

## Article

# Effect of Bovine Somatotropin Administration on Mastitis Incidence and Body Condition Score in Dairy Buffaloes

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**Abstract:** The objective of the current study was to assess the impact of recombinant bovine somatotropin (rbST) on mastitis incidence and body condition score (BCS) in Nili Ravi buffaloes. In a double-blinded clinical trial, 50 buffaloes (primiparous and multiparous) at + 60 days post-calving were divided into treatment (n = 25) and placebo control (n = 25) groups. The treatment group received ten doses of 500 mg rbST with 14-day intervals, while the control group received a placebo with the same regimen. Data on dry matter intake, milk yield and milk composition, mastitis incidence, and body condition score were collected. Repeated measures ANOVA was applied to the weekly averages of milk yield, dry matter intake, and milk composition. Individual incidences of mastitis were recorded throughout the study period. The results revealed that milk production significantly increased in both primiparous and multiparous buffaloes treated with rbST. However, the relative risk of mastitis increased by 3.0 times (95% CI: 1.62-6.88) in the bST-treated group compared to the control, with a 5-fold higher risk of multiple mastitis episodes (95% CI: 1.2–20.6). BCS in primiparous buffaloes was lower in the rbST-treated group, but there was no significant change in BCS dynamics between the rbST and control groups for either primiparous or multiparous buffaloes. These findings suggest that while rbST enhances milk yield in Nili Ravi buffaloes, its use may compromise animal welfare due to increased mastitis risk.

**Keywords:** dairy buffaloes; rbST; milk yield; welfare; mastitis; BCS

**Citation:** Imran, M.; Tausif, M. A.; Waseem, M. Effect of Bovine Somatotropin Administration on Mastitis Incidence and Body Condition Score in Dairy Buffaloes. *Insights Anim. Sci.* **2024**, *1*(2), 18–24.  
<https://doi.org/10.69917/ias.01.02-05>


Received: November 30, 2024

Accepted: December 20, 2024

Published: December 21, 2024

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**Publisher:** Insights Academic Publishing (IAP), Lahore, Pakistan.

## 1. Introduction

Water buffalo (*Bubalus bubalis*) ranks as the second major milk-producing animal globally [1]. Countries like India and Pakistan rely heavily on buffalo milk, which constitutes over 50% of total milk production [2]. Despite the importance of dairy buffaloes, long-term selection strategies to enhance individual animal production remain underutilized. As an alternative, farmers often turn to recombinant bovine somatotropin (rbST) injections to boost milk output.

Recombinant bovine somatotropin (rbST), a peptide hormone, is widely used to enhance milk production in both cows and buffaloes. Numerous studies have consistently demonstrated that rbST administration significantly increases milk production in dairy cattle [3, 4] and buffaloes [5–7]. However, the use of rbST remains controversial due to its potential implications for animal welfare, particularly concerning body condition score (BCS) [7] and mastitis [8], both of which are critical welfare indicators [9, 10]. Changes in BCS post-parturition are closely associated with production, reproduction, and health in cows [11]. Meanwhile, mastitis not only causes pain and discomfort in animals but also poses public health concerns due to the increased use of antibiotics for treatment, especially in regions lacking proper monitoring systems [12]. Although the use of rbST is banned in Pakistan, it continues to be utilized in neighboring countries like India.

Despite the widespread use of rbST, research focusing on its effects on welfare in dairy buffaloes remains limited. Therefore, this study aimed to investigate the impact of rbST administration on mastitis incidence, and BCS changes in Nili Ravi buffaloes.

## 2. Materials and Methods

### 2.1. Study Site, Animals, Housing, and Management

Fifty Nili Ravi lactating buffaloes (primiparous and multiparous) were enrolled from the herd of Buffalo Research Institute, Punjab, Pakistan. The buffaloes at  $60 \pm 2$  d post calving were enrolled from September 2014 to November 2014. The baseline parameters like BCS, days in milk, parity, weight, and milk yield of pre-experimental week have been presented in Table 1.

The buffaloes were housed in a naturally ventilated shed and tied at the mangers during the day, with free access to an outdoor open area at night. They were individually fed in mangers, and water was provided in rubber tubs adjacent to the mangers. All buffaloes were fed a fixed amount of concentrate and wheat straw (1 kg concentrate/2 kg milk + 3.5 kg straw per day) and had ad libitum access to seasonal green fodder. Feeding occurred once daily at 0900 h. The concentrate contained 17.5% crude protein with an estimated metabolizable energy of 2.6 Mcal/kg. Hand milking was performed twice daily at 0500 and 1600 h.

**Table 1.** Baseline parameters of Nili Ravi buffaloes in control (CTL) and recombinant bovine somatotropin treated (rbST) group.

Parameters	Treatment groups <sup>1</sup>		<i>p</i> Value
	CTL	rbST	
Sample size, n	25	25	
Days in milk IM	$60 \pm 1.3$	$62 \pm 0.9$	0.91
Body weight, kg			
Primiparous	$480 \pm 23$	$499 \pm 39$	0.43
Multiparous	$541 \pm 18$	$556 \pm 12$	0.37
Body condition score			
Primiparous	$3.30 \pm 0.05$	$3.40 \pm 0.13$	0.36
Multiparous	$3.12 \pm 0.08$	$3.21 \pm 0.13$	0.31
Milk production, kg			
Primiparous	$7.5 \pm 0.5$	$7.6 \pm 0.6$	0.90
Multiparous	$8.2 \pm 0.4$	$8.1 \pm 0.4$	0.89

<sup>1</sup> The rbST group received 10 doses of 500 mg rbST administered at 14-day intervals, while the CTL group received 10 placebo injections at the same intervals. Values are presented as means  $\pm$  SE.

### 2.2. Study Design and Treatment Groups

The fifty animals were blocked by parity (10 primiparous and 40 multiparous) and sequentially randomized into two groups: 1) treatment group (rbST;  $n=25$ ), and 2) control group (CTL;  $n=25$ ). The rbST group received 10 doses of 500 mg rbST prefilled injection (Somatech, Elanco Animal Health, USA) subcutaneously in the neck area with a 14-day interval. The CTL group received 10 placebo injections at the same interval. The administration of injections started at  $60 \pm 2$  days post-calving and continued until all 10 doses were completed. During the trial, the staff was blinded to the identity of treatments.

### 2.3. Dry Matter Intake, Milk Yield and Milk Composition

Dry matter intake was measured weekly by weighing feed offered and refused for 24 hours. Milk yield was recorded daily at each morning and evening milking. For milk composition, weekly samples (50 ml) collected from each buffalo during morning and evening milking were analyzed for fat, protein, lactose, solid not fat (SNF), and total solids (TS). The milk analysis was done using portable milk analyzer (Ultrasonic milk analyzer

Milkotester Master LTD, Belovo, Bulgaria) at Farm and Health Laboratory of Buffalo Research Institute.

### 2.3. BCS and Mastitis Incidence

Monthly BCS for each buffalo was recorded using a 5-point BCS chart on a scale of 1–5 with 0.25 increments, as validated by Magsi et al. [13]. The animals were individually moved to an open area around 1100 hours for BCS measurements.

Incidence of clinical mastitis (CM) was determined when an animal exhibited clinical signs, such as swelling of quarters and abnormal milk. CM was identified at milking by the milkers and verified by the farm veterinarian. Multiple occurrences of mastitis in an animal were also recorded. A new CM incidence was considered if it occurred more than 14 days after the previous episode, following the criteria of Barkema et al. [14]. Mastitis was treated according to the standard protocols of the farm.

### 2.4. Statistical Analyses

All statistical procedures were performed using SAS (SAS for Academics, SAS Institute Inc., Cary, NC). Data were analyzed separately for primiparous and multiparous buffaloes. Milk yield values were averaged weekly for statistical analysis. The effect of rbST on dry matter intake, milk yield, milk composition and BCS was assessed using Proc Mixed procedure of SAS. The effect of bST on mastitis incidence was analyzed using Proc Genmod procedure of SAS, and the results were presented as relative risk. Significance was declared at  $p \leq 0.05$ .

## 3. Results and Discussion

### 3.1. Dry Matter Intake, Milk Yield and Milk Composition

Dry matter intake, milk production, and composition results are presented in Table 2. Dry matter intake tended to increase in rbST-treated buffaloes compared to buffaloes in the CTL group ( $p = 0.06$ ). Dry matter intake was 15.0 and 16.0 kg/day for buffaloes in the CTL and rbST group, respectively.

Milk production increased with the administration of rbST in both primiparous ( $p < 0.0001$ ) and multiparous ( $p = 0.0004$ ) buffaloes. Milk production was 7.50 and 7.80 kg/day for primiparous buffaloes and 8.30 and 9.10 kg/day for multiparous buffaloes in the control and rbST-treated groups, respectively. The findings of the current study regarding the increase in milk yield align with previously reported results, demonstrating that the use of rbST effectively enhances milk production in buffaloes [6, 7, 15]. The response was more evident in multiparous buffaloes compared to primiparous buffaloes.

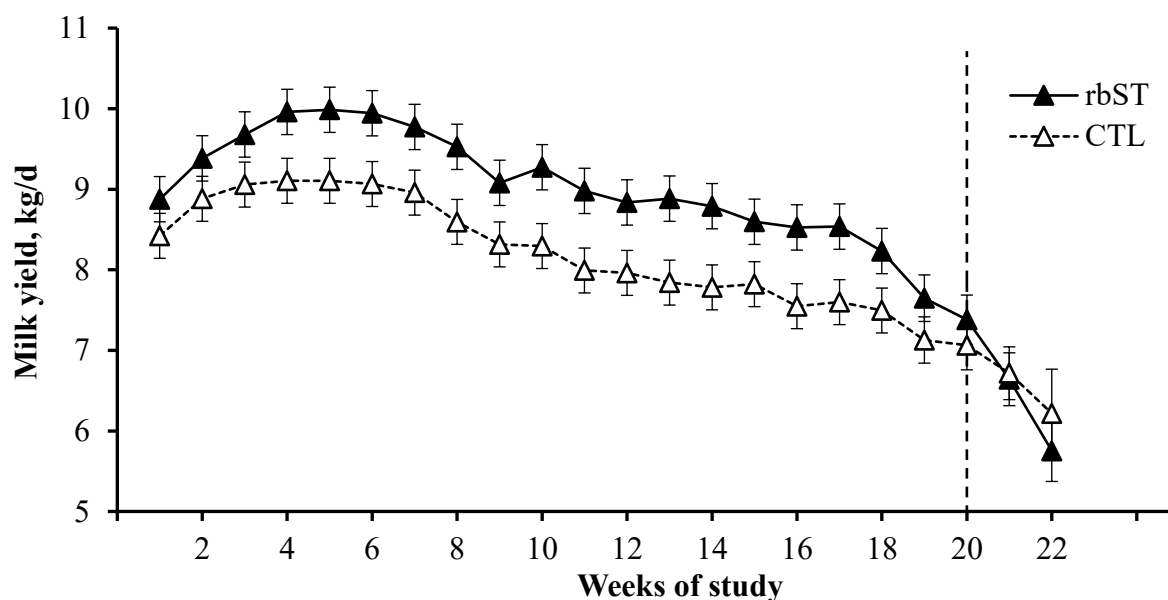
**Table 2.** Effect of rbST on DMI, milk production, and composition in Nili Ravi buffaloes.

Variable	Treatment groups <sup>1</sup>		Mean difference	SEM <sup>2</sup>	<i>p</i> Values
	CTL	rbST			
DMI, kg/d	15.0	16.0	1.0	0.62	0.06
Milk yield, kg/d					
Primiparous	7.50	7.80	0.30	0.06	0.0003
Multiparous	8.30	9.10	0.80	0.08	0.0004
Milk composition, %					
Fat	7.00	6.73	-0.27	0.12	0.023
Protein	4.00	3.98	-0.02	0.032	0.38
Lactose	5.40	5.39	-0.01	0.037	0.86
Solid not fat	10.30	10.18	0.12	0.06	0.06
Total solids	17.30	16.89	0.41	0.16	0.014

<sup>1</sup>The rbST group received 10 doses of 500 mg rbST administered at 14-day intervals, while the CTL group received 10 placebo injections at the same intervals. <sup>2</sup>SEM: Pooled standard error of the mean.

The administration of rbST did not affect the protein or lactose content in milk; however, fat content decreased with rbST administration ( $p = 0.023$ ). Fat content was 7.0% in milk of buffaloes in the CTL group and 6.7% in the rbST group. Solids-not-fat were not influenced by the rbST treatment ( $p = 0.06$ ). The decrease in total solids in rbST-treated buffaloes compared to the control group could be due to the reduction in fat content in these buffaloes ( $p = 0.014$ ). While rbST is not known to influence fat metabolism, the observed average 0.3% decrease in milk fat in the rbST-treated group could potentially be attributed to a dilution effect, as the buffaloes in the current study exhibited higher milk yields compared to those reported in the published literature [7]. The average total solids in the current study were slightly higher compared to the values reported in the region [16, 17]. This could be attributed to the fact that the buffaloes in the trial were likely better fed compared to animals raised under field conditions.

Figure 1 shows the weekly milk yield data over the entire study period. The decline in the lactation curve of buffaloes administered with rbST was particularly interesting after the cessation of its administration. Post-study data indicate that the rbST-treated buffaloes experienced a numerically faster reduction in milk yield compared to the control group. This observation highlights the need for further research to investigate the impact of rbST after its discontinuation.



**Figure 1.** Lactation curve of Nili Ravi buffaloes during the study period (n=50). The rbST group received 10 doses of 500 mg rbST administered at 14-day intervals, while the CTL group received 10 placebo injections at the same intervals. Error bars indicate the standard error of the mean (SEM). The vertical dashed line indicates the end of the rbST administration.

### 3.2. Mastitis Incidence and BCS

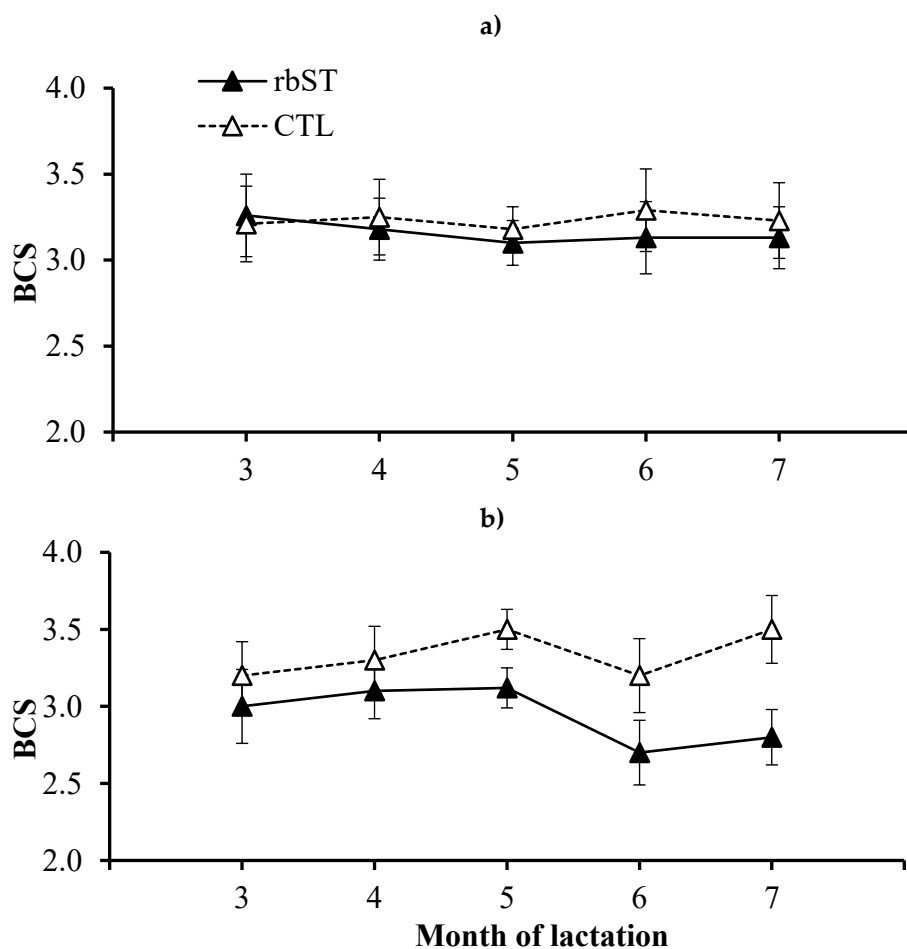
The mastitis results are summarized in Table 3. The relative risk of overall mastitis incidence was higher in the rbST group compared to the CTL group, with the rbST group showing a 3.3 times greater incidence of mastitis. A total of 20 cases of mastitis were observed in the rbST group, compared to only 6 cases in the CTL group. A single occurrence of mastitis was recorded in 4 buffaloes in the CTL group and in 10 buffaloes in the rbST group. Multiple occurrences of mastitis were observed in 2 buffaloes in the CTL group and in 10 buffaloes in the rbST group. The use of rbST significantly increased the incidence of mastitis, consistent with findings reported in the literature [15].

**Table 3.** Relative Risk of mastitis incidence in Nili Ravi buffaloes treated with rbST.

Mastitis Incidence	Treatment groups <sup>1</sup>	N	Relative risk <sup>2</sup>	95% CI <sup>3</sup>	p Value
Overall occurrence	CTL	6/25	1.0	Ref. <sup>4</sup>	0.001
	rbST	20/25	3.3	1.62 – 6.88	
Single occurrence	CTL	4/25	1.0	Ref.	0.08
	rbST	10/25	2.5	0.90 – 6.92	
Multiple occurrence	CTL	2/25	1.0	Ref.	0.025
	rbST	10/25	5.0	1.2 – 20.6	

<sup>1</sup>The rbST group received 10 doses of 500 mg rbST administered at 14-day intervals, while the CTL group received 10 placebo injections at the same intervals. <sup>2</sup>Relative risk is the relative probability of developing mastitis. <sup>3</sup>CI is the confidence interval. <sup>4</sup>Reference group.

The monthly BCS values of buffaloes are presented in figures 2. The BCS of multiparous buffaloes was similar between the treatment groups (Figure 2a). However, the primiparous buffaloes in the rbST group had lower BCS than the CTL group (Figure 2b).



**Figure 2.** Monthly body condition score (BCS) of multiparous (a) and primiparous (b) buffaloes. The rbST group received 10 doses of 500 mg rbST administered at 14-day intervals, while the CTL group received 10 placebo injections at the same intervals. Error bars indicate the standard error of the mean (SEM).

The current findings partially align with those of Fluck et al. [4] and De Morais et al. [18], who reported lower BCS in rbST-treated cows compared to the control group. In our study, only primiparous buffaloes exhibited lower BCS. This could be attributed to the

dual challenges faced by primiparous buffaloes, as they must simultaneously support both growth and lactation, potentially leading to greater depletion of body fat reserves. Additionally, the absence of an effect in multiparous buffaloes could be due to their lower milk yield compared to cows.

#### 4. Conclusions

In conclusion, the use of recombinant bovine somatotropin (rbST) significantly enhances milk production in both primiparous and multiparous Nili Ravi buffaloes. However, its administration is associated with a markedly increased risk of mastitis, including a higher likelihood of multiple episodes. While no significant differences in body condition score (BCS) dynamics were observed between rbST-treated and control groups, primiparous buffaloes in the rbST group exhibited lower BCS, likely due to the combined metabolic demands of growth and lactation. These findings highlight the need to carefully weigh the benefits of increased milk yield against potential compromises in animal welfare when considering rbST use in buffaloes.

**Author Contributions:** Conceptualization, M.I. and M.A.T.; methodology, M.I.; formal analysis, M.A.T. and M.W.; resources, M.A.T.; data curation, M.I.; writing—original draft preparation, M.I., M.A.T. and M.W.; writing—review and editing, M.A.T. and M.W. All authors have read and agreed to the published version of the manuscript.

**Acknowledgments:** The authors would like to thank the staff at the Livestock Experiment Station Bhuneki. They also extend their gratitude to Dr. Amjad Riaz for his support of the study.

**Funding:** This research was conducted with the support of external funding, the details of which are withheld at the authors' request.

**Institutional Review Board Statement:** All experimental procedures were undertaken in compliance with the Advanced Studies and Research Board of the University of Veterinary and Animal Sciences, Lahore (das/167 dated 24 January 2014).

**Data Availability Disclosure:** Data can be obtained from the corresponding author upon reasonable request.

**Conflicts of Interest:** The authors declare no conflict of interest.

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