



Effect of Bypass Fat on Genital Status and Blood Biochemical Profiles in Pubertal Swamp Buffalo Heifers and Puerperal Swamp Buffalo Cows

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ABSTRACT

Background: The interaction between nutrition and reproduction has long been known to have important implications for the reproductive performance in animal. The present study highlights the effect of by-pass fat supplementation on genital changes and blood biochemical profile in pubertal swamp buffalo heifers and puerperal swamp buffalo cows.

Methods: 24 heifers of 2-2.5 years of age and 24 puerperal cows on the day of parturition, irrespective of parity were used as experimental animal. The analytical method was based on the treatment protocols of 3 groups in both heifers and cows (n=8 in each group) viz., treated with oral bypass fat alone (I), oral bypass fat, mineral mixtures and injectable phosphorus (II) and control (III) respectively. Genital status, metabolic hormone (leptin, Ghrelin and IGF-1), reproductive hormone (estrogen and progesterone) and mineral constituents (calcium, phosphorus, zinc and copper) were analyzed on day 0, day 15 and day 30 in heifers and day 15, day 30 and day 45 in cows respectively.

Result: Feeding of bypass fat alone or bypass fat fortified with minerals and injectable phosphorus was not found to induce significant genital changes in heifers and cows. The serum leptin and IGF-1 levels were increased significantly ($p<0.05$) in heifers at day 30 in Group I and II. However, a significant rise ($p<0.05$) of leptin was recorded at day 45 in group I and II cows. IGF-1 level was recorded increased significantly only in cows treated with fortified bypass fat (Group II). With the progression of treatment the serum phosphorus level was increased significantly ($p<0.05$) in Group I and II cows. Serum estrogen levels were increased in both heifers and cows with progression of treatment in both Group I and II. However, serum progesterone levels were increased significantly only in heifers of Group I and II with progression of treatment.

Key words: Estrogen, IGF-1, Leptin, Phosphorus, Swamp buffalo.

INTRODUCTION

The swamp Buffaloes have 48 diploid no. of chromosome (2N) and are distributed mostly in upper Brahmaputra valley of Assam. Better adaptability of buffalo to tropical climate and higher disease resistance ensure their place in the future world agriculture facing the challenge of global warming. Nevertheless, not much attention has been paid for improving the indigenous swamp buffaloes through planned breeding programme. Low reproductive efficiency (delayed puberty, longer intercalving period, silent estrus, anestrus etc.) is a serious constraint to buffalo reproduction (Mahadevan, 1978). The age at first calving and post partum estrus interval in swamp buffaloes of Assam were 1841.75 ± 95.18 and 262.90 ± 30.27 days respectively (Deka *et al.*, 2018). The incidence is much higher in swamp buffaloes due to poor reproductive hormone profile and negative energy balance after parturition. The interaction between nutrition and reproduction has long been known to have important implications for the reproductive performance in animal. In this light, plethora of researchers have documented supplementation of fat, minerals and vitamins in the diet led to improvement in reproductive efficiency in cattle (Sahoo *et al.*, 2016; Dutta *et al.*, 2019; Rahman *et al.*, 2021) and buffaloes (Butani *et al.*, 2016; Deka *et al.*, 2019; Khadda *et al.*, 2024).

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Minerals are the essential nutrients bearing a significant role in the animal reproduction. Deficiency of essential minerals may result in failure of the homeostasis mechanism, affecting the productive and reproductive potential of animals. It is also known that energetic status of the animal modulates secretion of hormone that may play key role in growth of ovarian follicle, ovulation, corpus luteum formation and fertilization. Bypass fat technology protects the nutrient from degradation and bio-hydrogenation in rumen with increasing the energy density

of diet, thus enabling the animals to meet their energy and essential fatty acid requirements and improving reproductive and lactating performance. Staples *et al.* (1998) demonstrated that positive effect of bypass fat was due to fatty acids which act as a precursor of progesterone via cholesterol and prostaglandins. Acute negative energy balance during lactation can be minimized by feeding of bypass fat. Therefore, it is believed that addition of fat, minerals and vitamins in the diet improve body condition of the animals. Leptin, ghrelin and IGF-1 are metabolic hormones which have been considered as indicators of energy balance in dairy animals. Higher level of leptin and lower level of ghrelin was found to be silent indicators of positive energy balance (Nowroozi-Asl *et al.*, 2016). Certain macro-elements like Ca, P and trace elements including Zn, Cu have been found to be crucial for normal animal reproduction (Akhtar *et al.*, 2009). Very little information is available on characteristic genital changes in relation to supplementation of bypass fat in pubertal heifers and puerperal buffalo cows as well as variations in different blood biochemical constituents. In view of the above fact, the present study was designed to study the effect of bypass fat feeding on genital status and blood biochemical profile in pubertal heifers and puerperal cows.

MATERIALS AND METHODS

The experimental animals included in the present study comprised 24 pubertal heifers of 2-2.5 years of age weight ranging from 150 to 200 kg (176.79 ± 3.17) and 24 puerperal cows irrespective of parity on the day of parturition weight ranging from 300 to 400 kg (352.67 ± 6.21) maintained at different villages of Assam. The buffaloes were fed with concentrate feed and paddy straw once daily. Water was given *ad libitum*. The animals were let loose for grazing in the open field and vaccinated against foot and mouth disease, hemorrhagic septicemia and black quarter at 6 months interval. Deworming was done at 6 months interval. The analytical part was carried in the Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Science, AAU, Khanpara-781022, Guwahati, India from the period of 1st September 2022 to 31st August 2023.

The heifers and cows were grouped separately and divided into 3 groups of 8 animals each and assigned to three treatment plan.

Swamp buffalo	Treated with oral Bypass fat	Treated with oral bypass fat, mineral mixture and injectable phosphorus	Control
Pubertal heifer	Group I	Group II	Group III
Puerperal cow	Group I	Group II	Group III

Bypass fat alone: Bypass fat (Fatomax, Intas Pharmaceuticals limited, Ahmedabad, India) @ 100 gm per day per animal with concentrate mixture orally daily for 30 days continuously commencing from the day of treatment (day 0).

Bypass fat fortified with minerals and phosphorus: Therapeutic choice 1 + Mineral mixture (Minfa Gold, Intas Pharmaceuticals limited, Ahmedabad, India) @ 30 gm per animal daily with concentrate mixture for a period of 30 days + Injectable Phosphorus (Tonophosphan Vet, containing sodium salt of 4-dimethyl amino-2-methyl phenyl-phosphinic acid, MSD-Animal Health, Pune, India) @ 2 gm intramuscularly for three occasions on alternate days commencing from the day of treatment (day 0).

The heifers attained the pubertal age were given both treatment regimes and the 1st day of examination was considered as day 0. In case of puerperal cows, both the treatment regimens commenced from the day 15 of parturition.

Rectal palpation of the genital organs was done in each experimental heifer at day 0, day 15 and day 30 and in each puerperal cow at day 15, day 30 and day 45 after calving. On each day of examination the vulva, vagina, cervix, uterus and ovaries of the animals were examined to record the changes of the genital organs and the percentage frequencies were worked out separately for heifers and cows. The vulvar lips were drawn apart and the mucous membrane was examined for colour. The vulva was recorded as congested when the vulvar mucous membrane appeared hyperaemic. Vaginal mucus was recorded as discharge present or absent. Patency of the cervix was recorded as open or closed. Uterine tone was recorded as tonic or atonic. Ovaries were examined for presence of graffian follicle or corpus luteum and recorded as active ovary. From each animal under the study 5 ml of blood was collected on the day of rectal palpation, from the jugular vein using plastic syringe and 18 gauge needles and transferred to vacutainers with serum clot activator (Becton, Dickinson and Company, New Jersey, USA). The vacutainers were kept in slanting position at room temperature for about two hours for clotting. It was then transported to the laboratory and serum was separated from the coagulated blood by centrifugation at 3000 rpm for 10 minutes. The separated serum samples were collected in sterilized screw-capped vials, labeled and stored at -20°C for analysis.

The energy balance in the experimental animal was studied on the basis of levels of metabolic hormones *viz.* Leptin, Ghrelin and IGF-1 in blood collected from each experimental animal on each day of examination. ELISA techniques were used to estimate the serum leptin (Cloud-Clone Corp. marketed by 1304 Langham Creek Dr, Suite 226, Houston, TX 77084, USA), ghrelin (Diagnostics Biochem Canada Inc. marketed by 41 Byron Avenue, Dorchester, Ontario, Canada) and IGF-1 (Cloud-Clone Corp. marketed by 1304 Langham Creek Dr, Suite 226, Houston, TX 77084, USA) in the collected samples. The blood mineral constituents of serum samples were estimated by using quality reagent kits of calcium, phosphorus, zinc (Greiner Diagnostic GmbH- UnterGereuth 10-D-79353 Bahlingen-Germany) and Copper (Coral Clinical systems, Kolkata India). The level of the reproductive hormone estrogen and

progesterone in the serum samples collected from the experimental animals was estimated by using a solid phase enzyme linked immunosorbant assay kit (Estradiol ELISA kit and Progesterone ELISA kit LDN, Germany).

The frequency of occurrence of genital changes in buffalo heifers and cows at different days of treatment with different treatment regimen was expressed in percentage. Serum level of leptin, ghrelin, IGF-1, calcium, phosphorus, zinc, copper, estrogen and progesterone in pubertal heifers and puerperal cows at different days of treatment with different treatment regimes were expressed as Mean $\pm$ SE (standard Error). Means were analyzed using one way analysis of variance followed by post hoc test to determine the significance difference using the SPSS (Version 20.0, SPSS, Chicago, IL, USA). Difference with the values of $p < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Genital changes observed in vulva, vagina, cervix, uterus and ovaries of pubertal buffalo heifers and cows in different groups examined on different days of treatment have been presented in Table 1-2. In the present study feeding of

bypass fat alone or bypass fat fortified with minerals and injectable phosphorus was not found to induce significant genital changes in pubertal heifers and puerperal cows. Ovaries appeared smooth or inactive in 75 to 100 per cent pubertal heifers. In case of puerperal buffalo cows the ovaries were active indicating presence of palpable corpus luteum until 45 days of parturition, which might not be due to the effect of bypass fat feeding as the same was observed in the control group of cows also. The corpus luteum felt on day 15 of parturition as observed in the puerperal buffalo cows might be the corpus luteum of pregnancy, which was reported to take as long as 22 days for regression (Usmani *et al.*, 1985). Corpus luteum felt on day 30 and day 45 after parturition might be cyclic. Feeding of bypass fat in cattle and buffalo was found to enhance puberty in heifers (Lammoglia *et al.*, 2000), improve pregnancy rate (Sahoo *et al.*, 2016), increase oestrus response rate (Deka *et al.*, 2019), reduce post partum oestrus interval (Ramteke *et al.*, 2014) and shorten service period (Khan *et al.*, 2016).

Average levels leptin, ghrelin, IGF-1, calcium, phosphorus, zinc, copper, estrogen and progesterone in pubertal buffalo heifers and puerperal cows at different

Table 1: Frequency of occurrence of different genital changes in pubertal buffalo heifers at different days of treatment with different treatment regimens.

Genital organ	Changes Observed	Frequency of occurrence (%)								
		Group I (n=8)			Group I (n=8)			Group I (n=8)		
		0 day	day 15	day 30	0 day	day 15	day 30	0 day	day 15	day 30
Vulva	Oedematous	-	-	-	-	-	-	-	-	-
	Pale	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Vagina	Discharge present	-	-	-	-	-	-	-	-	-
	Discharge absent	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Cervix	Relaxed and open	-	-	-	-	-	-	-	-	-
	Closed	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Uterus	Tonic	-	-	-	-	25.00	-	-	-	-
	Atonic	100.00	100.00	100.00	100.00	75.00	100.00	100.00	100.00	100.00
Ovary	Active	-	-	-	-	25.00	25.00	-	-	-
	Smooth	100.00	100.00	100.00	100.00	75.00	75.00	100.00	100.00	100.00

Table 2: Frequency of occurrence of different genital changes in puerperal buffalo cows at different days of treatment with different treatment regimens.

Genital Organ	Changes Observed	Frequency of occurrence (%)								
		Group I (n=8)			Group I (n=8)			Group I (n=8)		
		day 15	day 30	day 45	day 15	day 30	day 45	day 15	day 30	day 45
Vulva	Oedematous	-	-	-	-	-	-	-	-	-
	Pale	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Vagina	Discharge present	-	-	-	-	-	-	-	-	-
	Discharge absent	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Cervix	Relaxed and open	-	-	-	-	-	-	-	-	-
	Closed	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Uterus	Tonic	-	-	-	-	-	-	-	-	-
	Atonic	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Ovary	Active	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	Smooth	-	-	-	-	-	-	-	-	-

days of treatment with different treatment regimens have been presented in Table 3-4 respectively.

Serum leptin

Feeding of bypass fat alone or fortified with minerals and injectable phosphorus for a period of 30 days led to an increase in serum leptin level in both pubertal heifers and puerperal cows. The increase was significant at day 30 of treatment in heifers and day 45 in post-partum cows. In the control groups serum leptin levels remained unchanged in both heifers and cows. The mean serum leptin level in all the groups at different days of treatment were found to be lower in the present study than the normal leptin levels (7.30 ± 0.19 ng/ml) in buffaloes as reported by Tajik and Nazifi (2011). The lower level of leptin resulted in negative energy balance and hamper fertility (Jayaprakash *et al.*, 2016). Threshold leptin levels are needed for proper activation and function of reproductive axis (Casanueva and Dieguez, 1999).

Serum ghrelin

There appeared no change in serum ghrelin level in pubertal heifers due to feeding of bypass fat alone or bypass fat fortified with minerals and injectable phosphorus up to 30 days of treatment as the mean values were similar to that observed in control heifers. The serum ghrelin level in bypass fat treated cows was significantly lower than that observed in the control cows during the period of day 15 to day 45 after parturition. This was indicative of a better energy position in the cows treated with bypass fat. However, fortification of bypass fat with mineral and injectable phosphorus did not have beneficial effect over feeding bypass fat alone. Studies conducted on different species of animals and human indicated that ghrelin was the peripheral signal for energy insufficiency and its level increased in conditions of negative energy balance (Repaci *et al.*, 2010). Higher level of leptin and lower level of ghrelin indicated positive energy balance (Nowroozi-Asl *et al.*, 2016).

Serum IGF-1

Bypass fat alone or bypass fat fortified with minerals and injectable phosphorus for a period of 30 days led to an increase in the serum IGF-1 level in pubertal heifers and the increase was significant from 15 to 30 days of treatment. In the untreated heifers serum IGF-1 level remain unchanged and lower than that recorded in the treated heifers. Another important observation was that there was no significant difference in serum IGF-1 level between the two treatment regimens. These findings clearly indicated that feeding of bypass fat led to improvement in energy status of pubertal heifers and fortification with minerals and phosphorus had no beneficial effect. Observations on the effect of the bypass fat treatment regimens on serum IGF-1 level in puerperal cows were not similar to that observed in pubertal heifers. Serum IGF-1 level increased significantly at day 45 post partum in those buffalo cows treated with bypass fat fortified with minerals and phosphorus. The serum IGF-1 level remained unchanged until 45 days post partum

Table 3: Level (Mean $\pm$ S.E) of biochemical profiles in the serum of pubertal heifers at different days of treatment with different treatment regimen.

Parameters	Group I (n=8)			Group II (n=8)			Group III (n=8)		
	Day 0	Day 15	Day 30	Day 0	Day 15	Day 30	Day 0	Day 15	Day 30
Leptin (ng/ml)	2.44 ^a $\pm$ 0.07	2.69 ^a $\pm$ 0.20	3.59 ^a $\pm$ 0.16	2.17 ^a $\pm$ 0.18	2.72 ^a $\pm$ 0.19	3.00 ^b $\pm$ 0.19	2.30 ^a $\pm$ 0.15	2.23 ^a $\pm$ 0.06	2.35 ^b $\pm$ 0.03
Ghrelin (pg/ml)	294.69 $\pm$ 36.43	293.50 $\pm$ 12.99	297.88 $\pm$ 7.82	283.15 $\pm$ 19.10	286.16 $\pm$ 7.37	285.29 $\pm$ 29.90	264.25 $\pm$ 2.45	264.63 $\pm$ 7.72	265.25 $\pm$ 2.45
IGF-1 (ng/ml)	60.99 ^a $\pm$ 1.62	76.98 ^b $\pm$ 1.24	99.00 ^b $\pm$ 0.90	68.51 ^a $\pm$ 7.61	70.80 ^{ab} $\pm$ 6.69	96.34 ^b $\pm$ 3.97	61.91 ^a $\pm$ 0.53	61.02 ^b $\pm$ 0.68	60.99 ^b $\pm$ 1.62
Calcium (mg/dl)	9.69 ^a $\pm$ 0.32	9.97 ^a $\pm$ 0.15	9.89 ^a $\pm$ 0.29	9.77 ^a $\pm$ 0.14	9.70 ^a $\pm$ 0.31	9.96 ^a $\pm$ 0.49	8.70 ^b $\pm$ 0.29	8.03 ^b $\pm$ 0.29	8.59 ^b $\pm$ 0.15
Phosphorus (mg/dl)	3.53 $\pm$ 0.10	3.58 $\pm$ 0.12	3.80 $\pm$ 0.19	3.40 $\pm$ 0.19	3.58 $\pm$ 0.15	3.73 $\pm$ 0.03	3.71 $\pm$ 0.02	3.63 $\pm$ 0.22	3.88 $\pm$ 0.19
Zinc (μ g/dl)	259.79 $\pm$ 24.19	260.88 $\pm$ 24.43	273.68 $\pm$ 12.19	290.93 $\pm$ 26.71	293.28 $\pm$ 23.64	293.93 $\pm$ 24.20	292.75 $\pm$ 2.10	292.21 $\pm$ 26.86	293.90 $\pm$ 24.26
Copper (μ g/dl)	62.96 $\pm$ 3.26	62.68 $\pm$ 4.78	63.82 $\pm$ 0.79	64.65 $\pm$ 4.28	66.00 $\pm$ 4.40	67.80 $\pm$ 3.99	66.32 $\pm$ 2.83	66.31 $\pm$ 4.43	66.03 $\pm$ 0.27
Oestrogen (pg/ml)	3.00 ^a $\pm$ 0.18	3.09 ^a $\pm$ 0.18	3.90 ^a $\pm$ 0.21	3.59 ^b $\pm$ 0.14	6.81 ^b $\pm$ 0.81	4.62 ^b $\pm$ 0.28	3.00 ^a $\pm$ 0.18	3.09 ^a $\pm$ 0.18	3.59 ^b $\pm$ 0.14
Progesterone (ng/ml)	2.73 ^a $\pm$ 0.10	2.29 ^a $\pm$ 0.28	4.27 ^b $\pm$ 0.51	2.61 ^a $\pm$ 0.11	1.12 ^b $\pm$ 0.14	3.14 ^b $\pm$ 0.12	2.71 ^a $\pm$ 0.06	2.77 ^a $\pm$ 0.25	2.79 ^a $\pm$ 0.05

*P< 0.05

^{a,b}Means bearing similar superscript in a column do not differ significantly.

^{A,B,C}Means bearing similar subscript in a row within the parameter do not differ significantly.

Table 4: Level (Mean± S.E) of biochemical profiles in the serum of puerperal buffalo cows at different days of treatment with different treatment regimen.

Parameters	Group I (n=8)			Group II (n=8)			Group III (n=8)		
	Day 15	Day 30	Day 45	Day 15	Day 30	Day 45	Day 15	Day 30	Day 45
Leptin (ng/ml)	3.06 ^a ± 0.13	3.05 ^a ± 0.16	3.65 ^a ± 0.12	2.65 ^b ± 0.12	2.70 ^{ab} ± 0.07	3.31 ^a ± 0.18	2.72 ^{ab} ± 0.09	2.50 ^b ± 0.09	2.43 ^b ± 0.13
Ghrelin (pg/ml)	243.28 ^a ± 8.79	241.04 ^a ± 2.06	242.20 ^a ± 2.77	244.81 ^a ± 2.29	243.86 ^a ± 1.26	243.05 ^a ± 2.60	256.25 ^b ± 3.03	256.75 ^b ± 2.74	257.50 ^b ± 2.52
IGF-1 (ng/ml)	62.74 ^a ± 4.42	74.70 ^a ± 4.03	74.85 ^a ± 2.50	68.20 ^a ± 2.36	74.64 ^a ± 4.00	89.63 ^b ± 0.69	63.44 ^a ± 0.32	64.65 ^a ± 0.66	64.65 ^c ± 0.66
Calcium (mg/dl)	9.58 ± 0.25	9.35 ± 0.47	9.49 ± 0.47	9.56 ± 0.35	9.27 ± 0.28	10.13 ± 0.09	9.09 ± 0.29	9.28 ± 0.30	9.72 ± 0.15
Phosphorus (mg/dl)	3.41 ^a ± 0.17	3.62 ^a ± 0.13	4.67 ^b ± 0.22	3.20 ^b ± 0.08	4.57 ^b ± 0.03	4.90 ^c ± 0.07	3.43 ^a ± 0.06	3.49 ^a ± 0.14	3.65 ^b ± 0.09
zinc (µg/dl)	235.44 ± 4.23	234.05 ± 2.10	234.00 ± 2.25	235.25 ± 2.20	235.70 ± 1.70	236.10 ± 2.00	235.00 ± 1.70	235.12 ± 1.10	235.55 ± 1.20
Copper (µg/dl)	54.28 ± 0.85	55.07 ± 0.71	55.25 ± 1.65	53.96 ± 0.66	54.81 ± 0.90	55.04 ± 2.05	54.17 ± 0.76	54.45 ± 1.83	55.44 ± 2.80
Oestrogen (pg/ml)	2.89 ^a ± 0.34	3.26 ^b ± 0.24	3.48 ^b ± 0.48	3.26 ^a ± 0.24	4.09 ± 0.63	4.73 ^b ± 0.88	2.53 ^a ± 0.08	2.71 ^a ± 0.06	2.99 ^a ± 0.27
Progesterone (ng/ml)	1.80 ± 0.11	1.52 ± 0.08	2.14 ± 0.45	2.22 ± 0.26	1.98 ± 0.17	1.99 ± 0.47	1.94 ± 0.21	1.68 ± 0.05	2.00 ± 0.06

*P < 0.05

^{a,b}Means bearing similar superscript in a column do not differ significantly.^{A,B,C}Means bearing similar subscript in a row within the parameter do not differ significantly.

in the bypass fat treated and control cows. In conclusion, it can be mentioned that feeding of bypass fat fortified with minerals and phosphorus was effective for improvement of energy status in post partum buffalo cows which usually suffered from production stress.

Serum calcium

Feeding of bypass fat alone or bypass fat fortified with mineral and injectable phosphorus for a period of 30 days did not improve level of serum calcium in both heifers and puerperal cows under study. However, mean serum calcium level in both the groups of bypass fat treated heifers was significantly higher than that in control heifers. This difference might not be due to the effect of bypass fat treatment, rather individual variations could be the reason as the level recorded in the control heifers was lower than that in the treated heifers even on day 0 of treatment. The mean serum calcium levels in all the treatment groups were within the normal range in normal cycling buffaloes i.e. 8.73-11.753 mg/dl as reported by Jayachandran *et al.* (2013).

Serum phosphorus

Result obtained in the present study clearly indicated that bypass fat feeding with or without fortification for a period of 30 days led to increased serum phosphorus level in puerperal buffalo cows but not in the pubertal heifers and the increase was more marked from 30 to 45 days post partum in the cows treated with bypass fat + minerals + injectable phosphorus. Values obtained in the present study in both cows and heifers at different days of treatment was lower than the normal phosphorus level (5.56 to 6.98 mg/dl) in cyclic buffaloes as reported by Jayachandran *et al.* (2013). Phosphorus deficiency may cause early embryonic death resulting in the repeat breeding and anoestrus condition (Kumar *et al.*, 2010).

Serum zinc

In the present study it was observed that in both pubertal heifers and puerperal cows serum zinc level did not increase due to treatment with bypass fat alone or bypass fat, minerals and injectable phosphorus together for a period of 30 days. In both bypass fat treated and control swamp buffalo heifers and puerperal cows mean serum zinc level recorded at different days of treatment ranged from 234.00 ± 2.25 to 293.93 ± 24.20 µg/dl, which was similar to that reported by Kumar *et al.* (2016) in normal cyclic Murrah buffaloes (2.86 ± 0.46 ppm).

Serum copper

Serum copper levels were also not influenced by feeding bypass fat alone or bypass fat fortified with mineral and injectable phosphorus for a period of 30 days in pubertal heifers as well as puerperal cows. Perusal of literature revealed very little information about the influence of bypass fat feeding on serum levels of macro and micro minerals for effective comparison of the results obtained in the present study and to comment on the factors affecting the above mentioned parameters. However periods of feeding of bypass

fat to animals might be one of the factors determining relationship of bypass fat feeding with serum levels of minerals in treated animals.

Serum estrogen

Results obtained in the present study showed that feeding of bypass fat alone or bypass fat with mineral and injectable phosphorus for a period of 30 days led to improvement in serum estrogen level in both pubertal heifers and puerperal cows. The increase was more marked at day 30 of treatment in heifers and at day 30 to 45 post partum in puerperal cows. The increase in the serum estrogen level might be an indication of increased follicular development in the ovaries of pubertal heifers as well as puerperal cows. When the two treatment regimens were compared it was observed that serum estrogen level in the heifers treated with bypass fat fortified with minerals and injectable phosphorus was higher than that in heifers treated with bypass fat alone. However in case of puerperal cows, serum estrogen level did not vary with the treatment regimen.

Serum progesterone

Levels of serum progesterone in pubertal heifers treated with bypass fat alone as well as with bypass fat fortified with minerals and injectable phosphorus showed a fluctuating trend within 30 days treatment period. In both the groups, serum progesterone levels dropped down at day 15 of treatment after which it was elevated to a significantly higher level at day 30. This fluctuation in serum progesterone level might be an indication of rise and fall of the luteal activity in the bypass fat treated heifers during the treatment period. On clinical examination, 25 per cent of pubertal heifers treated with bypass fat, minerals and injectable phosphorus showed an active ovary (luteal activity) on day 15 and day 30 of treatment, which supported the fluctuating level of serum progesterone observed in the present study. In case of puerperal cows, there was no significant variation due to treatment with bypass fat either alone or fortified with minerals and injectable phosphorus. Hence, it could be concluded that feeding of bypass fat alone or fortified with minerals and injectable phosphorus was beneficial to increase ovarian activity in pubertal heifers.

CONCLUSION

Feeding of bypass fat alone or fortified with minerals and injectable phosphorus for a period of 30 days was ineffective for inducing genital changes in both pubertal heifers and puerperal cows.

Conflict of interest

All authors declare that they have no conflicts of interest.

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