BOVINE SOMATOTROPIN

IDENTITY

Chemical name:

Synonyms:	bST Bovine Growth Hormone Somagrebove (American Cyanamid) Somidobove (Elanco) Sometribove (Monsanto) Somavubove (Upjohn)
Structural formula:	Pituitary-derived bST (one of four natural variants) see Figure 1
Products:	**Amino-Terminal Substitution of Ala (191)**
Somagrebove	Met-Asp-Gln
Somidobove	Met-Phe-Pro-Leu-Asp-Asp-Asp-Asp-Lys
Sometribove	Met
Somavubove	None
Molecular formula:	$C_{976}H_{1533}N_{265}O_{286}S_8$ (Figure 1)
Molecular weight:	21,812

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

Appearance:	white odorless powder
Melting point:	decomposition to black precipitate

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Bovine Somatotropin (bST) is a genetically engineered protein hormone either identical or similar to the natural bovine pituitary product. Its primary function is to increase milk production in lactating dairy cattle.

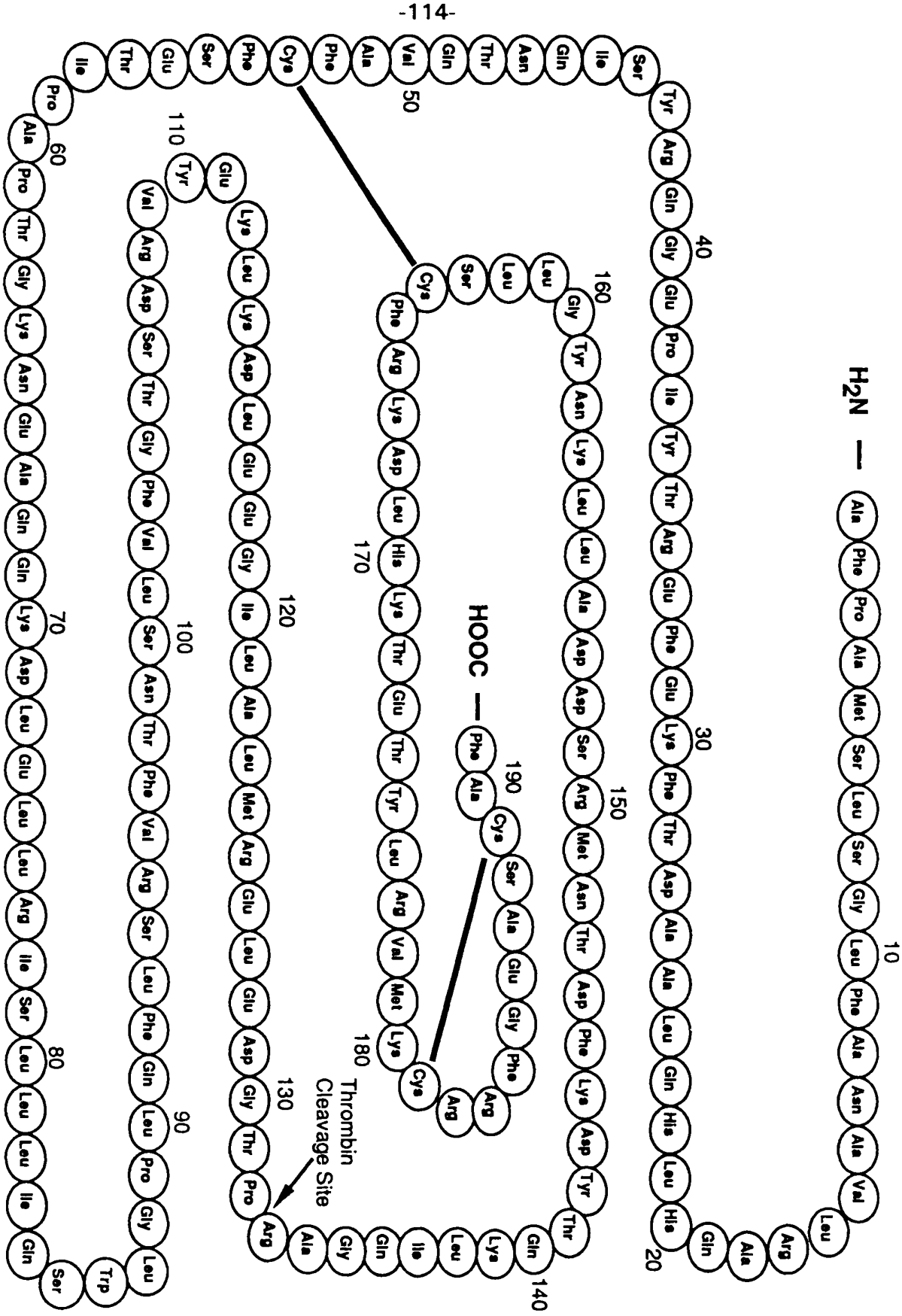

Figure 1. The Amino Acid Sequence of Bovine Somatotropin

Dosages

Bovine ST is administered to cattle via subcutaneous or intramuscular administration. A continuous application of the drug is proposed beginning approximately 50-90 days post-partum until the end of lactation. The products are administered either as a daily injectable or a 14-28 day sustained release injectable. The proposed dosage calculated on a daily basis ranges from 10-35 mg/day.

Other Residues

Since many of the effects of bST are known to be mediated by insulin-like growth factors, especially insulin-like growth factor I (IGF-I), IGF-I concentrations following bST-treatment have been determined. IGF-I is an endogenous polypeptide containing 70 amino acids (see Figure 2). Bovine and human IGF-I have the same amino acid sequence.

METABOLISM

Pharmacokinetics

Bovine ST

A study was conducted to determine if there was a difference in the pharmacokinetics of methionyl bST (sometribove) and a naturally occurring variant of bST (ALA-VAL bST) in lactating Holstein cows (Birmingham et al., 1988). A 25 mg IV bolus dose was administered to each of nine animals in a random cross-over design. Blood samples were collected over a 12-hour period and analyzed for somatotropin concentration using a homologous radioimmunoassay. A decline in serum somatotropin levels with time followed a biexponential curve described by a two-compartment open model. The pharmacokinetic estimates of both variants of bST were not significantly different ($P>0.05$). Distribution half-lives of ALA-VAL bST and sometribove averaged 0.17 and 0.12 hours, and the terminal half-lives were 0.66 and 0.52 hours, respectively. Total body clearances were 66.99 and 68.14 L/h and volumes of distribution averaged 22.83 and 18.91 L for ALA-VAL and sometribove, respectively. These results indicate that the pharmacokinetics of methionyl-sometribove are indistinguishable from a naturally occurring somatotropin variant, ALA-VAL bST.

Two studies demonstrated that treatment with recombinant bST (rbST) increased the concentration of bST in plasma. In one study (Schams et al., 1988a), fourteen Simmental dairy cows were administered 500 mg of sometribove/ injection in the form of slow-release formulation to determine its effect on peripheral blood concentrations of bST. The cows were divided into 2 groups and sometribove was administered according to a cross over design. Group A was treated 5 times (10 weeks) at two-week intervals beginning 10 weeks after parturition, and group B was given a placebo. Three weeks after this treatment period (weeks 20-23 postpartum), group B was treated for 10 weeks with bST, and group A received a placebo (weeks 24-33). Blood for hormone analysis was sampled at weeks 13, 17, 23, 27 and 31 for 24 hour periods at 30 minute intervals. Bovine ST was analyzed by RIA.

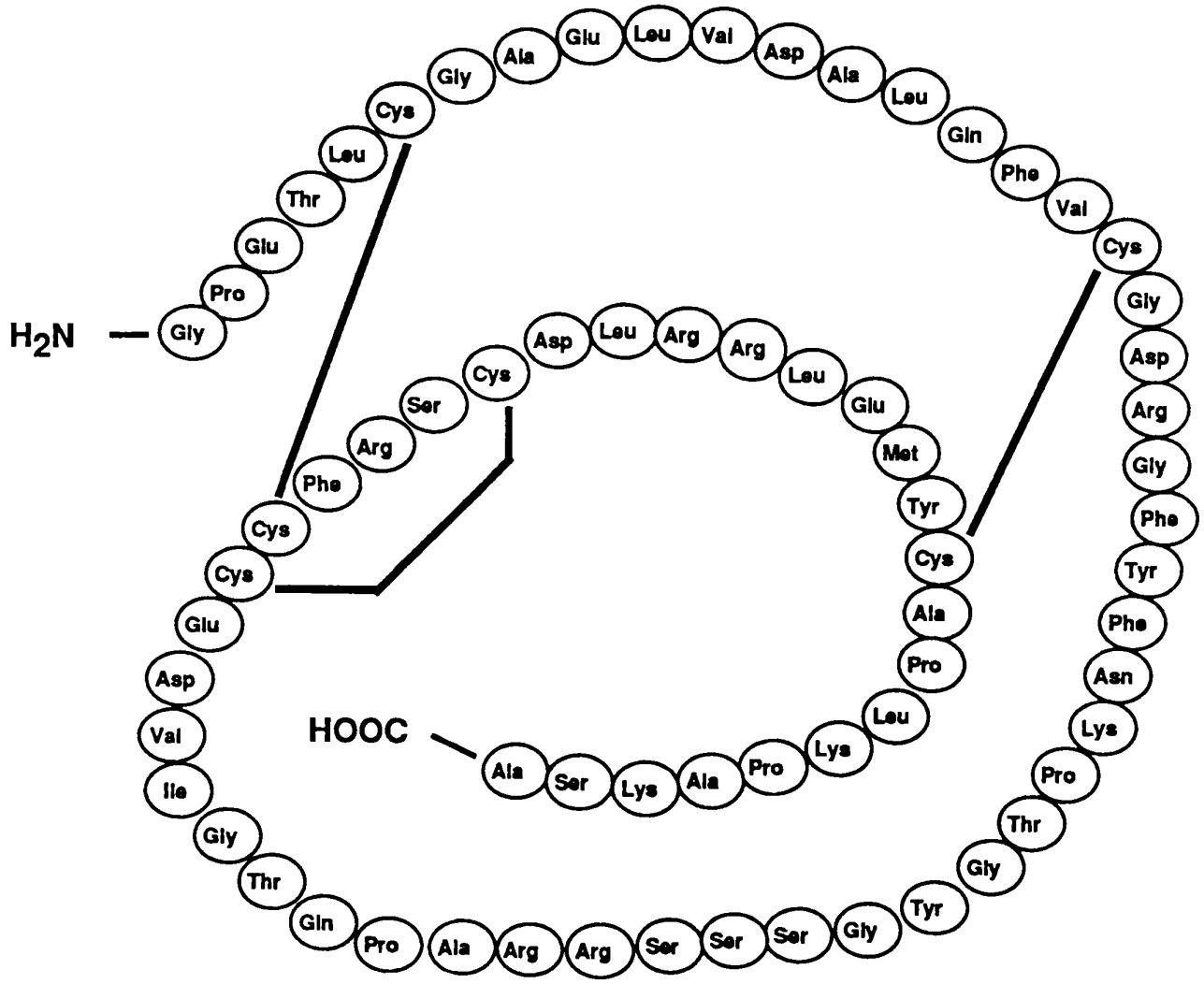

Figure 2.The Amino Acid Sequence of Insulin-like Growth Factor 1

The means of bST blood concentrations in untreated cows for the different time courses varied between 8.9 and 13.2 ng/ml. For both groups treatment with bST increased concentrations of bST about 2.4-3.2 fold to mean values of 25.8-36.1 ng/ml. There was a high variability in bST concentrations within both groups of animals.

In a series of 3 experiments, Schams and Karg (1988b) demonstrated that an increase in plasma bST levels was acheived after the animals were treated with somidobove. In the first experiment, eight cows (4 controls and 4 treated) of different breeds were used as either untreated controls or injected subcutaneously with 640 mg of somidobove. The mean blood bST levels in the controls were approximately 10 ng/ml. Bovine ST concentrations increased after the injection of bST, a maximum was reached on day 3 (42.6 ng/ml) and levels decreased to baseline by day 13. Similar results were obtained in their second experiment.

In their last experiment, forty-eight dairy cows, divided in four treatment groups, were used. The four treatment groups, containing 12 cows each, were made up of: control, high dose of somidobove (960 mg), medium dose (640 mg), and low dose (320 mg). From each animal, plasma samples were taken each week for four weeks before treatment, each week for 24 weeks after the first treatment and for four weeks after the last treatment. In general, maximum concentrations of bST were obtained during the first and second week of treatment with the high and medium dose with levels of 90 ng/ml and 50 ng/ml, respectively. For the low dose, the increase was more moderate (35 ng/ml) during the second week. Average control values were approximately 10 ng/ml. For bST, there is a tendency toward decreased plasma concentrations with an increasing number of bST treatments.

IGF-I from bST-treated Cattle

In a study conducted for Monsanto (Schams et al., 1988a), fourteen Simmental dairy cows were administered 500 mg of sometribove/injection in the form of a slow-release formulation to determine its effect on peripheral blood concentrations of IGF-I. The cows were divided into 2 groups and bST was administered according to a cross over design. Group A was treated 5 times (10 weeks) at two-week intervals beginning 10 weeks after parturition, and group B obtained a placebo. Three weeks after this treatment period (weeks 20-23 postpartum), group B was treated for 10 weeks with bST, and group A received a placebo (weeks 24-33). Blood for hormone analysis was sampled at weeks 13, 17, 23, 27 and 31 for 24 hour periods at 30 minute intervals. Each fifth sample was analyzed by RIA for IGF-I.

The means of IGF-I concentrations in untreated cows for the different time courses varied from 261-320 ng/ml. After bST treatment, the levels of IGF-I increased 3.1-3.9 fold to mean values of 941-1163 ng/ml. There was a high variability in IGF-I concentrations within both groups of animals.

METABOLISM IN CATTLE

Monsanto conducted studies (Rogan et al., 1988; Mehta, 1988) to isolate and characterize the bST metabolites in bovine serum. From 4 cows treated with 15 g of sometribove on day 0 and day 7, the serum was collected 7 days after injection. Bovine ST concentrations were determined by using a particle concentration sandwich fluorescent immunoassay, which measures intact bST. The results of the bST analysis of the serum were found to be 184, 208, 213, and 122 ng/ml for the individual cows.

Bovine ST and its metabolites were subjected to SDS-PAGE electrophoresis. Silver staining revealed 8-10 protein bands that ranged from 7 kd to 95 kd. Proteolytic fragments of bST produced by thrombin and intact bST were included in the electrophoresis run so that the molecular weight of any metabolites could be defined. Under reduced conditions two immunoreactive bands were seen in the isolate. The migration of the bands was identical to that of the reduced thrombin-clipped bST (7 kd and 14 kd). The intact bST band was also present but the band at 14 kd was the most intense. Under the oxidized conditions the binding pattern of the thrombin-clipped bST and the isolate were also identical. The band at 14 kd was again the most intense.

A total of 14 micrograms of bST metabolite was isolated from the 4 cows, which allowed for amino acid sequencing. The sequence analysis was done by using an automated Edman degradation system to determine the N-terminal protein sequence. An aliquot of each successive degradation was analyzed for PTH-amino acid derivatives. The resulting N-terminal amino acid sequence of the material through 15 positions showed 2 bST sequences. The sequences were present at close to an equimolar ratio. One sequence was homologous to the N-terminus of the bST protein, and the other sequence represented a fragment produced by cleavage at the same site as the thrombin cleavage site as shown in Figure 1. Thus, the exact position of the cleavage site was between amino acid 132 and 133, which is identical to the thrombin cleavage site. Therefore, the major identified metabolite of bST in plasma following sometribove administration was the same as the thrombin-clipped bST.

RESIDUE STUDIES

Bovine Somatotropin

Cattle Milk

Several milk residue studies were conducted to determine if bST concentrations are increased when animals were treated with somidobove. In the first experiment (Schams and Karg, 1988b), eight cows (4 controls and 4 treated) of different breeds were used. Treated animals were injected subcutaneously with 640 mg of somidobove every 28 days. Bovine ST milk concentrations in the controls and all treated animals were below the detection limit of the assay of 0.5 ng/ml in skim milk.

In an overdosing study (Kline et al., 1987), seventeen lactating Holstein heifers, divided in four treatment groups, were used. The four treatment groups were made up of: control (n=5), 960 mg of somidobove (n=8), 2880 mg of somidobove, and 4800 mg of somidobove. Milk samples were taken from each animal on two occasions before the injection and at 2 day intervals until 28 days after injection. The milk was assayed for bST content by RIA, with a sensitivity limit of 1.6 ng/ml bST in milk. Bovine ST concentrations in milk samples were below the sensitivity limit of the assay for all control animals and all animals treated with 960 mg of somidobove. One milk sample from a heifer treated with 2880 mg of somidobove was assayed at 2.2 ng somatotropin/ml and four samples from the animals treated with 4800 mg were assayed at somatotropin levels up to 2.75 ng/ml.

In the last study (Smith et al., 1985), six lactating dairy cows were given either no treatment (3 controls) or a subcutaneous injection containing 960 mg of somidobove in the sustained-release formulation. Milk samples were collected pre-treatment and at daily intervals for 14 days following treatment. The milk was assayed for total somatotropin content by RIA with a test sensitivity of 0.9 ng/ml. No somatotropin was detected above the sensitivity limit of the method in any milk sample from the treated cows.

In these residue studies, levels of bST in the milk of cows treated with up to 960 mg somidobove every 28 days did not result in detectable residues of somatotropin as measured by the RIA with a sensitivity of 0.9-1.6 ng/ml. These studies demonstrate that the use of bST will not lead to any detectable residues in milk above 0.9-1.6 ng/ml.

Cattle Tissues

Five lactating dairy cows were administered 500 mg of sometribove every 14 days. While on treatment, the muscle and liver of the cows were biopsied at each of the following times: after the first injection, 7 days after the first injection, after the second injection (day 14), 7 days after the second injection (day 21), and after the third injection (day 28). The samples were analyzed using validated RIA procedures. The results are summarized in Table 1. (Hammond et al., 1990).

Table 1. Concentration of bST (ppb) in Biopsied Tissues of Dairy Cattle Injected with 500 mg of Sometribove

Withdrawal	Muscle		Liver	
Time (days)	Control	Treated	Control	Treated
0	2.6 ± 2.1*	2.8 ± 1.3	13 ± 2.5	16 ± 3.8
7	2.1 ± 1.9	3.1 ± 1.7	11 ± 2.1	24 ± 9.5
14	2.9 ± 1.8	4.0 ± 2.2	12 ± 2.6	18 ± 7.4
21	3.7 ± 2.7	4.2 ± 2.2	11 ± 3.6	25 ± 5.6
28	2.1 ± 1.7	3.7 ± 0.7	9 ± 3.0	16 ± 6.8

*Values are means ± SD.

Based on the data, sometribove treatment of cows leads, at most, to a 2-fold increase in bST concentrations in muscle and liver.

INSULIN-LIKE GROWTH FACTOR-I

Cattle Milk

Monsanto conducted several studies to determine the average baseline concentration of IGF-I in untreated cow's milk. White et al. (1989a) estimated the range of IGF-I concentration typically found in bulk tank milk from dairy cows.

A survey of of 100 raw bulk tank milk samples from a commercial processing plant was conducted to provide additional data on the naturally-occurring range of IGF-I concentrations in milk. The mean IGF-I concentration in these samples was 4.32 ng/ml with a range of 1.27-8.10 ng/ml.

Collier et al. (1988) estimated the range of IGF-I concentrations found in milk from Missouri dairy cows. Milk samples from 408 untreated cows from five Missouri dairy herds were assayed for IGF-I concentrations by RIA, which had a sensitivity of 0.05 ng/ml. The highest mean concentration of IGF-I in milk was detected in early lactation (days 6-15, 6.2 ng/ml) after which milk concentrations declined. Lowest values were detected at mid-lactation (days 150-210, 1.85 ng/ml) after which they increased slightly (210+ days, 2.22 ng/ml). Older animals had significantly higher mean milk IGF-I concentrations (2.83 ng/ml) than first lactation animals (2.15 ng/ml). However, stage of lactation effects were detected in both parities and the effect of parity was apparent at all stages of lactation.

Considerable variation in milk IGF-I concentrations was related to the farm that the milk was collected from (range of farm means, 0.74-4.21 ng/ml). This significance was not related to an unequal representation of parities, stage of lactation or level of milk production from each of the farms.

The survey studies show that the levels of IGF-I in milk of untreated cows is quite variable ranging from 0.7 to 8.1 ng/ml, depending on parity and stage of lactation of the cow.

Schams and Karg (1988b, 1988c) investigated the increase in IGF-I concentrations in the milk of cows treated with somidobove. In the first experiment, eight cows (4 controls and 4 treated) of different breeds were used. The treated cows were injected subcutaneously with 640 mg of somidobove every 28 days. Milk was collected in the morning before the 3rd injection and further on days 1, 3, 6, 8, 10, 13, 15, 17, 20, 22, 24, 27 and after the 4th injection of bST on days 1, 3, 6, 8, 10 and 13.

After somidobove injection, mean IGF-I levels in the treated animals are always higher than those found in the controls. The average IGF-I milk concentration found in the control animals was 28.4 ng/ml, and the average IGF-I milk

concentration in the 640 mg somidobove-treated animals was 35.5 ng/ml. Therefore, in this study an increase of approximately 25% of the mean was found in the somidobove-treated animals. It should be noted that concentrations of IGF-I in skim milk from treated cows are less than 5% of those measured in the blood plasma (1000 ng/ml).

In a study conducted for Elanco (Davis et al., 1989), thirty-six cows (24 Friesian, 12 Jersey), weighing approximately 420 kg, that had completed at least one full lactation, were used. Somidobove was given in a single subcutaneous injection at three different doses to the three different groups (12 cows/group): zero (vehicle), 320 mg and 640 mg. A composite milk sample was taken for IGF-I determination at 3, 10, 17 and 24 days after treatment at consecutive p.m. and a.m. milkings.

The concentration of IGF-I in milk was higher by day 3 in cows treated with 320 and 640 mg of somidobove relative to the control cows. The values at day 10 and thereafter were not significantly different between treatment groups. In the day 3 milk samples, the IGF-I concentrations ranged from 8-14 ng/ml in control cows, 6-32 ng/ml in the 320 mg dose group, and 9-19 ng/ml in the 640 mg dose group. One cow in the 640 mg dose group showed consistently high values for milk IGF-I content. Values on days 3, 10, 17 and 24 were 27, 58, 26 and 16 ng/ml, respectively. After somidobove treatment in this study, the levels of IGF-I in the milk increased less than 50% relative to the milk IGF-I content in the control cows.

Torkelson et al. (1988) compared concentrations of IGF-I in untreated cows to IGF-I concentrations in sometribove-treated cows. To assess the impact of bST treatment on milk IGF-I, samples were collected from 9 control cows and 9 cows treated with 500 mg of sometribove every 14 days in a prolonged-release formulation. Milk samples were collected 7 days after each of the 3 consecutive treatments. After each of the 3 doses, mean milk IGF-I in controls was 3.22, 2.62 and 3.78 ng/ml and in treated cows was 3.80, 5.39 and 4.98 ng/ml, respectively. Differences between treated and control groups was significant after the second and third doses. However, concentrations of IGF-I in milk from bST-treated animals were within the range of values detected in milk from untreated cows.

White et al. (1989b) conducted a study to provide additional data relative to the effect of exogenous administration of oil-formulated sometribove on milk concentrations of IGF-I. Eighteen lactating Holstein cows were randomly divided into two groups of nine each and administered subcutaneous injections of 500 mg of sometribove in a prolonged-release formulation or a sham injection at a 14-day interval.

Milk IGF-I concentrations also significantly increased in the sometribove treated animals although the increases were numerically small and occurred only in injection cycles two and three of treatment. The overall range of concentration for both treatment groups were similar with the control group having a range of 2.16-8.15 ng/ml and the sometribove treatment group having a range of 1.56-8.83 ng/ml. These results are summarized in Table 2.

Table 2. Least-squares means for ln and actual milk IGF-I concentrations and the numerical range of IGF-I levels (ng/ml)

Sample	Treatment	Ln Conc.* ± SEM	Mean**	Range
Pretreatment	Control	1.62 ± 0.11	5.05	3.01 - 9.04
	500 mg rbGH	1.37 ± 0.11	3.95	0.84 - 7.53
Day 7	Control	1.15 ± 0.08	3.17	2.85 - 4.29
	500 mg rbGH	1.25 ± 0.07	3.50	1.56 - 7.05
Day 21	Control	1.21 ± 0.14	3.34	2.04 - 5.79
	500 mg rbGH	1.67† ± 0.14	5.33†	2.67 - 8.83
Day 35	Control	1.21 ± 0.11	3.35	2.16 - 8.15
	500 mg rbGH	1.54† ± 0.11	4.68†	3.23 - 7.38

*Least-squares means ± SEM of least-squares means; **Mean = Antilog of log concentration; †These means are significantly different from the control values ($P < 0.05$).

Miller et al. (1989a) assessed the potential carryover of IGF-I in processed milk. IGF-I concentrations were measured in raw and pasteurized milk and in milk treated using conditions similar to those used in the preparation of infant formula (autoclaving).

Daily milk samples were obtained before and after pasteurization from a local commercial processing plant. The milk was pasteurized using a standard procedure. Conditions used to process milk for infant formula, i.e. heating in a retort at 250°F for 15 minutes, can be simulated in the laboratory by autoclaving milk at similar temperatures for comparable times. Raw (unpasteurized) and pasteurized milk samples were subjected to conditions simulating retorting and then assayed for IGF-I content. These results were then compared to measured IGF-I in actual Similac® infant formula milk.

The raw milk and pasteurized milk samples contained measurable levels of IGF-I of 5.6 and 8.2 ng/ml, respectively. The infant formula contained only trace amounts of IGF-I of 0.7 ng/ml. These results suggest that IGF-I is not destroyed by the pasteurization process but the heating of milk for the preparation of infant formula denatures IGF-I.

When the raw or pasteurized milk samples were heat-treated using a process similar to that of infant formula, the amount of IGF-I remaining in the milk was below detection and at least one-fifth of the preheat-treatment concentrations.

American Cyanamid conducted an extensive study of the effect of stage of lactation, diet composition and daily injections of somagrebove to Holstein cows on concentrations of IGF-I in milk (Schingoethe and Cleale, 1989). Twenty multiparous cows were assigned to one of four treatments with each treatment group containing 5 cows each. The four treatments consisted of two control groups, one fed a normal diet and the other fed a high energy and protein diet with both groups receiving excipient only, and the other two treatment groups, one fed a normal diet and the other fed a high energy and protein diet with both receiving 10.3 mg somagrebove daily. The treatments began 28-35 days postpartum and continued for 16 weeks.

Milk samples were collected every Monday p.m. and Tuesday a.m. of the treatment period and assayed for IGF-I content by RIA. Concentrations of immunoreactive IGF-I were not statistically different ($P > 0.89$) in milk of somagrebove-treated cows collected from consecutive p.m. and a.m. milkings. The mean IGF-I concentration throughout the study in the control animals was 9.67 ng/ml and in the somagrebove-treated animals was 9.06 ng/ml. Concentrations of IGF-I were significantly higher ($P < 0.05$) in the milk from the cows that consumed the high energy and protein diet than those that received the normal diet. Therefore, this study demonstrates that there is no increase in IGF-I concentration in the milk of cows treated with up to 10.3 mg of somagrebove when tested for a 16 week period.

Elanco also conducted a study to determine the concentration of IGF-I in the raw milk of control cows and the raw, pasteurized and heat-treated milk of cows receiving sustained release somidobove. In addition, the concentration of IGF-I was determined in commercially available pasteurized milk and infant formulae (Coleman et al., 1990).

Six multiparous and six primiparous Holstein cows were used in the study. Primiparous cows ranged from two to four years of age and weighed from 553 to 607 kg. Multiparous cows ranged in age from four to eight years and weighed from 649 to 689 kg. Animals were randomly assigned to treatment or control groups based on parity. The treatment group (three primiparous and three multiparous cows) received 640 mg somidobove in a sustained release vehicle in two doses, 28 days apart.

The milk from each cow was collected before injection and on days 3, 10, 17 and 24 after each injection. Raw milk samples were refrigerated pending analysis. The concentrations of IGF-I in the milk samples were determined using an RIA procedure.

In addition to the raw milk samples, milk from supplemented cows collected on days 3 and 10 of the second treatment period was pasteurized and heat-treated, and analyzed for IGF-I using the RIA method. The method had a sensitivity limit of 1 ng/ml. Pasteurized milk was heated to 63 ± 2°C for 30 minutes *as per* the Pasteurized Milk Ordinance. Heat-treated milk was autoclaved at 121 ± 2°C, 19 ± 2 psi for five minutes. Heat-treated samples were analyzed at 1, 6 and 24 hours after heat treatment to demonstrate that IGF-I was permanently denatured by the treatment and did not renature after cooling to ambient temperature.

IGF-I concentrations in the milk of treated and control cows for each specified day through two injection periods are shown in Table 3.

Table 3. Concentration of IGF-I (ng/ml) in the Milk of Control and Somidobove-treated Cows for Each Specified Day Through Two Injection Periods

Injection Period	Day Post-injection	IGF-I [mean ± SD]	
		Control	Somidobove
Pretreatment	- -	19.8 ± 4.0	22.2 ± 7.6
1	3	18.0 ± 3.1	22.2 ± 4.7
1	10	21.6 ± 3.4	26.7 ± 6.3
1	17	22.7 ± 4.8	22.8 ± 5.8
1	24	21.3 ± 4.9	18.4 ± 3.6
2	3	21.0 ± 2.8	23.8 ± 4.8
2	10	26.3 ± 6.6	30.8 ± 8.6
2	17	25.3 ± 6.8	23.4 ± 4.5
2	24	24.4 ± 5.8	23.0 ± 3.7

It should be noted that IGF-I concentrations in raw milk samples are similar for cows of different parities and different treatment-parity combinations. Table 4 demonstrates the effect of pasteurization and heat treatment on the IGF-I levels in the milk of somidobove-treated cattle. Table 5 contains information on the concentration of IGF-I in 3 brands of commercially available pasteurized milk and 3 brands of infant formulae.

Table 4. IGF-I Concentrations (ng/ml) in the Milk of Somidobove-treated Cattle Following Pasteurization and Heat Treatment

Milk Treatment	Hours Post-treatment	IGF-I [mean ± SD]
None	0	24.1 ± 4.3
Pasteurized	1	23.9 ± 4.1
	6	29.5 ± 5.1
	24	27.5 ± 4.0
Heat-treated	1	14.3 ± 2.6
	6	14.2 ± 1.9
	24	12.9 ± 2.4

The sponsor concludes that pasteurization has no effect on the concentration of IGF-I in milk. Autoclaved milk, simulating processing for infant formulae, had significantly reduced (35 - 48%) levels of IGF-I compared to raw milk.

Table 5. Concentration of IGF-I (ng/ml) in Commercially Available Pasteurized Milk and Infant Formulae

	IGF-I [mean ± SD]
3 Brands of Milk	
A	25.5 ± 6.5
B	25.0 ± 4.4
C	23.6 ± 4.6
Brand of Formula	
1	13.6 ± 3.4
+ 50 ng IGF-I/ml	83.2 ± 9.7
2	16.3 ± 1.6
+ 50 ng IGF-I/ml	78.2 ± 9.3
3	7.3 ± 2.2
+ 50 ng IGF-I/ml	69.7 ± 5.3

Monsanto also conducted a milk residue study to determine if an increase in IGF-II concentrations existed in sometribove-treated cows. Sixty-four lactating Holstein cows (21 primiparous, 43 multiparous) were used in the study and the animals either received 500 mg sometribove in an oil-based prolonged release formulation or excipient via intramuscular or subcutaneous injection at 14 day intervals. Treatments were administered from 60 ± 3 days postpartum until a minimum of

74 days prior to expected calving or until a cow's average daily milk production for an injection cycle dropped below 5 kg/day (when dry-off occurred). Milking was done twice daily at 0600 and 1800 hours. Composite milk samples from each cow were collected on day -7 of the pretreatment period and on day 7 of injection cycles 1-10.

Milk concentration of IGF-I was increased across the 10 injection cycles. The average increase in milk IGF-I concentration was 2.2 ng/ml and there was no parity by treatment interaction. There was no increase in milk IGF-II concentrations in any of the sampling periods (Table 6). (Miller et al., 1989b)

Table 6. The effect of 500 mg of sometribove administered intramuscularly (IM) or subcutaneously (SC) on milk concentrations of IGF-I and IGF-II (*least squares means ± SEM).

Sampling Period	Primiparous Cows	Multiparous Cows
	Milk IGF-I Concentration* (ng/ml)	
Overall Cycle 1-10		
Control	3.5 (± 0.67)	3.9 (± 0.39)
IM	5.9† (± 0.59)	5.9† (± 0.37)
SC	6.1† (± 0.60)	5.6† (± 0.39)
	Milk IGF-II Concentration* (ng/ml)	
Overall Cycle 1-10		
Control	106.6 (± 9.11)	97.8 (± 6.21)
IM	116.3 (± 8.47)	107.2 (± 5.99)
SC	116.4 (± 8.36)	94.5 (± 5.95)

†These means are significantly different from the control values ($P < 0.05$, protected t-test).

In summary, the biweekly injection of 500 mg of sometribove in lactating cattle increased milk IGF-I concentrations. However, there was no increase in milk IGF-II concentrations in milk from bST-treated animals.

Cattle Tissues

Five lactating dairy cows were administered 500 mg of sometribove every 14 days. While on treatment, the muscle and liver of the cows were biopsied at each of the following times: after the first injection, 7 days after the first injection, after the second injection (day 14), 7 days after the second injection (day 21), and after the

third injection (day 28). The samples were analyzed using validated RIA procedures. The results of this study are summarized in Table 7. (Hammond et al., 1990).

Table 7. Concentration of IGF-I (ppb) in Biopsied Tissues of Dairy Cattle Injected with 500 mg of Sometribove

Withdrawal Time (days)	Muscle		Liver	
	Control	Treated	Control	Treated
0	80 ± 16[a]	91 ± 26	77 ± 6.2	72 ± 9.0
7	272 ± 160[b]	312 ± 130	72 ± 9.1	162 ± 36
14	252 ± 141	152 ± 62	72 ± 15	112 ± 11
21	68 ± 20	126 ± 58	70 ± 8.3	142 ± 52
28	215 ± 173	135 ± 19	70 ± 14	92 ± 15

[a]Values are means ± SD
[b]Elevated IGF-I levels are associated with wound healing as biopsies at these intervals were collected from the same anatomical locations (Jennische et al., 1987).

Human Milk

Human milk concentrations of IGF-I were measured during the first 9 days postpartum (Baxter et al., 1984). The mean IGF-I concentration at 1-day postpartum was 17.6 ng/ml, at 2-days postpartum was 12.8 ng/ml and at 3-days postpartum was 6.8 ng/ml. After 3-days postpartum, the IGF-I concentration stabilized over the following week at 7-8 ng/ml. In a subsequent article (Corps et al., 1988), IGF-I concentrations in human milk were measured and ranged between 13 and 40 ng/ml at 6-8 weeks postpartum with a mean of 19 ng/ml. It was determined that IGF-I concentrations in human milk were 2- to 3-fold higher at 6- to 8-weeks postpartum than that 3-7 days postpartum.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

The analytical methods used to determine the amount of bST and IGF-I in the plasma, milk and tissue of the cow were exclusively immunoassay procedures, primarily RIA (Collier et al., 1991; Malven et al., 1987; Torkelson et al., 1987). Each sponsor developed their own immunoassay; none could distinguish between the natural bST and their recombinant bST product. All procedures were validated and extensively evaluated. The 2-3 fold difference seen in the concentrations of bST and IGF-I between companies reflect differences in the antisera and differences in the purity of the reference standard used (Grings et al., 1988). When rbST standard is used, values are approximately 3.3 times lower than values ontained with NIH standards.

APPRAISAL

There are 4 different recombinantly derived bST (rbST) products being evaluated by the Committee. They either slightly differed in the N-terminal portion of the protein from pituitary bST or are identical in amino acid sequence. Treatment of lactating dairy cows with rbST causes an increase in plasma bST concentrations

and milk production which is physiologically indistinguishable from the changes induced with pituitary-derived bST. The analytical methods used to determine the concentration of bST in plasma, milk or tissues do not differentiate between rbST and endogenous bST. Thus, when concentrations of bST are given, they are total bST concentrations.

Milk residue studies demonstrate, even at exaggerated doses, that the proposed use of rbST will not lead to any detectable concentrations of bST in milk above those normally present in untreated cows (0.9-1.6 μg/L). Additionally, the major metabolite identified in the serum was the same as the bST fragment cleaved by thrombin, between amino acid 132 and 133.

In regard to tissue residue data, rbST treatment of cows leads, at most, to a 2-fold increase in bST concentrations to levels of 4.2 μg/kg in muscle and 25 μg/kg in liver.

Some studies suggest that rbST treatment may produce a slight increase in the average milk IGF-I (insulin-like growth factor I) concentration; however, the most definitive and comprehensive studies demonstrate that IGF-I concentrations are not altered after rbST treatment. Additionally, IGF-II concentrations in cows' milk are also not affected by rbST treatment.

Further results indicate that rbST is denatured after pasteurization, but IGF-I is not destroyed by the pasteurization process. However, the heating of milk for the preparation of infant formula reduces the amount of IGF-I by at least 50%. Additionally, human breast milk contains IGF-I concentrations which are similar to those found in control and rbST-treated cows.

IGF-I concentrations in the biopsied muscle and liver of rbST-treated cows increase at most 2-fold to 300 and 160 mg/kg, respectively. However, these elevated IGF-I concentrations in the muscle may not be related to rbST treatment but to wound healing.

The effects of rbST on the major components of milk, if any, are minor and primarily occur early in the treatment period prior to adjustments in dry matter intake by the cow. Furthermore, milk composition of treated cows is well within the normal variation observed during the course of a lactation. Thus, there appears to be no significant impact of rbST treatment on the nutritional and processing qualities of milk.

APPENDIX

Effects of bST Treatment of Cows on Milk Composition

Milk and dairy products can serve as major source of nutrients for both children and adults and are particularly high in protein, calcium, phosphorus, and several vitamins. The unique biophysical properties of milk also impact its processing into various dairy products. Thus, any production drug, feed, or management technique proposed for dairy cows would be of questionable value if it adversely altered the nutritive and processing qualities of milk.

The physiology and biochemistry of milk synthesis in dairy cows do not appear to be significantly changed due to bST. Studies evaluating the energy metabolism of dairy cows treated with rbST suggest that nutrient digestibility, maintenance requirements, and the utilization of metabolizable energy for milk production are unchanged. Rather, more nutrients are directed toward milk synthesis as opposed to tissue deposition (Bauman et al., 1985; Tyrrell et al., 1988; Kirchgessner et al., 1989; Sechen et al., 1989; McGuffey et al., 1991).

Early experiments reported in the literature (c. 1980-1985) suggested no direct effects of short-term bST treatment of dairy cows on fat, protein, lactose, calcium, and phosphorus content of milk. However, evaluation under proposed conditions for use is an important aspect of the review of production drugs. For this reason the drug sponsors were requested to submit data from repeated sampling and analysis of milk composition throughout full lactation studies of effectiveness and animal safety of rbST.

Numerous reports of experiments which evaluated the effects of rbST on milk production and general composition have been published. Some researchers have also examined in detail the chemical composition and processing qualities of milk from rbST-treated cows. This section will focus primarily on published reports in addition to abstracts which specifically address the composition of milk from rbST-treated cows.

Lipid Components. The majority of the lipid component of bovine milk is composed of triglycerides (97-98%). Remaining lipids include diglycerides, monoglycerides, free fatty acids, phospholipids, cholesterol, and other components in minute quantities. Fat content in fresh milk is the most variable of its major components and is dependent upon a number of factors such as diet, stage of lactation, season, and environment. Differences also exist among breeds of cattle. Holsteins, the predominant breed in the U.S., have the lowest milk fat content (about 3.6%), whereas Jerseys produce milk with the highest concentration (about 5.0%; Jenness, 1985).

Dietary changes are probably the easiest way to manipulate milk fat content. The main precursors of bovine milk fat are affected by diet through changes in rumen fermentation and availability of endogenous fatty acid sources. In particular, feeding rations high in readily fermentable carbohydrates (grain) and low in fiber (forage) to increase energy content of the ration may depress rumen pH. Rumen fermentation in turn is altered such that fatty acid precursors are reduced. In

extreme cases, milk fat percent may be reduced as much as 60 percent. The physical form of forages in the diet also has an influence of fat percent; for example, finely ground, immature, or low fiber forages may depress rumen pH and reduce milk fat content. Some of these negative effects of highly fermentable feeds on milk fat percentages may be minimized by adding buffers to diets to increase rumen pH (Linn, 1988).

Concentrations of milk fat also vary in cows over stage of lactation. Milk fat percent of colostrum is relatively high (>5%) and then decreases until about 5 to 10 weeks postpartum (about 3.6%), at which point fat content increases until the end of lactation (approximately 45 weeks) to about 4% (Jenness, 1985). Part of this variation can be attributed to the high grain diets typically fed to cows in early lactation to maximize energy consumption during peak lactation. However, the high milk volume in early lactation also dilutes fat concentration.

Other factors influencing milk fat composition include age and seasonal changes. Fat content of milk decreases as cows become older and on average is 0.4 percentage units lower during summer months than in cooler seasons, although diet may confound seasonal effects (Jenness, 1985; Linn, 1988).

Peak daily milk yield in the dairy cow occurs at approximately 4 to 8 weeks postpartum and then gradually decreases. Voluntary feed intake follows a similar pattern, but maximum intake occurs between 10 and 14 weeks postpartum. Thus, cows are typically in negative energy balance in the first two months of lactation and mobilize considerable amounts of adipose tissue to supply the tremendous amounts of energy needed to sustain high levels of milk production during early lactation. Increased circulating concentrations of nonesterified fatty acids (NEFA) due to mobilization of adipose tissue can increase milk fat percentages. Adipose tissue stores are typically replenished in late lactation as daily milk yields diminish over time (NRC, 1989).

Milk that is commercially available does not reflect the natural variation in fat content among cows since standardization to various fat levels is a regular part of the commercial processing of milk.

Effects of bST on milk fat percent are dependent upon the energy balance of the treated animal. An increase in milk yield typically occurs within 5 days after initiation of treatment. When milk production response causes the animal to be in negative energy balance, milk fat percent is usually increased due to mobilization of adipose tissue (Bauman et al., 1988) and increased plasma concentrations of NEFA (Eppard et al., 1985a; Peel and Bauman, 1987; Bauman et al., 1988; Lough et al., 1988; Van den Berg, 1989). In contrast, when bST-treated cows were consuming sufficient quantities of nutrients to meet the energy needs for additional milk synthesis, body lipid mobilization did not increase, but lipid synthesis was instead reduced (Sechen et al., 1989). In this scenario, blood levels of NEFA were unchanged, and milk fat content was also unaffected (Eppard et al., 1985a; Peel and Bauman, 1987; Sechen et al., 1989; Van den Berg, 1989).

Long-term administration of rbST to dairy cows usually results in increased voluntary dry matter intake after approximately 5 to 10 weeks of treatment to

support increased milk synthesis (Bauman et al., 1985; 1989; Elvinger et al., 1988; Soderholm et al., 1988). Depending on the stage of lactation when treatment is initiated and dose of rbST administered, cows will typically be in negative, or at least reduced, energy balance in the first few weeks of treatment until the adjustment in intake (Bauman et al., 1985; 1989; Elvinger et al., 1988; Baer et al., 1989), and milk fat percent may be increased during this period (Baer et al., 1989). Most studies indicate that milk fat percentages averaged over the entire rbST treatment period are no different than controls (Bauman et al., 1985; 1989; Elvinger et al., 1988; Soderholm et al., 1988; Baer et al., 1989; Van den Berg, 1989). Lean et al. (1991) found that cows treated with relatively high doses of a daily injectable rbST product in their previous lactation tended to have lower milk fat percentages than controls in the early postpartum period (i.e., before rbST treatment resumed). This was probably due to the fact that the formerly treated cows had maintained higher dry matter intake and energy balance than controls and lower serum NEFA concentrations during the postpartum period.

In most mammalian tissues the predominant fatty acid synthesized is palmitic acid (16:0). However, enzymes in the mammary gland are modified to permit synthesis of shorter chain fatty acids. The fatty acids between 4 and 15 carbons in length as well as about 50% of C16 fatty acids are synthesized de novo within the mammary gland. The remaining C16 and all of the longer fatty acids are derived from dietary sources or body lipid stores. The net effect is that approximately 50% of milk fatty acids are between 4 and 16 carbons in length, and the remaining fatty acids are predominantly 18 carbons long. Greater than 95% of these fatty acids in bovine milk are found in triglycerides (Jenness, 1985).

Similar to fat percent, effects of rbST treatment on fatty acid composition in milk appears to depend on energy balance of the treated cow. In general, when production responses due to bST treatment result in negative energy balance, adipose tissue mobilization increases, resulting in a smaller proportion of short- to medium-chain fatty acids (less than 16 carbons in length) and more long-chain fatty acids (16 and 18 carbons) in milk fat. If energy consumption is sufficient to maintain positive energy balance, proportions of fatty acids do not change (Bitman et al., 1984; Eppard et al., 1985b; Lough et al., 1988; Van den Berg, 1989). Lynch et al. (1988a) demonstrated that shifts in fatty acid composition of milk with stage of lactation were the same in both treated and control cows (treatment beginning at 60 days postpartum). In early lactation when both groups of cows were in negative energy balance, the percentage of short-chain fatty acids in milk was lower, whereas long-chain fatty acids were higher in concentration. As cows returned to positive energy balance after 8 to 12 weeks of lactation, these changes were reversed. The changes due to stage of lactation were independent of rbST treatment. Differences in proportions of fatty acids in milk of treated cows as compared to controls are only evident early in the treatment period prior to adjustments in feed intake (Lynch et al., 1988a; Baer et al., 1989; Van den Berg, 1989). Lean et al. (1991) reported lower ratios of long-chain to short-chain fatty acids in milk during the early subsequent lactation period in cows treated with high doses of bST on a daily basis in their previous lactation compared to controls. The formerly treated cows were consuming more feed and were in higher energy balance that controls during this period and presumably mobilizing less body lipid.

The normal cholesterol content of milk lipid is approximately 0.2 to 0.4 percent (Jenness, 1985). Bitman et al. (1984) observed a significant decrease in milk cholesterol content in cows treated with rbST for 14 days and in negative energy balance (0.34 vs. 0.27% of milk lipid). However, over a longer period of treatment (days 60 through 305 of lactation), Lynch et al. (1989a) found that cholesterol content of milk fat was similar between control and rbST-treated cows, 33.1 and 34.6 mg per cup of whole milk, respectively, or approximately one-ninth of the recommended daily intake of cholesterol (300 mg/day). Once again, stage of lactation had the greatest effect on milk cholesterol content; levels increased in both control and treated cows as lactation progressed.

To summarize, treatment of cows with rbST does not create new and unusual fatty acids in milk, nor does it affect cholesterol content. During long-term treatment with rbST, changes in proportions of fatty acids are minor and return to control levels once dry matter intake adjusts and nutrient requirements to support increased milk yields are met. Stage of lactation has a much greater effect on fatty acid and cholesterol content in milk than rbST treatment.

Total Protein (Nitrogen) Fraction. Total protein in milk includes various caseins, whey proteins (e.g., lactoglobulin, lactalbumin, albumin, immunoglobulins) and nonprotein nitrogen (e.g., peptides, amino acids, urea, ammonia). Total protein percent of fresh milk (i.e., nitrogen times 6.38) often shows considerable variation, although not to as great an extent as fat. Among breeds, Holsteins average 3.2% protein, whereas milk from Jerseys is typically around 3.8%. Total protein content in bovine colostrum is high (about 16%), primarily due to increased immunoglobulin concentrations. This quickly reduces to approximately 3.5% within 5 days postpartum. Milk protein content reduces to approximately 3.0% at 5 to 10 weeks postpartum, and then increases to 4.0% towards the end of lactation, thus corresponding inversely to milk yield (Jenness, 1985).

Insufficient amounts of dietary protein will reduce milk protein concentration, although the source protein in the diet may also have an effect. Similar to fat percent, protein in milk tends to decrease as cows age. High temperatures also have a negative effect on milk protein content (Jenness, 1985; Linn, 1988).

The percent total protein in milk of cows treated with bST appears to be dependent primarily upon resultant nitrogen balance. A number of studies have demonstrated that if treatment results in a negative or significantly reduced nitrogen balance, protein content of milk is reduced, indicative of a limited supply of amino acids (Eppard et al., 1985a; Peel and Bauman, 1987; Bauman et al., 1988; Baer et al., 1989; Van den Berg, 1989). In contrast, if positive nitrogen balance is maintained, the protein content of milk usually does not change (see Eppard et al., 1985a; Peel and Bauman, 1987; Lough et al., 1988; Sechen et al., 1989; Van den Berg, 1989). In long-term treatment with daily injectable rbST products (5 to 40 mg/day), feed intake adjusts to meet nutrient requirements for extra milk production, and milk protein percent over the lactation is not significantly altered from controls (Bauman et al., 1985; Elvinger et al., 1988; Leonard et al., 1988; Soderholm et al., 1988; Baer et al., 1989). An exception was the study by Kindstedt et al. (1991) in which Jersey cows treated with 500 mg bST/14 days starting 8 weeks postpartum until

the end of lactation had significantly lower total milk protein than controls (3.92 vs. 4.12%) averaged over the entire treatment period despite being higher in average protein balance during the same period. Nevertheless, stage of lactation had a greater effect on milk protein percent that bST treatment (Elvinger et al., 1988; Leonard et al., 1988; Kindstedt et al., 1991).

Milk protein percent (and milk fat content to a lesser extent) in cows treated with sustained release rbST products shows a cyclic pattern; the percent tends to be higher in the latter phase of the injection cycle, i.e., the last few days of 14-day cycles and week 4 with 28-day sustained release products (Bauman et al., 1989; Van den Berg, 1989). Milk yield also had a cyclic pattern with these sustained release products, with peak yield occurring during the first half of each cycle. Since milk protein percent during the "valleys" of cycles in rbST-treated cows did not fall below control levels the changes did not appear to be related to nitrogen balance. Rather, different half-lives for enzymatic processes related to milk synthesis may vary (Bauman et al., 1989). For example, if enzymatic changes related to lactose production (and therefore milk volume, see below) had shorter half-lives as compared to those related to fat and protein synthesis, expected changes in milk volume would result in the observed patterns. Indeed, milk lactose concentration was relatively constant during injection intervals (Bauman et al., 1989).

Because of the cycling of total protein percent in milk of cows treated with sustained release rbST products, interpretation of the effect on protein content may be influenced by the days in which milk samples are collected. Bauman et al. (1989), who sampled milk on days 5 and 12 of each 14-day cycle, reported increased content of total protein in milk (3.34 vs. 3.25%) when cows were treated with 500 mg rbST/cycle. Kindstedt et al. (1991) found total milk protein to be lower in Jersey cows injected every 14 days with 500 mg rbST. Milk was sampled on day 8 of the cycle, when milk protein was expected to be lowest in treated cows. Nevertheless, Ozimak et al. (1989) observed the opposite effect despite a similar sampling schedule as Kindstedt et al. (1991); total protein content was higher in milk of treated cows than in milk of controls (350 mg rbST/14 days; 3.52 vs. 3.27%) with sampling on day 7 of the 14-day cycle. Lenoir and Schockmel (1987) measured no differences in total protein of milk from cows treated with rbST on a biweekly basis versus controls. However, milk was sampled once per month on a calendar basis over 6 months, and therefore, was not synchronized with injection cycles. This suggests that cycling of milk protein with sustained-release products would likely have little influence on commingled milk from different farms since cows are at different stages of lactation and would be on different injection schedules.

Approximately 95% of the total nitrogen fraction in bovine milk is found in true proteins, and in general, this fraction provides the majority of the nutritional quality of milk with respect to protein. Milk proteins are generally subdivided into two classes: caseins and whey proteins.

Caseins are unique to milk and make up approximately 80% of the true protein in bovine milk (Jenness, 1985). There are several types of caseins including alpha- (several variants), beta-, gamma-, and kappa-casein which, along with fat content,

determine the potential yield and quality of cheese produced from a volume of milk. For example, curd formation is triggered by specific proteolysis of kappa-casein, and adequate separation of whey from curds is dependent upon beta-casein (see review by Pearse and Mackinlay, 1989).

A number of studies examining casein content, expressed as a percent of milk, in cows treated with rbST over a full lactation demonstrated no differences from control levels (Lenoir and Schockmel, 1987; Leonard et al., 1988; Barbano et al., 1988; Baer et al., 1989; Van den Berg, 1989). Ozimek et al. (1989) found a significant increase in both true protein (3.17 vs. 2.88%) and casein content (2.63 vs. 2.38%) of cows treated every 14 days with 350 mg rbST. However, Kindstedt et al. (1991) reported lower true protein (3.74 vs. 3.95%) and casein (3.11 vs. 3.34%) in treated Jerseys (500 mg rbST/14 days). Baer et al. (1989) noted that true protein and casein percent tended to decrease in the first 2 weeks of treatment when cows were in negative nitrogen balance, but then returned to control levels as the lactation progressed. Indeed, stage of lactation and animal variability had more significant effects on casein composition of milk than rbST treatment (Van den Berg, 1989).

When expressed as a percent of true protein, there has been a trend toward lower casein content, e.g., 78.8 vs. 80.2% (Baer et al., 1989) and 83.38 vs. 84.52% (Kindstedt et al., 1991) in rbST treated vs. control cows, although not in all cases (Lynch et al., 1988a). This might be explained by an increase in total whey protein content in milk (Baer et al., 1989). Nevertheless, both Baer et al. (1989) and Kindstedt et al. (1991) estimated that the minor reductions in casein content they observed as a percent of true protein would amount to no more than a 0.1% lower theoretical yield of Cheddar cheese per weight of milk. Concentrations and proportions of the various casein fractions in milk of cows treated with rbST over a lactation were not different from controls (Lenoir and Schockmel, 1987; Leonard et al., 1988; Lynch et al., 1988a; Van den Berg, 1989).

Noncasein proteins in milk fall into the broad category of whey (or serum) proteins. Two of the principal whey proteins are specific products of the mammary gland: alpha-lactalbumin and beta-lactoglobulin. Other whey proteins include immunoglobulins, albumins, about 50 different enzymes, and many other compounds (Jenness, 1985).

Alpha-lactalbumin is part of the lactose synthetase complex, a rate-limiting enzyme in the synthesis of lactose, the major milk carbohydrate. It is secreted along with lactose into milk. Beta-lactoglobulin is the most abundant protein in bovine whey, but its biological function is unknown (Jenness, 1985).

Whey protein percentages in milk of cows treated with daily injectable (Leonard et al., 1988), 14-day sustained release (Lenoir and Schockmel, 1987; Kinstedt et al., 1991) or 28-day injectable rbST products (see review by Van den Berg, 1989) were not significantly different than controls. In contrast, whey protein percent was higher in a study using a daily injectable product (0.71 vs. 0.65%; Baer et al., 1989) and a 14-day injectable (0.54 vs. 0.49%, Ozimek et al., 1989). Although not measured, Baer et al. (1989) theorized that higher alpha-lactalbumin content in milk of treated cows may have increased the percent of whey protein and could

explain the increase in total lactose, and consequently yield of milk, in rbST-treated cows. Indeed, Eppard et al. (1985) and Ozimek et al. (1989) reported increased alpha-lactalbumin content in milk of rbST-treated cows, although Lynch et al. (1988a) found no change as a percent of true protein. Relative percents of beta-lactoglobulin were unchanged (Lynch et al., 1988b) or higher (Ozimek et al., (1989) in rbST-treated cows.

Nonprotein nitrogen (NPN) content of bovine milk is approximately 6% of total milk nitrogen and includes amino acids, ammonia, and urea (Jenness, 1985). Nonprotein nitrogen percent or its percent of total nitrogen was higher (4.61 vs. 4.26%, Kindstedt et al., 1991; 0.179 vs. 0.172%, Barbano et al., 1988; 0.40 vs. 0.35%, Ozimek et al., 1989) or not different (Baer et al., 1989; Lenoir and Schockmel, 1987) in rbST-treated cows as opposed to controls.

Overall, results to date concerning the protein composition of milk have shown only slight, if any, alterations due to rbST treatment of cows. The type of rbST product and influence due to cycling (i.e., daily injectable vs. sustained release) and milk sampling schedule may influence interpretations. Some studies indicate a trend toward slightly increased whey proteins and NPN and decreased milk casein in rbST-treated cows. A question which remains is whether such effects are typical for cows which naturally produce more milk. Most studies over full lactations report considerably more variation due to stage of lactation than rbST treatment. The differences in milk protein composition have been small, and it appears that they would have virtually no impact on nutritional quality of milk and only minor, if any, effects on processing characteristics.

Lactose. The principle carbohydrate in the milk of most species is lactose, a disaccharide composed of glucose and galactose. Bovine milk contains approximately 4.6% lactose, and although it does vary slightly with breed of cow, stage of lactation, and climate, it is generally very constant due to its role in maintenance of milk osmolality. Overall, bST treatment has not altered lactose content in milk in short-term studies (Eppard et al., 1985; Tyrrell et al., 1988) and in studies carried out over full lactations (Bauman et al., 1985; Barbano et al., 1988). Baer et al. (1989), observed a higher concentration of lactose in milk of cows treated over a lactation (4.80 vs. 4.71%), but the minute difference is of questionable biological significance.

Minerals. While calcium and phosphorus are generally considered the most important minerals in milk, over 30 minerals have been detected. The total ash content of bovine milk averages about 0.75% (Jenness, 1985), and this was not changed in milk from cows treated with rbST over an entire lactation (Bauman et al., 1989).

Bovine milk contains approximately 1.2 g calcium and 1 g phosphorus per kg (NRC, 1989). Calcium and phosphorus content of milk of bST-treated cows are within normal ranges and show no consistent changes due to treatment (Eppard et al., 1985b; Lenoir and Schockmel, 1987; Bauman et al., 1989; Pikus et al. 1989; Van den Berg, 1989). This has similarly been demonstrated with concentrations of sodium, potassium, iron, magnesium, copper, manganese, and zinc in milk of cows treated with bST (Eppard et al., 1985b; Pikus et al., 1989; Van den Berg, 1989).

Vitamins. Milk is an important source of thiamine (vitamin B-1), riboflavin (vitamin B-2), pyridoxine (vitamin B-6), pantothenic acid, biotin, vitamin B-12, and choline. It also provides moderate amounts of niacin, folic acid, vitamins C, D, and E, and is very low in vitamin K. The bovine mammary gland cannot synthesize vitamins, so it is dependent upon its blood supply for vitamins secreted into milk. Vitamins A, D, E, and K are fat-soluble and are associated with the fat component of milk.

The B-vitamins and vitamin C are water soluble and therefore associated with the skim fraction of milk (Jenness, 1985).

Hartnell et al. (1987) measured the content of several vitamins in milk of 42 cows treated with either a placebo or sustained release rbST (500 mg/14 d). Morning milk samples collected on several days within an injection cycle were pooled per treatment group from cows at about 30 weeks postpartum and on treatment 18 to 24 weeks. Content of the following vitamins was not different in milk of rbST-treated cows as compared to controls: vitamin A, thiamine, riboflavin, pyridoxine, vitamin B-12, choline, and pantothenic acid. Biotin levels were significantly increased in milk of treated cows (0.0017 vs. 0.0019 mg/100 g), however, the small change was of questionable biological significance. Brown (1988) also determined that concentrations of vitamin A in milk of cows which were treated with rbST for a second consecutive lactation were not different than in controls.

Processing qualities and acceptability of dairy products. Van den Berg (1989) reviewed a number of U.S. and European studies evaluating the yield and quality of cheese produced from milk of rbST-treated cows. It was concluded that the manufacturing properties of milk plus yield and quality of cheeses were not affected by rbST treatment of cows.

Azzara et al. (1987), Baer et al. (1989), Lenoir and Schockmel (1987), Lynch et al. (1988b, 1989a, 1989b), and Marshall and Cartledge (1988) evaluated a number of characteristics important in the processing and stability of milk, including protease and lipase activity, starter culture growth, pH, freezing point, coagulation times, thermal properties of fat, and susceptibility to rancid flavor. No differences were detected between milk of controls and rbST-treated cows over treatment periods ranging from 6 months to a full lactation.

Taste panel evaluation of milk and cheese from rbST-treated cows has demonstrated no significant differences in taste, odor, texture, and acceptability compared to products from nontreated animals (Baer et al., 1989; Lynch et al., 1989b, Van den Berg, 1989).

Summary. The physiology and biochemistry of milk synthesis in dairy cows is not significantly altered by rbST treatment. To date, the effects of rbST on the major components of milk, if any, are minor and primarily occur early in the treatment period prior to adjustments in dry matter intake by the cow. Furthermore, milk composition of treated cows is well within the normal variation observed during the course of a lactation. Thus, there appears to be no significant impact of rbST treatment on nutritional and processing qualities of milk.

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