleeding

may be a problem. If there are too many platelets, there is a risk of a blood clot forming in a blood vessel. Platelet counts can be performed manually or using automated cell counters. Platelet clumping lowers the platelet count when determined by any method. Platelet clumping is usually due to a sample collection problem and can be minimized by collecting blood from a large peripheral vein (jugular). The blood should be mixed with an anticoagulant as soon as possible after collection, by gentle rotation or inversion. Platelet clumping increases with time, so platelet counts should be done as soon as possible after collection to maintain accuracy. In lactating buffaloes mean values in different seasons were about 251.8 and 201 10^3/µl, without significant differences (Fagiolo et al., 2004).

Mean Platelet Volume (MPV) - Individual platelets can vary markedly in size within a given sample. Little is documented in literature regarding the clinical interpretation of this parameter. In very general terms, increased MPV might be expected in "regenerative" thrombocytopenia, i.e., that caused by increased peripheral loss, destruction, or utilization of platelets and accompanied by increased production of platelets by marrow (megakaryocytic hyperplasia). Accelerated thrombopoiesis tends to result in the release of larger platelets (which also have enhanced functional capabilities). Fagiolo et al. (2004) found the following mean values in early lactating buffaloes in different seasons: 8.8 and 9.7 fl.

The immune system

The immune system is an incredibly intricate mechanism that prevents infections and diseases by moderating malignant and foreign cells within the body. To evaluate the immune responses of water buffalo to infectious agents and potential vaccines, it is necessary to characterize the immune system and elucidate the changes in the immune response that account for the development of protective immunity (Davis et al., 2001).
The immune system shows two types of response: non-specific (innate) and specific (adaptive/acquired).
The first one mainly depends upon monocytes, neutrophils (granulocytes, eosinophils, basophil), complement, interferon and lysozyme activity whereas the second one relies on T-cells and B-cells production. The immune system is composed of several organs and systems, as well as various types of immune cells. Organs that constitute the immune system - known as lymphoid organs -include the spleen and the thymus. Additional components of the immune system are lymph nodes and bone marrow. Within the bone marrow, lymphocytes (white blood cells) are created which function as immune cells. Neutrophils are the main circulating white blood cells, which seek out invaders when they are summoned into action by other immune cells. They secrete toxins that kill antigens (invading proteins) and devour them. The two major classes of lymphocytes are B-cells, which mature in the bone marrow and reside in the lymph system, and T-cells, which mature in the thymus and circulate throughout the body. B-cells are responsible for producing antibodies (immunoglobulins), which are proteins designed to recognize and mark a specific antigen, while T-cells are charged with destroying antigens that are tagged with an antibody.
There are three types of T-cells: cytotoxic T-cells, helper T-cells and suppressor T-cells. Cytotoxic T-cells attach themselves to malignant or infected cells. They secrete interferons, which stop viruses from reproducing. Helper T-cells including TH1 and TH2 assist cytotoxic T-cells by recognizing an attack on the body, at which point cytotoxic T-cells are sent to fight the infection. Helper T-cells also help the body's B-cells produce antibodies. Suppressor T-cells are responsible for regulating the body's immune response to invasions; they stop cytotoxic T-cells from releasing cytokines (immuno-regulatory substances) and prevent the production of immunoglobulins. Five types of immunoglobulins are normally distinguished but in water buffalo and in the cow four classes exist.

IgA, IgG1 and IgG2, IgM and IgE - IgA is responsible for holding off invaders or pushing them out of the body, which is why it exists in tears, milk, sweat and saliva. IgG is specifically designed to kill certain bacteria and viruses, and activates enzymes that digest invaders. Tripaldi et al., (2003) reported an IgG titre of 0.07 ± 0.03 OD_{450} in adult buffalo cows.

IgM circulates in the blood stream to kill bacteria. IgE plays a remarkable role in ruminant colostrum, since this class is transmitted to newborn calves during the first days of lactation providing a passive immunity against several parasite species.
Additional immune cells are scavenger cells called phagocytes which include granulocytes and macrophages. These cells seek out and devour invading cells. Macrophages are usually stationary and protect a specific area, although they are also known to travel to a point of infection to assist in warding off an antigen. They release pyrogen, a substance that alerts the body to increase temperature to induce fever, which is often useful for killing pathogens. Another scavenger, the natural killer (NK) cell, destroys invaders without seeking out B-cells' tags. NK cells search for foreign cells and kill them by releasing toxic enzymes and interferons. Just as NK cells secrete interferons, other immune cells secrete interleukins, another type of cytokine, or immuno-regulatory substance.

These substances, which include monokines (secreted by monocytes) and lymphokines (secreted by lymphocytes), are responsible for regulating the body's immune response, such as the magnitude of an inflammation. Interleukins, which are secreted by macrophages, monocytes and some T-cells, include more than 30 types. Interleukin-1 is produced by macrophages and is involved in inducing fever, which can kill or slow down a virus or bacterium. Interleukin-2 assists helper T-cells in encouraging cytotoxic T-cells to kill invaders. Interleukin-4 enhances the B-cells' ability to produce antibodies (IgG and IgE in particular), and it stimulates helper T-cells and cytotoxic T-cells. Interleukin-6 is released by macrophages, monocytes and some T-cells, and induces B-cells to produce antibodies. Tumour necrosis factor (TNF) is released by macrophages to induce fever. It can kill cancer cells and promotes the production of lymphokines. With all of these cells and processes for fending off invading microbes, fungi, bacteria and viruses, the immune system has its work cut out for it, and there are numerous external factors that can disrupt the body's process for fighting off infection such as anxiety and depression, as well as malnutrition. Since malnutrition adversely affects immune function, ensuring proper nutrition through the diet is essential for protecting and maintaining the immune system. In addition to nutrient intake, several botanical and nutritional substances work to enhance the various activities that make up immune function (Abbas-Lichtman, 2000). Analysis of leukocytes in peripheral blood of young and adult buffaloes has revealed that the composition of leukocyte populations in water buffalo is similar to that in cattle. One of the unique features to emerge from the study of the immune system of cattle is the presence of two complex sub populations of gdT cells, one sub population that is similar to gdT cells as observed in humans and other species and a second sub population that has only been identified in sub orders of Artiodactyla, Ruminantia, Suiformes, and Tylopoda. As in cattle, the WC1+ population of gdT cells in buffaloes was comprised of subsets that express the WC1-N3 and WC1-N4 isoforms. The frequency of WC1+ gdT cells was high in young animals and low in adults. There were corresponding differences in the frequency of CD2+ abT cells in young and adult animals. There was no apparent correlation in the frequency of B cells with the frequency of WC1+ gdT cells in young and adult animals (Davis et al., 2001). Recently, Tripaldi et al., (2003), studying the effect of two different housing systems on a range of behavioural and physiological variables, found no difference in the immune response following a percutaneous injection of phytohaemagglutinin (7.73 ± 0.4 mm - 6.32 ± 0.4 mm); 40 days after injection, the IgG titre increased from 0.07 ± 0.03 OD_{450} to 1.33 ± 0.03 OD_{450}.

Lysozyme - Lysozyme is a protein present in many tissues and secretions which was able to interfere with the growth of some specific bacterial colonies. This lysing element was called "lysozyme" by Fleming himself who went on to study its different characteristics and in 1922 isolated the enzyme from hen egg white, other tissues and biological secretions of living organisms. Some years later the bactericidal activity of lysozyme was widely substantiated and after 1930 many studies revealed how in nature every living organism, both in the animal and the plant kingdoms, produces lysozyme. The term "lysozyme" (or rather lysozymes considering their ubiquity and their various structural differences) refers to an enzyme with well-defined hydrolasic activity. In nature different types of lysozyme exist with different characteristics according to their origin. It is difficult to distinguish between human lysozyme, which is

contained in various secretions such as tears and saliva, and the lysozyme present in products belonging to the animal and vegetable kingdom. In animal serum lysozyme destroys bacteria, mostly Gram positive. In fact, this enzyme preferably hydrolyzes the b1,4 glucosidic linkages between N-acetylmuramic acid and N acetylglucosamine which occur in the mucopeptide cell wall structure of Gram positive microorganisms. An elevated level of serum lysozyme signifies a good non specific immune response. Cavallina et al. (2003) found a decreasing trend in buffalo calves' lysozyme from the first to the third month of age and following weaning, with mean values ranging from 3.8 to 0.33 µg/ml. In water buffalo during lactation, Fagiolo et al. (2004) detected seasonal differences with higher lysozyme levels in summer (2.7 µg/ml) than in winter (0.84 µg/ml).

Bactericide activity - Among aspecific immune responses there is bactericide activity. This particular serum activity permits the elimination of some antigens, mostly Gram negative microrganisms.
Furthermore, these antibodies may be prevalently produced from birth as a response to stimulations, both from intestinal flora and heterogenic antigens of non-bacterial origin.
Naturally the defensive power and therefore the natural resistance of an organism during first infection, depend upon the casual affinity that the pathogenic strain shows towards this antibody. In any event these antibodies show their best disposition against Gram negative bacteria.
The bactericide test is based on the principle that exposing numerous Gram negative strains to human and animal serum cause the lysis of a percentage of germs. In this procedure, serum samples are tested with E. coli culture, in S phase and incubated at 37°C for four hours.
Serum bactericide activity is determined by measuring the percentage of eliminated microrganisms and comparing these results with the starting culture percentage (Poli, 1996). In buffalo calves bactericide activity significantly increases from 41 percent in the first month of age to 64 to 68 percent over three months (Cavallina et al., 2003). In lactating buffaloes Fagiolo et al. (2004) did not find any seasonal variation with mean values ranging from 52 to 56 percent in winter and summer, respectively.

Electrophoresis

Electrophoresis is the migration of charged molecules like proteins in an electrical field. The separation of proteins in an electrical field is based on the size, shape, and charge (Figure 2). The charges are provided by the side chains of the amino acids of which the proteins are composed. The charge of the protein depends on the pH of the protein and the pH of the surrounding buffer. Most electrophoretic methods use a supporting media, such as starch, paper, polycrylamide, or Agarose. It should be remembered that the actual environment through which the proteins migrate is composed of a 50 percent buffer solution. The term zone electrophoresis refers to electrophoresis which is carried out in a supporting medium, whereas moving-boundary electrophoresis is carried out entirely in a liquid phase. When proteins are visualized on gels and the migration distances are compared to standards the isoelectric pH (isoelectric focusing) and molecular weights (SDS electrophoresis) of various proteins can be measured. The isoelectric pH and molecular weights are useful in identifying and purifying proteins.
Serum proteins are separated into albumin and globulins. In other words, total protein (albumin + globulin). Albumin is the protein with the highest concentration in the serum. It carries many small molecules, but it is also of prime importance in maintaining the oncotic pressure of the blood.
Globulins are roughly divided into alpha-1, alpha-2, beta, and gamma globulins. These can be separated and quantified in the laboratory by electrophoresis and densitometry.
Usually, alpha-1 and alpha-2 protein levels increase in the presence of inflammation. The beta fraction includes transferrin, plasminogen, and beta lipoproteins. The gamma fraction includes the various types of antibodies (immunoglobulins M, G and A). Using this method we can detect any change in the protein fractions concentration due to several health alterations.
Buffalo calves (aged four to six months) serum protein electrophoresis evidenced the following

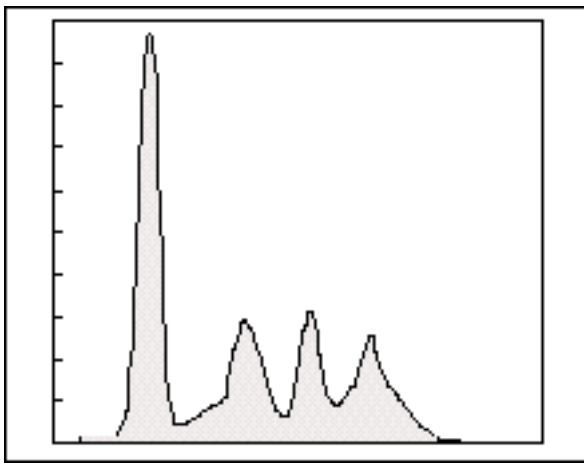

Figure 2. A Buffalo serum protein electrophoresis as a pattern in polyacrilamide gel (IZS Latium and Tuscany).

percentage results in females: albumin 45; α-globulin 16; β-globulin 17; γ-globulin 22, and in males: albumin 44.8; α-globulin 15.5; β-globulin 14.9; γ-globulin 24.9. In adult buffaloes the following percentage results were found: albumin 35.5; α-globulin 25.5; β-globulin 11; γ-globulin 28 (females), and albumin 37.7; α-globulin 27.4; β-globulin 8.5; γ-globulin 26.4 (males) (Satija et al., 1979).

Flow cytometry

Flow cytometry is a method to measure certain physical and chemical characteristics of cells or particles as they travel in suspension (blood, bone marrow, body fluids or tissue cell suspensions) one by one past a sensing point.
Flow cytometry employs instrumentation that scans single cells flowing past excitation sources in a liquid medium. The technology can provide rapid, quantitative, multiparameter analyses on single living (or dead) cells based on the measurement of visible and fluorescent light emission. Flow cytometry is a widely used method for characterizing and separating individual cells. Physical characteristics such as cell size, shape and internal complexity can be measured and, of course, any cell component or function that can be detected by a fluorescent compound can be examined.
A number of different antibody panels are used, depending on the clinical question to be resolved.
The relative fluorescence intensity of the positive cells indicates the amount of antibody bound to specific binding sites on the cell.
The specific panel of antibodies used is selected by the laboratory based on the patient's clinical history, referring physician information, and the morphologic appearance of the cells present in the specimen. Antibodies of particular interest may be requested by the referring physician. In addition, printouts of flow histograms will be provided upon request.
So the applications of flow cytometry are numerous, and this has led to the widespread use of these instruments in biological and medical veterinary fields.
The following analyses can be executed using flow cytometry:

- Immunophenotyping
- Tumour Antigen Markers
- Apoptosis
- Enzyme activity
- DNA/RNA content and Cell Cycle Analysis
- Cytokine receptors and their synthesis
- Phagocytosis
- Identification of WBC in milk

It has been used for an initial screening in buffaloes on leukocyte differentiation molecules that are differentially expressed on one or more lineages of leukocytes: 200 monoclonal antibodies (mAbs) yielded 138 with patterns of reactivity similar or identical to those in cattle (Davis et al., 2001).

Clinical applications

Immunophenotyping has proved to be an important tool in the diagnosis and classification of leukemias and lymphomas. Morphology and immunophenothyping generally provide sufficient information for making a reliable diagnosis in patients with a clinically suspect lymphoproliferative disease.
With the advent of monoclonal antibody technology and availability of fluorochromes, immunophenotyping of leukocyte subsets in animals has become more routine. Flow cytometry is also used to analyse mastitic milk and to define a leukemic population.
All these applications can be employed in the examination of buffalo peripheral blood and milk.

Hormonal parameters

Cortisol - Cortisol is a corticosteroid hormone synthesized in the zona fasciculata of the cortex of the adrenal glands. It is synthesized from cholesterol and its production is stimulated by the pituitary adrenocorticotropic hormone (ACTH) which is regulated by the corticotropin releasing factor (CRF). ACTH and CRF secretions are inhibited by high cortisol levels in a negative feedback loop. In plasma a preponderance of cortisol is connected with a high affinity to corticosteroid binding globulin (CBG or transcotin). Cortisol acts through specific intracellular receptors and affects numerous physiologic systems including immune function, glucose counter regulation, vascular tone and bone metabolism.
Cortisol production has an ACTH-dependent circadian rhythm with peak levels in the early morning and a nadir at night. The factor controlling this rhythm has not been completely clarified and can be disrupted by a number of physical and psychological conditions. ACTH and cortisol are secreted independently from the circadian rhythm in response to physical and psychological stress. For this reason it has attracted widespread attention as the so-called "stress hormone." Serum cortisol levels fluctuate in response to a number of different variables, apart from ACTH levels, including psychological stress and such physiological strains as hypoglycemia, illness, fever, trauma, pain, fear, physical exertion or extremes of temperature. Cortisol is usually released in response to long-term stress. In a previous study, Borghese et al., (1991) found cortisol levels in buffaloes significantly correlated with estradiol-17b (r=0.45) and with the quantity of milk produced (r=0.38), with values of 13-14 ng/ml in the early stage of lactation and 4-6 ng/ml in the subsequent stages. Bertoni et al., (1994b) clarified the pattern of changes of cortisol level showing that it varied before (10.4 ng/ml) and after meal (9.3 ng/ml). Later, Bertoni et al., (1997) and Terzano et al., (unpublished data) confirmed the significant cortisol level reduction following a meal (4.7 ng/ml vs 3.3 ng/ml; 3.67 ng/ml vs 2.97 ng/ml, respectively). Zia-Ur-Rahman et al. (1997), in a study on hormonal and haematological profiles in buffaloes subjected to different stress conditions, found higher serum cortisol levels before and after transport (23.1 *vs* 80.6 mmol/l, respectively), before and after handling (23.1 *vs* 86.0 mmol/l, respectively) and before and after slaughter (23.1 *vs* 92.0 mmol/l, respectively). The findings were indicative of different hormonal changes during different types of stress in buffaloes.

Triiodothyronine (T3) and Thyroxine (T4) - The thyroid hormones, thyroxine (T4) and triiodothyronine (T3), are tyrosine-based hormones produced by the thyroid gland. They act on the body to increase the basal metabolic rate, heat production and the O2 consumption; moreover they affect carbohydrate absorption, glycogenolysis, gluconeogenesis, lipid metabolism, protein synthesis and increase the body's sensitivity to catecholamines (such as adrenaline). The major type of thyroid hormone in the blood is T4. This is converted within cells to the active T3 by deiodinases. The thyroid is directly regulated by the anterior pituitary and indirectly regulated by the hypothalamus. The hypothalamus secretes TRH, which stimulates

the anterior pituitary to synthesize and secrete TSH. TSH enters the blood stream and eventually stimulates the thyroid gland to undergo hyperplasia, increase iodine uptake, and synthesize and secrete thyroid hormones (T3 and T4) into the blood stream where they act on tissues. The plasma thyroid hormone levels are highly affected by various factors: species, breed, sex and individual characteristic (Borghese et al.,1991). The authors reported significantly higher plasma T3 values in buffalo cows less than five years old (1.39 ng/ml) than the oldest (1.08 ng/ml). Moreover the same authors found that the calving period had a significant effect on T4 values, these latter values also increased during lactation and resulted inversely correlated with milk production (r=-0.30). Moreover their levels depend on physiological phases (Campanile et al.,1997), the nutritive value of diets (Bertoni et al., 1997), housing systems and environmental conditions. Montemurro et al. (1995b) reported the following T3 plasma values in heifers bred in two different farms (0.91-1.88 ng/ml), the same values which were found by other authors (Ghaly, 1987; Dixit et al., 1984; Sharawi et al., 1987). The same authors also reported the mean value of T4 plasma levels which range between 3.35-5.27 g/dl. Terzano et al., (unpublished data) found different plasma T3 levels between, before and after meals (1.70 ng/ml *vs* 1.0 ng/ml). The circadian variation of serum T3 and T4 concentrations was investigated by Vulcano et al., (1997) in pregnant and non pregnant Murrah buffalo cows; they found no differences in serum T3 and T4 concentrations during the cycle in any group. The serum T3 concentration was higher at 2 a.m. (1.88 ng/ml) in non-pregnant cows and serum T4 concentration was higher at 2 p.m. (5.68 g/dl) and 6 p.m. (6.0 g/dl) in pregnant cows, confirming the effect of pregnancy and circadian rhythms on thyroid activity. Zia-Ur-Rahman et al. (1997), in their study found higher serum T3 levels after transport (3.5 pmol/l,), handling (3.8 pmol/l,) and slaughter (3.8 pmol/l,), as compared to before transport (2.2 pmol/l).

Reproductive hormones

Gonadal steroid hormones - Steroid hormones are secreted by the ovary, testes, placenta and adrenal cortex. The two classes of hormones produced by the ovaries are *progestins and estrogens* and have cholesterol as a common precursor.

- *Progestins* are a group of hormones with similar physiological activity, the most important being progesterone (P4). This latter has a dominant role in regulating the oestrus cycle. In buffaloes, during the normal cycles, plasma P4 levels drop sharply two or three days before the luteinizing hormone (LH) peak, start to rise two to four days after the LH surge reaching the highest characteristic mid-luteal phase (dioestrus) levels (ranging between 5 and 12 ng/ml in Mediterranean buffalo cows or between 4-6 ng/ml in Murrah buffalo cows). P4 blood concentration can change during the seasons: Srivastava et al. (1999) during the fresh season reported the highest progesterone levels of the luteal phase in comparison to that recorded during the hot dry or hot wet seasons. Hattab et al. (2000) have shown that the P4 metabolites levels measured in faeces are a good medium for the monitoring of the corpus luteum function, reflecting the blood P4 levels with a high significant correlation coefficient (r=0.77). Therefore providing a simple tool for determining luteal status in buffalo. Moreover P4 have been reported to hasten the transport of oocytes in the oviduct, to have a dominant role in the maintenance of pregnancy, particularly during the early stage, and to be able to synergize with estrogens in stimulating the udder alveolar development and growth. P4 blood level is considered a good indicator of the onset of puberty and heifers are usually considered to have achieved puberty when plasma P4 levels exceed 1.5 ng/ml. A number of environmental factors have a pronounced effect on the age at puberty. In general, any factor which slows growth rate, thus preventing expression of full genetic potential, will delay puberty. A buffalo heifer on a good level of nutrition will reach puberty at about 19 months (Terzano et al., 1993, 1997; Borghese et al., 1994).

- *Estrogens* are hormones produced by the ovary and are transported in the body by binding proteins. Estrogens act on the Central Nervous System in order to induce behavioural oestrus in females and the most important of these hormones is estradiol. In buffalo the general pattern

of secretion of the estradiol-17ß indicates a surge which takes place on the day preceding the LH peak (Singh et al., 2001; Malfatti, 2003) or frequently very close to the LH peak with blood levels ranging between 9 and 13 pg/ml (Seren et al., 1994) respectively in Murrah and Mediterranean buffaloes. Following the LH surge, there is a rapid decline in estradiol secretion and this results in the cessation of oestrus after a relatively constant time interval (about 12 hours). The basal estradiol levels during the luteal phase of the cycle range between three and eight pg/ml, but some minor elevations can be observed in the early luteal phase, up to 1.3 pg/ml (Singh et al., 2001; Malfatti, 2003). Oestrus symptoms are much less obvious than in cattle; it is significant to note that in Italian studies silent oestrus was recorded in more than two thirds of the animals, all presenting endocrine changes comparable to that of the cattle showing overt oestruses.

Pituitary gonadotrophins - The *luteinizing hormone* (LH) is important in studies of ovarian activity since its preovulatory surge is responsible for the rupture of the follicle wall and ovulation. The peak values are always well defined in respect to the basal blood levels; the duration of LH surge was estimated by different authors and was calculated at 6 to 12 hours (Seren et al., 1994) or 6 to 9 hours (Maurel et al., 1995) or 8 to 12 hours (Barile et al., 1998) in Mediterranean buffalo cows. Buffalo cows are characterized by the occurrence of two peaks of the hormone during the oestrus time; this phenomenon was recorded by Seren et al. (1994) in 25 percent of the 24 observed oestruses. The double LH peak was frequently followed by double ovulation that also occurred after a single peak in an additional 8 percent of the animals. LH peak time (in relation to oestrus symptoms and to ovulation time) is more important than the LH peak. Seren et al., (1994), monitoring ovulation time by ultrasound, found that the mean time of LH peak-ovulation was 35.5 hours (in buffalo cows with single ovulation) and 60 hours (in buffalo cows with double ovulation). Moioli et al. (1998), in spontaneous oestruses of buffalo cows, found a mean interval LH peak-ovulation (this latter detected by rectal palpation considering follicle changes from turgid to flaccid) of 25.2 ± 13.1h and of 46.1 ± 18.8h respectively in oestruses followed by pregnancy and in those not followed by pregnancy. The same authors found that the mean time of LH peak end of the continuous courtship was 2.4 ± 10.4h and 14.7 ± 15.2h respectively in the two groups. Several authors (Barile et al., 1998, Malfatti et al., 2003) studying buffalo cows after PRID + PMSG treatment, found that the mean time from PRID removal to LH peak ranged from 54.7 ± 12.3h to 61.0 ± 12.05h and that it was nearly 85 hours from PRID removal to ovulation time. Malfatti et al. (2003), evaluating the LH peak in buffalo cows synchronized with two different hormonal protocols for fixed time artificial insemination, found no difference between the PRID + PMSG group and PRID + GnRH group, in the interval from PRID removal to LH peak (57.3 ± 13.4h and 54.4 ± 9.6h respectively) nor in the interval from PRID removal to ovulation time (86.4 ± 13.1h and 90.0 ± 11.1h respectively). Following this previous trial, Malfatti et al. (2004), evaluated LH peak and ovulation time in buffalo cows submitted to two different hormonal protocols (PRID and Ovsynch) for fixed time artificial insemination, during the lower breeding season; no differences were found between treatments. Particularly average LH peak values occurred at 51.30 ± 13.94h and ovulation at 85.14 ± 13.63h and the mean time interval detected between LH peak and ovulation was 33.71 ± 4.30h. Moreover in the PRID group the times of LH peaks and ovulations were notably more scattered and highly significant than in the Ovsynch group (CV%= 37.50 vs 3.55 and 21.41 vs 6.73). The authors concluded that both treatments were able to synchronize oestrus and ovulation in buffaloes and that the Ovsynch protocol appears more suitable for use in fixed time artificial insemination programmes due to a better LH peak and ovulation synchronization. The basal level of LH did not register differences during pregnancy. The *follicle stimulating hormone* (FSH) promotes follicle growth and estrogen production through granulosa cells in the ovarian follicles. This hormone has been studied by several authors in buffalo cows over the past few years. Seren et al. (1994) found the FSH surge (1.6-6.0 ng/ml) to be coincident with the LH peak and to have a duration time of six to nine hours, with a lack of evident additive peaks during the luteal phase of the oestrus cycles, when the hormone levels ranged between 0.2-1.5 ng/ml. Singh et al. (2001) also found the FSH surge to be coincident with the LH peak, averaging near 25 ng/ml, but reported the major surge of this hormone on the tenth day after oestrus and a minor increase on days 4 and 15 after

oestrus. Palta and Madan (1995, 1997a) recorded FSH peak means of 70-80 ng/ml and a duration time of 5.8 ± 0.07h (during pregnancy) and 5.5 ± 0.02h (during postpartum) after GnRH stimulation. It has now been established also in buffalo that a transient rise in serum concentration of FSH begins each follicular wave (Baruselli et al. 1997; Singh et al. 2001; Presicce et al. 2003) and a decreased episodic secretion of LH is associated with loss of dominance and with the end of a nonovulatory follicular wave. Palta and Madan (1995, 1997a) found alterations of the gonadotrophins release by hypophysis in pregnant and postpartum buffalo cows. The basal level of FSH presented significant differences during pregnancy; in fact it was higher at day 60 than at day 240 of pregnancy. During postpartum the basal levels of LH and FSH increased significantly from day 2 to day 20, but only the LH levels were higher at day 35 in comparison to day 20. The authors pointed to a progressive reduction of the hypophysial responsiveness to the GnRH during pregnancy and this could be due to the chronic negative feedback exerted by gonadal steroids, together with a probable reduction of GnRH receptors in the pituitary, both verified in other ruminants (Nett, 1987; Schoenemann et al., 1985). After delivery a progressive reestablishment of the positive feedback by estrogens was observed and a rise of the basal gonadotrophins levels was recorded.
Prolactin synergizes with LH by increasing LH receptor sites in the corpus luteum. It also has a stimulating effect on the development of the mammary gland and the synthesis of milk. In buffalo cows prolactin blood concentrations during the oestrus cycle have not been extensively studied. Seren et al. (1994) reported a pulsatile secretion starting before the luteolysis and lasting during the oestrus, ending near the ovulation time. The plasma levels were very variable, ranging from 10-20 to 150-200 ng/ml.

Inhibin - Inhibins are heterodimeric protein hormones secreted by the granulosa cells of the ovary in the female and Sertoli cells of the testis in the male. It is recognized that inhibin plays an important role in regulating FSH secretion (Baird et al., 1993; Singh et al., 2001) and also has local paracrine actions in the gonads (Burger H.G., 1992). Two forms of biologically active inhibin (inhibin A and B) have been identified and most data regarding changes in circulating inhibin concentrations come from human studies (Fried et al., 2003; Eldar-Geva, 2000). These hormones have been studied in buffalo cows by only a few authors over the past few years. Palta et al. (1996) validated a specific RIA method to assay immuno reactive inhibin (ir-inhibin) in the plasma of buffalo cows measuring the hormone blood levels during the oestrus cycle. They observed an increase of inhibin concentrations during the follicular phase with a peak occurring on day 0 (oestrus day defined by the lowest P4 value); the differences occurring between the concentrations recorded during the cycle were not significant, ranging from 0.40 ± 0.07 to 0.67 ± 0.13 ng/ml. Based on their tissue localization (Muttukrishna et al., 1994), it is believed that inhibin A is a product of large follicles, whereas inhibin B is produced by a cohort of antral recruited follicles. These data suggested that inhibin A may provide an indication of follicular development (Lockwood et al., 1996), whereas inhibin B may be a suitable marker for ovarian follicle reserve (Seifer et al., 1997). The inhibin concentrations increase notably when superovulatory treatments are performed, due to the greater number of large follicles growing. Palta et al. (1997b) recorded concentrations up to 1.01 ± 0.31 ng/ml (nearly double the physiological values at oestrus) when PMSG superovulatory treatment was performed. Terzano et al. (2004a) evaluated the relationship of plasma inhibin A (analyzed in duplicates by a human sandwich type immunoassay) to ovarian follicular development in prepuberal Mediterranean Italian buffaloes subjected to two different ovarian stimulation protocols. The data suggested that the medium/large follicles are the most important source of hormone production and that serum inhibin A determined during FSH treatment may provide a useful marker in the control of ovarian hyperstimulation.

Pregnancy-associated glycoproteins (PAGs) - The pregnancy-associated glycoproteins constitute a large family of glycoproteins synthesized by trophoblast binucleate cells and released in the maternal blood circulation after implantation until birth. During the last decade they have been isolated from the placenta of various ruminant species (Terzano et al., 2004b). In recent years, it has become evident that there may be more than 100 PAG genes and that many of them are expressed (Gonzalez et al., 2000). Among these the best known is the

Pregnancy Specific Protein B (PSPB), first detected in bovine placenta (Butler et al., 1982), and now widely used for pregnancy diagnosis in most animals. It represents, as do other PAGs, a reliable "foetal-placental welfare marker" and could be a very useful technique for early detection of animals with a high risk of pregnancy failure (Willard et al., 1995; Zoli et al., 1995; Garbayo et al., 1998). The first study of the presence of PAGs in pregnant buffalo cows was by Debenedetti et al. (1997), and this investigation showed that its blood concentration is clearly pregnancy-linked. Malfatti et al. (2001), through a double antibody RIA (Humblot et al., 1998) utilizing bovine PSPB antibody and standards, found that in this species the hormone became detectable in 33 percent of animals between the 20th and 25th day after fertilization. On the 30th day it resulted measurable in all animals (1.6 ± 1.1 ng/ml) and on the 35th day, 91 percent of the animals had PAGs blood level > 1.0 ng/ml. The hormone concentration reached values of 6.6 ± 3.2 ng/ml on the 50th day and at the end of pregnancy the values were similar (6.28 ± 1.87). The PAGs decreased markedly after birth: five days after, their levels were 45 percent less. The successive decrease was much slower: the mean calculated halving time is nearly 10 days; 50 days after birth the PAGs plasma concentrations were not detectable (< 0.3 ng/ml). Barbato et al. (2003) have described the first isolation and the partial characterization of PAGs from the buffalo placenta which enabled a study of the PAGs blood concentration and its trend during pregnancy and led to a reliable pregnancy test for the buffalo species.

Melatonin - Melatonin is a brain hormone, produced and stored in the pineal gland during daylight and then secreted during darkness, beginning a short time after sunset and ending at sunrise. Its secretion constitutes the endocrine signal of the light-dark rhythm in the environment. The best known role of melatonin is the regulation of the circadian as well as of the annual rhythms in many species, from the more primitive species to man. In the endocrinology of ruminants its role has been extensively explored with regard to the induction of seasonal ovarian cyclicity in ewes and goats, especially at high latitudes. Buffalo is considered a seasonal species and this seasonality does not seem to depend on the diet, food availability or metabolic status, while climate and photoperiod appear to play a pivotal role. The strong influence of photoperiod seems to be further corroborated by the finding that the period of higher reproductive efficiency is reversed in the two opposite hemispheres (Borghese et al., 1994; Zicarelli, 1997; Baruselli et al., 2001). Parmeggiani et al. (1993, 1994), assayed melatonin blood levels in buffalo cows reared on Italian farms where the reproductive activity was characterized by strong seasonality and on farms where parturition frequency tended to be more uniformly distributed throughout the year. The season-linked buffaloes evidenced a melatonin profile reflecting the photoperiodic changes, with hormone concentrations below 20 pg/ml during the light time and systematic rises after sunset (averages of near 60 pg/ml). Conversely the less season-linked buffaloes presented melatonin concentrations frequently high during the light time (30 40 pg/ml), with a lack of evident melatonin increases during the night. The author's view, is that the different melatonin trends are the result of extensive selection carried out in the latter farms aimed at eliminating the seasonal breeder cows (Seren et al., 1995). Borghese et al. (1995) assayed melatonin blood levels, every two hours for 24 hours at equinoxes and at solstices, in 16 buffalo cows and in 16 buffalo heifers in natural daylight conditions in order to better understand melatonin's role in hypothalamus-pituitary-ovarian axis processing. Blood melatonin level was less than 10 pg/ml during the day, while it was 30-100 pg/ml during the night, showing considerable differences between seasons and between cows and heifers. In March, in particular, the buffalo cows showed much higher melatonin values at night than heifers.

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