RESEARCH ARTICLE

Body Weight and Blood Biochemical Changes Following Nutritional Supplementation in Prepubertal Jaffrabadi Buffalo (*Bubalus bubalis*) Heifers

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Abstract

The high plan of nutrition right from an early age is believed to enhance ovarian activity and early onset of puberty in heifers. This study was therefore conducted to know the body weight, ovarian and blood biochemical changes in nutritionally supplemented and control groups of prepubertal Jaffrabadi buffalo (*Bubalus bubalis*) heifers. A total of 12 animals of 30 ± 2 month's age and the identical body weight of 380–410 kg from Cattle Breeding Farm, JAU, Junagadh were utilized for this study. They were randomly divided into two equal groups of 6 animals in each treatment and control groups. The heifers of the treatment group were supplemented with bypass fat (100 g), bypass protein (950 g) and chelated mineral mixture (50 g) over the routine farm feeding practices of control group, and its effect on body weight gain and blood biochemical profiles were studied at a monthly interval during 6 months of experimental period. The overall effect of nutritional supplementation on animals body weight, blood glucose and total cholesterol over the control group was statistically non-significant (p >0.05) yet beneficial. The plasma total protein levels in the supplemented group increased with a duration of supplement and levels were significantly (p <0.05) higher over the control group. The ovarian dynamics evaluated by transrectal ultrasound during last three months of the study revealed an increasing number of growing follicles >4 mm in diameters (5.00 ± 0.13 to 6.67 ± 0.40), without significant variation in diameters of largest follicles (9.14 ± 0.45 to 10.27 ± 0.85 mm) between groups or periods. However, none of the heifers exhibited behavioral signs of estrus during 6 months study, in spite of the establishment of cyclicity with the presence of developed follicles, or CLs suggesting silent ovulation, at 36 ± 2 months of age in this breed.

Keywords: Body weight, Blood glucose, Nutrients supplementation, Prepubertal Jaffrabadi heifers, Total cholesterol, Total protein.

Ind J of Vet Sci and Biotech (2019): 10.21887/ijvsbt.15.1.11

Introduction

Jaffrabadi is one of the heaviest buffalo breeds of the world, a native of Saurashtra region of Gujarat. Their heifers usually attain puberty when they reach about 55–60% of their mature body weight (~800 kg), but it can be highly variable, ranging from 18 to 46 months (Jainudeen and Hafez, 1993). Age at first heat, age at first calving, calving interval and service period of Jaffrabadi buffaloes on the farm were observed to be 1312.8 ± 39.1, 1622.7 ± 39.4, 502.2 ± 17.6 and 197.4 ± 17.7 days, respectively (Anonymous, 2016). The effects of underfeeding are greatest on pre-pubertal animals. Poor nutrition delays puberty reduces conception rate and increases pregnancy loses in heifers. The effects of poor nutrition differ depending on whether the main deficiency is in energy, protein, vitamins, minerals or trace elements. Most dairy animals in tropics are poorly fed, and improving their feeding can immediately increase their reproductive performance. Fat supplements based on calcium salts of palm fatty acids improved first service conception rate over controls in grazing dairy cows; however, no effect of treatment on overall pregnancy rate was observed (McNamara *et al*., 2003). The several potential mechanisms have been postulated by which polyunsaturated fatty acid (PUFA) supplements may improve fertility in dairy animals (Staples *et al*., 1998). The high growth rate has been reported through protein feeding, the young stock could attain early maturity to start the reproductive life (Tandon *et al*., 2008). Trace minerals (cobalt, copper, iron, manganese, selenium, zinc) and fat-soluble vitamins (vitamin A, D, E, and K) play an important role in the fertility of animals. This study was aimed to know the effect of nutritional supplementation on body weight change and blood biochemical profile in prepubertal Jaffrabadi buffalo (*Bubalus bubalis*) heifers.

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How to cite this article: Raval, R.J., Vala, K.B., Kalariya, V.A., Dhami, A.J., & Kavani, F.S. (2019). Body Weight and Blood Biochemical Changes Following Nutritional Supplementation in Prepubertal Jaffarabadi Buffalo (Bubalus bubalis) Heifers. Ind J Vet Sci and Biotech, 15(1):50-54.

Source of support: Nil

Conflict of interest: None

Submitted: 09/7/2019 **Accepted:** 12/7/2019 **Published:** 27/7/2019

Materials and Methods

The study was carried out at Cattle Breeding Farm (CBF), JAU, Junagadh, having a tropical climate. The experimental

Table 1: Mean (±SE) body weight of pubertal Jaffrabadi heifers (30 ± 2 months of age) under control and nutritionally supplemented (treatment) groups

Study period	*Body weight (kg)*		*Gain in bwt over control (kg)*
	Control group (N = 6)	*Treatment group (N = 6)*	
0 day	393.33 ± 22.57[a]	394.00 ± 13.76[a]	–
1st month	406.00 ± 22.21[ab]	410.17 ±15.58[ab]	+04.17
2nd month	430.50 ± 22.44[ab]	431.67 ± 15.65[abc]	+01.17
3rd month	459.00 ± 22.50[ab]	460.33 ± 21.02[abcd]	+01.33
4th month	473.50 ± 24.08[ab]	488.50 ± 20.90[bcd]	+15.00
5th month	491.33 ± 22.98[ab]	512.83 ± 22.63[cd]	+21.50
6th month	505.67 ± 22.77[b]	533.33 ± 24.14[d]	+27.66

Means bearing different superscripts within the column differ significantly (p <0.05)

protocol was approved by the Institutional Animal Ethics Committee (IAEC) of the College. A total of 12 animals of 30 ± 2 month's age and the identical body weight of 380 to 410 kg from CBF were utilized for this study. They were randomly divided into two equal groups of 6 animals each in treatment and control groups. All the experimental animals were fed as per ICAR (2013) to meet the nutrient requirement. Animals of the control group were managed as per routine farm feeding schedule, i.e., fed 10–12 kg green maize, 5.8–6.0 kg mature pasture grass, and 1.60 kg concentrate mixture (Amul brand) with 50 g mineral mixture daily, while those of treatment group received an additional 100 g bypass fat, 950 g treated cottonseed cake as bypass protein (replacing 50% of Amul Dan) and 50 g chelated mineral mixture (AAU, Anubhav brand) throughout the experimental period.

Monthly body weight of all animals was recorded. The blood samples (7–8 mL) were collected aseptically from the jugular vein in the separate sterile glass vials with anticoagulant at monthly interval. The samples were centrifuged at 4500 rpm for 15 minutes and plasma separated was stored in a deep freeze at –20°C with a drop of sodium Merthiolate (0.1%) until analyzed. Ovarian dynamics was evaluated by per rectal palpation as well as transrectal ultrasonography (5.0–7.5 MHz probe) at monthly interval during last three months of study to detect a number of follicles >4 mm size per ovary and the diameter of largest/dominant follicle and/or CL, if any.

The blood glucose level in fresh blood was directly measured by using digital Gluco One BG-03 Glucometer and strips (Morepen Laboratories Limited, Delhi). Plasma total cholesterol and total protein levels were determined by CHOD/PAP and Biuret methods, respectively, using assay kits and procedures described by Diatek Healthcare Pvt. Ltd., Kolkata, India. The data obtained were analyzed statistically using ANOVA, DNMRT and 't' test to know the effect of periods and groups on each trait (Snedecor and Cochran, 1994).

Results and discussion

Body Weight Change

The mean (± SE) values of body weight of prepubertal Jaffrabadi heifers (30 ± 2 month age) under control and nutritionally supplemented (treatment) groups are presented in Table 1. The mean body weights of buffalo heifers supplemented nutritionally and those of control groups were increased gradually and significantly (p <0.05) from start to the end of the study period of six months. The gain in body weight of supplemented group over control was apparent from 4th to 6th month, the supplemented group gained average 15 to 27 kg more weight during these three months compared to control group, suggesting a beneficial effect of nutritional supplementation in growing heifers. However, the effect of the treatment was statistically non-significant (p >0.05) (Table 1). Nutritional supplementation (bypass fat, bypass protein, mineral mixture and concentrate mixture) has also been reported to improve the body condition score and weight gain of dairy animals by many earlier researchers (Chatterjee and Walli, 2003; Tandon *et al*., 2008; Patel *et al*., 2012; Gajera *et al*., 2013). However, Ferguson *et al*. (1990) did not find any change in body weight of herds supplemented with calcium salt of long-chain fatty acids.

Blood Glucose

The monthly mean blood glucose values of buffalo heifers supplemented nutritionally were higher than those of the control group at different time intervals, but the differences were statistically non-significant (p >0.05) (Table 2). Similar observations have been reported earlier by Tyagi *et al*. (2010) and Savsani *et al*. (2013). However, greater values of blood glucose on supplementation of high energy diet were reported in buffaloes by Campanile *et al*. (2010) and Khan *et al*. (2016). Funston *et al*. (1995) reported that fat supplementation may increase glucose production through increased propionate production and this increase in glucose may have a positive effect on LH release.

Table 2: Mean (±SE) blood glucose, plasma total protein and total cholesterol profile of prepubertal Jaffrabadi buffalo heifers under control and nutritionally supplemented (treatment) subgroups

Pubertal period	*Blood glucose (mg/dL)*		*Plasma total protein (g/dL)*		*Total cholesterol (mg/dL)*	
	Control group (N = 6)	*Treatment group (N = 6)*	*Control group (N = 6)*	*Treatment group (N = 6)*	*Control group (N = 6)*	*Treatment group (N = 6)*
0 day	65.83 ± 3.27	68.50 ± 3.10	5.81 ± 0.11	6.04 ± 0.11^{a}	69.50 ± 3.42	72.33 ± 5.49
1st month	69.67 ± 1.94	75.17 ± 2.56	6.06 ± 0.16^{A}	6.49 ± 0.11^{Bab}	68.50 ± 4.62^{A}	83.17 ± 6.19^{B}
2nd month	76.00 ± 4.65	79.50 ± 2.06	6.06 ± 0.07^{A}	6.46 ± 0.11^{Bab}	74.17 ± 5.37^{A}	91.33 ± 6.27^{B}
3rd month	74.17 ± 3.27	79.17 ± 5.23	6.17 ± 0.19	6.49 ± 0.16^{ab}	76.83 ± 5.28^{A}	91.17 ± 4.67^{B}
4th month	74.17 ± 3.00	76.67 ± 3.57	6.20 ± 0.15	6.50 ± 0.05^{ab}	72.83 ± 3.43	83.50 ± 4.92
5th month	77.17 ± 1.68	79.50 ± 4.02	6.26 ± 0.21^{A}	6.73 ± 0.10^{Bb}	78.67 ± 3.65	90.67 ± 4.89
6th month	76.17 ± 2.30	79.67 ± 3.04	6.39 ± 0.16^{A}	6.80 ± 0.11^{Bb}	76.17 ± 2.02	86.17 ± 7.96

Means bearing different superscripts within the column (^{a,b}) and within the row (^{A,B}) differ significantly (p <0.05)

Plasma Total Protein

In the present study, the effect of the treatment was significant (p <0.05) during the first two and last two months of the experimental period, showing a beneficial effect of bypass protein and mineral supplementation. Further, the plasma total protein levels increased with advancing age or study period in both the groups, but the differences (p <0.05) in month wise mean plasma total proteins were observed to be significant only in heifers of the nutritionally supplemented group (Table 2). Supplementation of bypass protein in the diet is known to improve the circulatory blood plasma total protein concentration in the animals. The present overall mean values of total protein observed were close to the previous report of Selvaraju *et al*. (2017). Further, the values reported by Nayyar *et al*. (1996) and Akhtar *et al*. (2010) were higher than the present findings while Ahmed *et al*. (2010) reported the lower value of plasma total protein.

Plasma Total Cholesterol

The mean plasma total cholesterol levels of heifers in the treatment group were greater than those of heifers in control group at all periods, with a significant difference during first three months, indicating a beneficial effect of nutritional supplementation (Table 2). However, the rise in plasma total cholesterol concentration with advancing age was not significant (p >0.05) in heifers of any of the groups, though the rise was more in the treatment group. The relatively higher plasma total cholesterol concentrations in a nutritionally supplemented group of heifers could be due to the effect of bypass fat supplementation since degradation of fat provides precursors for cholesterol synthesis. The higher cholesterol synthesis is an indirect indication of initiation of ovarian follicular and luteal activity and thereby the reproductive cycle in such animals. Grummer and Carrol (1991) recorded an increase in plasma cholesterol concentration of animals under fat supplementation regimens. Tyagi *et al*. (2010) reported much higher value (238.73 mg/dL) of plasma cholesterol concentration in bypass fat supplemented group, while Nayyar *et al*. (1996) reported lower value (56.01 ± 2.80 mg/dL) than the present observations in prepubertal buffalo heifers. Raval *et al*. (2017) found a higher cholesterol level in Jaffrabadi buffaloes supplemented with calcium salt of palm fatty acids. Campanile *et al*. (2010) reported that heifers fed low energy diet had reduced plasma concentration of glucose, total cholesterol, and HDL cholesterol than heifers fed high energy diet. The lesser concentrations of metabolic substrates in heifers fed low energy diet can be explained by the reduced energy availability in these heifers.

Ovarian Dynamics

The ovarian dynamics evaluated during the last three months of study by monthly per rectal palpation and a transrectal ultrasound revealed an increasing number of growing follicles >4 mm in diameters. The total number of follicles per ovary ranged from 5.00 ± 0.13 to 6.67 ± 0.40 in the treatment group and 5.75 ± 0.42 to 6.42 ± 0.44 in the

Table 3: Mean follicular population and diameter of the largest follicle in prepubertal Jaffrabadi heifers under control and nutritionally supplemented groups

Experimental period	*Total number of follicles (N = 6)*		*Size of largest follicle (mm) (N = 6)*	
	Control group	*Treatment group*	*Control group*	*Treatment group*
4th month	5.92 ± 0.47	5.00 ± 0.13^{a}	9.66 ± 0.83	9.35 ± 0.39
5th month	5.75 ± 0.42	6.67 ± 0.40^{b}	9.14 ± 0.45	9.38 ± 0.18
6th month	6.42 ± 0.44	5.58 ± 0.33^{ab}	10.27 ± 0.85	9.51 ± 0.59

Means bearing different superscripts within the column (^{a,b}) differ significantly (p <0.05)

control group. Diameters of the largest follicles ranged from 9.35±0.39 to 9.51 ± 0.59 mm in the treatment group; and 9.14 ± 0.45 to 10.27 ± 0.85 mm in the control group. The mean (±SE) values shown for these traits for 4th, 5th and 6th month of the study revealed significant (p <0.05) increase in an overall number of a total number of follicles from 4th to 6th months of study in nutritionally supplemented heifers as compared to control animals. However, diameters of the largest follicles were non-significantly higher in the control group as compared to those of the treatment group (Table 3). These changes could probably be attributed to the effect of nutrition supplementation on ovarian growth, though it was not marked at this stage in Jaffrabadi heifers. Moreover, none of the nutritional supplement and control group of heifers in the present study exhibited behavioral signs of estrus during 6 months study, although monthly ultrasonography revealed establishment of cyclicity with the presence of developed follicles or CLs during later three months of study, suggesting the growth of reproductive organs and silent ovulation.

Bergfeld *et al*. (1994) observed that maximum diameter of dominant follicle was larger (p <0.05) in heifers receiving greater energy diet than in heifers receiving the diet with lower energy before the onset of puberty. Size of dominant follicles was larger (p <0.05) immediately preceding puberty as compared to the earlier prepubertal period in both lower and higher energy treatment groups. Campanile *et al*. (2010) reported identical ovarian follicular characteristics in both high energy and low energy supplemented groups of heifers, but heifers fed the low energy diet tended to have oocytes of reduced quality compared with heifers fed the high energy diet. Heifers fed high energy diet had greater concentrations of glucose and insulin in circulation which may have contributed to greater oocyte quality in these heifers. The present observations on a mean number of follicles and the size of largest follicle were in contrast to the observations of Singh (2000), who reported significantly higher values in buffalo heifers fed rumensin treated diet than the control diet. They found the mean size of the largest follicle as 7.65 ± 0.09 mm and 7.51 ± 0.15 mm in rumensin treated and control heifers, respectively.

From the study, it was concluded that the nutritional supplementation (bypass fat, bypass protein, and chelated mineral mixture) for 6 months from 30 ± 2 months of age in prepubertal Jaffarabadi buffalo (*Bubalus bubalis*) heifers had non-significant yet positive effect on body weight gain, ovarian dynamics, as well as plasma total protein, total cholesterol levels with silent ovulation, but behavioral signs of puberty, were not expressed even at three years of age by these animals.

Acknowledgment

Authors want to thank Principal and Dean, College of Veterinary Science and Animal Husbandry and Research Scientist (AGB), Cattle Breeding Farm, JAU, Junagadh for providing necessary facilities for carrying out this work.

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