Global Veterinaria 4 (4): 366-373, 2010
ISSN 1992-6197

The Effect of Recombinant Bovine Somatotropin Administration on Milk Production, Some Hemato-Biochemical Parameters and Reproductive Performance of Lactating Cows

[1]Azza, H. Abdelrahman, [2]A.S. Khalil, [3]H.T. EL-Hamamsy and [4]O.H. Ezzo

[1]Department of Clinical Pathology, Faculty of Veterinary Medicine, Beni-Sueif University, Egypt
[2]Farms' Quality Manager, Juhayna Food Industries, 6th October City, Giza, Egypt
[3]Department of Internal Medicine, Faculty of Veterinary Medicine, Cairo University, Egypt
[4]Department of Animal Reproduction and Artificial Insemination,
National Research Center, Dokki. Egypt

Abstract: An experiment was conducted to examine the effect of bovine somatotropin on the milk yield, hematology, some serum biochemicals values and some reproductive performance of primiparous and multiparous cows. Sixty Holstein-Friesian cows were divided into 2 groups; 30 primiparous and 30 multiparous, each group was further sub-divided into 3 subgroups each of 10 cows, the first group was served as control while the 2nd and 3rd groups were injected once every 2 weeks for 5 months with 250 and 500 mg of bovine somatotropin (rbST), respectively. For all groups, milk production/day was registered during the whole period of the experiment. Blood samples were collected for the hematological examination and some serum biochemical parameters. The reproductive performance as expressed by days-open and conception rate were recorded. Supplementation with rbST resulted in significant increase in the average milk production especially in multiparous cows given rbST. The hematological parameters revealed a non significant changes in the erythrogram while leucogram showed a non significant leucocytosis accompanied with neutrophilia and lymphocytosis, the activity of both AST and ALT and creatinine revealed non significant changes in both primiparous and multiparous cows given rbST. While the level of insulin, total protein, albumin and lipogrammes revealed non significant increase in both primiparous and multiparous cows given rbST, while, level of urea and calcium showed significant decrease and phosphorus level showed a reverse trend in cows received rbST. Regarding the days-open in months the study showed non-significant differences between subgroups for primiparous cows while there was apparent increase in multiparous cows receiving 250 and 500 mg bST. An improvement of conception rate (%) in all treated cows as compared with that of control was observed.

Key words: Somatotropin % Cows % Milk production % Blood metabolites % Reproductive performance

INTRODUCTION

Somatotropin is a key homeorhetic which is involved in the regulation of nutrient partitioning. It was found that, its administration to dairy cows increases milk production and improves the efficiency of milk synthesis [1]. Recombinant bovine somatotropin (rBST) is a synthetically derived hormone that may be identical to naturally occurring bovine growth hormone, or slightly modified by the addition of extra amino acids. The biological effects of rbST have been extensively investigated and the ability of this technology to enhance productive efficiency while maintaining the health and well-being of dairy cows is well established [2]. Previous studies have demonstrated that somatotropin is a key control of nutrient use. When exogenously administered, bovine somatotropin (bST), markedly improves productive efficiency in lactating cows [3]. Improvement of efficiency and economic return is an important goal in dairy farming, as in any agricultural enterprise. With the use of bST, a unit of milk is produced with less feed and protein supplement and with a reduction in animal excreta (manure, urine and methane). Nationally, the use of bST is simply reinforced, but it does

Corresponding Author: Azza, H. Abdelrahman, Department of Clinical Pathology,
Faculty of Veterinary Medicine, Beni- Suief University, Egypt

not fundamentally change, dairy industry trends of increased milk yield per cow, reduced number of cows and declining dairy farm numbers [3]. Administration of exogenous native pituitary or rbST significantly increases milk yield in dairy cattle, either in short [4], or long-term experiments [5]. Moallem *et al*. [6], concluded that daily milk intake (DMI) was significantly increased by bST treatment. During the first 3 weeks of treatment the increase in DMI of the bST treated cows was 46% more than that of controls. Phipps *et al.* [7], reported that milk composition in *Bos indicus* and its crossbreds was unaffected by the administration of bST. The treatment also significantly increased milk yield in Jersey (+ 2.9 kg per day), Friesian (+ 3.6 kg per day) and Holstein (+ 2.7 kg per day) cows. Burton *et al.* [8], suggested that somatotropin possesses a role in immune system of cows, it increased cow's immunity as judged by proliferative responsiveness of peripheral blood lymphocytes in culture. The consequences of bST treatment for fertility are not clear. In early studies, cows treated with bST had an increased calving to conception interval that was attributed, at least in part, to increased incidence of anestrus. [9], In contrast, bST either increased [10] or had no effect [11] on pregnancy rate per insemination when cows were inseminated using a timed artificial insemination (TAI) protocol. Treatment with bST increased pregnancy rate per insemination for repeat breeder cows bred at estrus [12] A lot of researchers, scientists and dairymen proclaimed the esteemed value of somatotropin in increasing beef and dairy production [3] Accordingly, the FDA allowed the commercial use of bST in February 1994. While the effects of rbST on milk production have been extensively studied and reported, less attention has been paid to the possible effects of the drug on target animal health.

The objective of this paper was to report the effect of two different doses of rbST supplemented in dairy cows on, milk yield, hematological picture, metabolic efficiency, Liver and Kidney functions and reproductive performance.

MATERIALS AND METHODS

Materials

Animals: The present study was conducted on Holstein-Friesian cows aging from 2.5 to 8 years and weighing at the start of the experiment from 450 to 550 kg. All animals under investigations were belonging to The Dairy Farm No.14 at El-Tal Al-Kabier military farm. The animals were fed during the period of study a formulated ration according to their production level as shown in Table 1a. The concentrates mixture given to the cows were manufactured by the military factory of concentrates and its composition is shown in Table 1b. The period of the administration lasted 5 months for each group.

Hormone: The hormone used in this study was Somatech™ which is a bovine somatotropin produced by Monsato and distributed by Elanco Animal Health. Somatech Is a Sterile Sometribove Zinc Suspension

Table 1a: Compositions of the Concentrate mixture given to cows during the study

Milk production	Concentrates*	Barseem**/ Darawa***	straw
4 - 6 kg	6 kg	26 kg	3
6 - 8 kg	7 kg	28 kg	3
8 - 10 kg	8 kg	28 kg	3.5
10 - 12 kg	9 kg	30 kg	3.5
12 - 14 kg	10 kg	33 kg	3.5
14 - 16 kg	11 kg	33 kg	4
16 - 18 kg	12 kg	33 kg	4

* Concentrates of military factory

** Tripholium alexandrinum (Egyptian clover)

*** Sorgum Sativum (maize)

Table 1b: Compositions of the Concentrate mixture given to cows during the study

Contents	%
Yellow corn	35
Sun flower seed meal	35
Bran	25
Minerals	0.1
Molasses	2.9
Limestone	1
Salt	1

Diagnostic Kits Used for Biochemical Studies: Serum insulin-coat-A-count® - was determined using radioimmunoassay (RIA) kits, which was obtained from Diagnostic Products Corporation. Diagnostic chemical kits used for determination of glucose, total protein, albumin, triglycerides, creatinine were obtained from Stainbio Laboratory USA, Serum Total lipids was measured using Biodiagnostic kits, while serum cholesterol, AST, ALT and urea were enzymatically determined using QCA kit, Spain. Calcium and inorganic phosphorus were measured using ELITECH kits.

Methodology

Experimental Design: Sixty Holstein-Friesian cows (30 primiparous and 30 multiparous), aging from 2.5 to 8 years were divided into 2 groups; primiparous (cows having calf for the first time) and multiparous (cows having calves for more than one time). Each group was further sub-divided into 3 subgroups; each contained 10 cows, 1st subgroup as a control group, the second injected with 250 mg rbST and the third subgroup injected with 500 mg rbST. The rbST (Somatech™) was subcutaneously injected in the tail depression, 60 days post calving and then every 14 days, the period of the study lasted 5 months for each group, [13].

Milk Production: Milk yield was daily recorded for all cows during the whole period of the experiment.

Hematological Studies: Red and total white blood cell count, packed Cell Volume (PCV) hemoglobin concentration and differential leukocytic count were estimated according to the techniques described by to Jain *et al*. [14].

Biochemical Investigation: Serum was analysed for the following biochemical assays: Insulin concentration was determined using RIA kit according to Bates[15], the sensitivity of the assay is <0.4ng /ml and the intra and interassay coefficients of variation are 6.4 and 10.9 respectively. Glucose level was measured enzymaticaly after Howantiz and Howantiz [16].Total protein was determined according to Weichselbaun [17] and albumin after Dumas and Biggs [18], total lipids, triglycerides and total cholesterol were estimated according to Zoliner and Krisch, [19], Wahlefeld [20]and Stein [21], respectively. Serum AST and ALT were assayed by the method of Reitman and Frankel, [22]. BUN, was determined enzymaticaly after Henry and Jodd [23] and creatinine was colorimetrically determined according to Fabiny and Eringshausen [24] serum calcium was carried out according to Corns and Ludmman, [25] and phosphorus was measured after Daly and Erthingshausen, [26]

Reproductive Performance: All cows were observed twice daily for detection of estrous signs and rectal palpation was carried out every 2 weeks for detection of ovarian structures (corpus luteum and follicles). Estrous cows were artificially inseminated and conception was c onfirmed. Some reproductive performance expressed by days-open and calving interval were registered for each animal during the whole period of the experiment.

Statistical Analysis: The obtained data were statistically analyzed using one way analysis of variance (ANOVA). According to Motulsky [27] using Graphpad prismstat computer software.

RESULTS

Milk Production: The average milk production per day (Table 2) showed non significant increases in primiparous cows and significant (p>0.05) increases in multiparous given bST, especially those treated with 500 mg bST dose.

Hemogram: The erythrogram of cows treated with 250 and 500 mg bST revealed non significant changes in values of RBCs and Hb concentration, PCV % (Table 3). The data of leucogram showed non significant leucocytosis associated with a non significant neutrophilia and lymphocytosis especially with multiparous cows given 500 mg bST. (p = 0.05), Table 4.

Biochemical Parameters: Values of serum biochemical parameters (Table 5) showed that insulin level revealed non significant increase accompanied with significant decrease in glucose level (p=0.05) in all treated groups as compared with control.

Values of total protein, albumin and A/G ratio showed a non- significant increase in both primiparous and multiparous cows receiving both doses of bST.

The estimation of liver and kidney function tests, revealed non significant changes in the activity of both AST and ALT and level of creatinin in all treated groups while, urea level showed significant (p=0.05)decrease in multiparous cows. Serum lipogrammes revealed non significant increase in total lipids and triglyceride and total cholesterol compared to control groups. (p=0.05).

Table 2: Average milk production (Kg/day) of primiparous and multiparous cows given 250 and 500 mg bST

	Primiparous			Multiparous		
Parameter	Control	250mg	500mg	Control	250mg	500mg
Average amount of milk (Kg/day)	11.57±0.84	11.62±0.79	11.70±0.78	12.33±0.85	13.84±0.91	14.81±0.56*

Table 3: Erythrogram of primiparous and multiparous cows given 250 and 500 mg bST

	Primiparous			Multiparous		
Parameter	Control	250mg	500mg	Control	250mg	500mg
RBCs count ($X.10^6/\mu L$)	5.32 ± 0.16	5.7 ±0.19	5.9 ±0.31	4.2 ±0.18	4.32 ±0.22	4.33±0.20
Hb (g/dL)	11.33±0.57	12.54±0.5	12.39±0.95	8.1±0.29	8.21±0.21	8.5±0.34
PCV (%)	29.3±1.28	30.2±1.27	28.2±0.77	25.5±0.91	25±0.56	24.3±0.94
MCV (fl)	54±2.4	53±2.2	54±2.9	60±1.5	59.7±2.1	58.9±2.9
MCH (pg)	21±0.6	22±0.6	21±0.7	19±1.3	19±0.8	20±0.8
MCHC (%)	39±1.9	42±2.1	44±1.5	31±1.04	33±0.94	38±1.56

Table 4: Leucogram of primiparous and multiparous cows given 250 and 500 mg bST

	Primiparous			Multiparous		
Parameter	Control	250mg	500mg	Control	250mg	500mg
WBCs count ($X.10^3/\mu L$)	6.4 ± 0.37	7.11±0.61	7.21±0.33	6.5±0.57	7.31±0.26	7.8±0.27
Neutrophils count ($x.10^3/\mu L$)	3.11±0.16	4.35±0.16	4.11±0.20	3.27±0.16	4.89±0.11	4.69±0.18
Lymphocytes count ($x.10^3/\mu L$)	3.15± 0.18	3.85±0.20	3.92±0.20	3.21±0.23	4.51±0.22	4.41±0.15
Monocyte count ($x.10^3/\mu L$)	0.372±0.1	0.372±0.1	0.334± 0.08	0.255±0.1	0.346±0.15	0.314±0.14
Eosinophils count ($x.10^3/\mu L$)	0.091±0.047	0.079±0.034	0.078±0.033	0.085±0.05	0.073±0.034	0.007±0.003

Table 5: Values of some serum biochemical parameters of primiparous and multiparous cows given 250 and 500 mg bST

	Primiparous			Multiparous		
Parameter	Control	250mg	500mg	Control	250mg	500mg
Insulin level (IU/mL)	6.95±0.75	8.95±0.66	9.07±1.9	5.9±0.67	7.46±0.7	8.1±1.2
Glucose (mg/dl)	62.87±3.56	40.22±2.04*	44.07±2.242*	59.23±3.75	41.31±3.225*	43.4±4.568
Total protein (g/dL)	9.7±0.49	10.8±0.18	10.6±0.22	10.1± 0.43	10.8±0.40	10.9±0.33
Albumin (g/dL)	4.2±0.08	5.2±0.11	5.5±0.11	4.1±0.09	4.8±0.05	5.1±0.11
Globulin (g/dL)	5.5±0.47	6.5±0.16	6.1±0.2	5.1±0.19	5.9±0.40	5.9±0.32
A/G %	0.72±0.10	0.78±0.02	0.81±0.029	0.71±0.07	0.87±0.07	0.91±0.06
AST (IU/L)	16.2±0.52	21.7±0.52	21.1± 0.88	25.1±0.61	22.5±1.5	21.1±0.5
ALT (IU/L)	20.7±1.4	23.2±0.8	21.9±1.6	21.6±1.13	22.7±1.7	22.5±1.2
Urea (mg/dL)	23.67±1.63	22.7±1.50	22.97±1.61	16.51±1.9	12.96±0.93*	12.44±0.75*
Creatinine (mg/dl)	1.52±0.01	1.17±0.05	1.15±0.01	1.38±0.06	1.13±0.01	1.2±0.04
Total lipids (mg/dl)	736±29	739±58	740±38	733±42	741±50	740±36
Triglycerids (mg/dl)	96±5	97.4±11	98±8	93.9±12	95±16	96±12
Cholesterol (mg/dl)	128±9.8	132±9.1	130±4.8	133±8	135±9	136±3
Calcium (mg/dL)	10.8±0.61	10.1 ±0.32	10.3 ±0.22	10.7± 0.25	8.4± *0.52	8.5±0.33 *
inorganic phosphorus (mg/dl)	4.93± 0.13	5.3 ±0.27	5.0±0.23	4.8±0.21	6.4±0.29 *	6.7±0.2 *

Table 6: Reproductive performance for primiparous and multiparous cows given 250 and 500 mg bST

	Primiparous			Multiparous		
Parameter	Control	250mg	500mg	Control	250mg	500mg
Days-open (month)	3±0.43	3.5±0.41	3.2±0.32	2.8±0.42	4.4±0.5	4.8±0.52
Conception rate (%)	60	80	90	50	60	80

The serum calcium level showed significant decrease (p=0.05) in all treated groups which was evident with 250 mg bST dose in primiparous cows while phosphorus level showed a significant increase (p=0.05) in all groups as compared with control groups.

Reproductive Performance: Table 6 reveals days-open (in months) for primiparous and multiparous groups as well as the conception rate. There were no significant differences among subgroups for primiparous cows while there were significant (p=0.05) increase in multiparous cows receiving 250 and 500 mg bST. Concerning conception rate (%) a significant (p=0.05) increase of conception rate (%) in cows given both 250 and 500 mg bST in comparison with the control cows was recorded.

DISCUSSION

From this study, it was evident that bST administration to cows resulted in definite increase in milk production. Primiparous cows showed tiny increases with either 250 and 500 mg, this condition may be explained in light that primiparous cows have non well-developed udder, multiparous cows showed significant increases especially cows received 500mg of bST. These results were in parallelism with a most array of researchers such as Capper *et al.* [2] and Gulay *et al.* [28]. Somatotropin is a homeorhetic controller that shifts the partitioning of nutrient so that more are used for milk synthesis [29]. The result obtained by Faulkner [30] indicated that there were increase in the availability of glucose within the mammary epithelial cells in response to growth hormone treatment that would result in increase in the rate of lactose synthesis and hence stimulation of milk production. In addition, somatotropin appears to promote milk production by a partitioning effect on absorbed nutrients, so to supply more substances for mammary synthesis and also the level of nutrition may influence yield responses for milk [31]. On the contrary to the present results, Witkar *et al.* [32] found that the most conspicuous increase in milk production occurred in primiparous than multiparous cows. However, Davis *et al.* [33] reported that bST did not affect milk production.

The present results of erythrogram revealed non significant changes in all groups received 250 and 500mg of bST, precisely, the present results were broadly in agreement with those obtained by Eppard *et al.* [34] and Sallam *et al.* [35]. Leukogram revealed non significant increase in total leukocyte counts accompanied with neutrophilia and lymphocytosis in all treated groups. The obtained values were concordat with Bulanova *et al*. [36] and Sallam *et al.* [35]. Burton *et al.* [37] gave reverse results, while Hoben *et al.* [38], claimed no change in the leukogrammes upon administration of bST to cows. This increases of total leukocyte counts, neutrophil and lymphocytes, may reflect the positive impact of bST on the immune system of the treated cows [37]. The obtained results of serum insulin revealed a non-significant increase in both groups with administration of bST. These results were in agreement with those obtained by Fike *et al*. [39], whereas; Gulay *et al.* [28] reported no effect of bST on serum insulin level of treated cows. The level of serum glucose showed a remarkable drop especially among primiparous cows injected with 250 and 500 mg bST and multiparous cows injected with 500 mg, these results were generally in agreement with those obtained by Arieli *et al.* [40], while Gulay *et al.* [28] reported no change in serum glucose level upon administration of bST. On the contrary, Sallam *et al.* [35], recorded an increased serum glucose concentration with administration of bST. A possible explanation of such hyperinsulinemia accompanied with hypoglycemia is claimed by Gong *et al.* [41] who stated that the action of bST may be mediated by increase synthesis and secretion of IGF-1 and insulin. The obtained results revealed that a non-significant increase of total protein and albumin was observed with bST injection in primiparous cows. These results were generally in agreement with Davis *et al.* [33], a possible cause of such changes is that bST has an anabolic function causing positive nitrogen balance with tendency toward such increase. The activities of serum ALT and AST which are commonly used as indicator of liver cell damage and death were not affected by treatment with bST. These results agreed with Graf *et al.* [42]. The obtained results for serum urea levels revealed a significant decrease in multiparous cows, while creatinine level showed non significant changes, Generally these results were in agreement with those obtained by Sallam *et al.* [35]. On the contrary Shin *et al*. [43] reported that cows given bST had higher concentration of blood urea and Davis *et al*. [33], reported no effect of bST on serum urea level. Science the serum level of urea is the index of the hepatic amino acids oxidation and of ruminal ammonia fermentation, therefore its decrease may reflect a higher amino acids removed from blood for milk protein biosynthesis. These findings indicated that bST has no adverse effect on renal function. Serum total lipids triglycerides and cholesterol showed a non significant increased in multiparous cows given 500 mg bST, On the other hand, Floris *et al.* [44] reported no change in

triglyceride or cholesterol upon administration of bST. It was claimed that somatotropin mobilizes large quantities of free fatty acids from adipose tissues to be used to supply most of the energy of the body, thus somatotropin acts as a potent carbohydrate and protein sparer [45]. The estimated values of serum calcium and phosphorus levels of cows treated with bST showed a significant decrease in calcium level accompanied with a significant increase in phosphorus in treated multiparous cows, These findings were odd as compared with Desmukh *et al.* [46], who reported that bST had no effect on serum calcium level and Shin *et al*. [43], who found increased values of serum calcium level in cows treated with bST. Increase milk yield upon bST administration might explain the cause of such decrease in serum calcium level. Effect of administration of bST (250 and 500 mg) on some parameters related to breeding and conception revealed that, the recorded results for the days-open of treated cows revealed no significant changes in between primiparous group, but there were apparent increase in average days open in multiparous cows. Concerning conception rate (%), it revealed an increase in cows given both 250 and 500 mg bST in both groups than control cows. Although bST increased days-open in multiparous cows, (possibly due to increased milk production), it simultaneously improved conception rate (%). Recently, Flores *et al.* [47], reported that bST increased GH in beef cattle and hypothesized that bST would alter other metabolic hormones and might influence ovarian follicles in postpartum. Meantime, somatotropin treatment increased concentrations of IGF-I in postpartum cows in the current experiment and is at least partly responsible for the increase in diameter of the largest follicle in anestrous post partum cow. These results - in general - were in agreement with the conclusion of *Bell et al.* [12]. While Silvia *et al.* [48], reported that bST had no effect on reproductive performances. On the contrary, Dohoo *et al.* [49] mentioned that bST altered the reproductive performance of treated cows.

In conclusion, the obtained results in the present study is in favor of using bST, whereas both used doses (250 and 500 mg) increase milk production, especially using 500 mg bSt in multiparous cows, had no deleterious effects on the general health condition of the cows as it had no harm effect on blood picture, did not alter metabolic efficiency of carbohydrate, protein, fats and minerals and did not have any negative effect on liver and kidney functions and the average days open and conception rate would be increased slightly by the use of bST.

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