



Original Research

Effect of Nutritional Supplementation on Treatment of Anoestrus Swamp Buffalo Cows and Heifers

R. Deka*, K. C. Nath, M. Bhuyan, N. C. Nath¹, G. C. Das², N. Deka and N. Das

Department of Animal Reproduction, Gynaecology and Obstetrics, College Of Veterinary Science, AAU, Khanapara-781022, Guwahati, Assam, INDIA

¹Department of Veterinary Physiology

²Department of Animal Genetics and Breeding

*Corresponding author: rajudeka110@gmail.com

Rec. Date:	Jun 17, 2018 10:08
Accept Date:	Oct 08, 2018 17:20
DOI	10.5455/ijlr.20180617100830

Abstract

A study was conducted on 8 Swamp buffalo cows and 8 heifers maintained at Network Project on Buffalo Improvement (Swamp), College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, to accumulate data on efficacy of bypass fat with or without minerals and vitamins fortification in the treatment of anoestrus. Efficacy of bypass fat in the treatment of anoestrus was studied on the basis of oestrus response, genital changes and level of some of the blood biochemical constituents. Bypass fat with minerals and vitamins was more effective than bypass fat alone for the treatment of anoestrus in Swamp buffalo cows and heifers resulting in higher oestrus response rate of 75 per cent. Level of serum leptin, calcium, phosphorus and cholesterol was found to be increased and that of ghrelin decreased with the period of treatment. Bypass fat fortified with minerals and vitamins could be effectively used for treatment of anoestrus in Swamp buffalo cows and heifers.

Key words: Anoestrus, Bypass Fat, Ghrelin, Leptin, Swamp Buffalo

How to cite: Deka, R., Nath, K., Bhuyan, M., Nath, N., Das, G., Deka, N., & Das, N. (2019). Effect of Nutritional Supplementation on Treatment of Anoestrus Swamp Buffalo Cows and Heifers. International Journal of Livestock Research, 9(2), 55-63. doi: 10.5455/ijlr.20180617100830

Introduction

Dairy industry in India is mainly buffalo oriented and buffalo milk is richer than cow's milk, especially in terms of fat content. Better adaptability of buffalo to tropical climates and higher disease resistance ensure their place in the future world agriculture facing the challenges of global warming. Buffaloes found in the state of Assam are swamp type. The buffaloes of Assam are hardy and suitable for agricultural work in wet lands and better adaptable to local hot humid climate. The interaction between nutrition and reproduction has long been known to have important implications for the reproductive performance in animals.





Supplementation of fat, minerals and vitamins in the diet led to improvement in reproductive efficiency in cattle (Ramteke *et al.*, 2004). Under nutrition results in the loss of body weight and body condition leading to negative energy balance (Bindari *et al.*, 2013). Bypass fats are commonly referred to as ruminal inert fat, associated with calcium ions instead of glycerol backbone. Bypass fat has low solubility in rumen and is less susceptible to biohydrogenation. However, in abomasum at acidic pH it is dissociated and set free fatty acids and calcium for absorption. Initially, the role of protected fat was considered only as an energy supplement during the transition period leading to improvement in reproductive performance, but later on, it was demonstrated that the effect was also due to fatty acids which act as a precursor of progesterone via cholesterol and prostaglandins (Staples *et al.*, 1998). The deleterious effect of acute negative energy balance on lactation can be avoided by feeding bypass fat. Leptin and ghrelin are the two metabolic hormones which have been consider 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). The present study was made to study the effect of nutritional supplementation on genital status in anoestrous buffalo cows and heifers.

Materials and Methods

The experimental animals included in the present study comprised of 8 buffalo cows and 8 buffalo heifers maintained at Network Project on Buffalo Improvement (Swamp), College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam for the period of September, 2016 to March, 2017.

Grouping of Animals

The experiment was conducted in buffalo cows and heifers separately. Breeding records of 24 cows and 10 heifers which were more than 4 years of age in September 2016 were checked. A heifer was considered to be anoestrus when she failed to show the external signs of oestrus even after 4 years of age. A cow was considered to be anoestrus when she failed to show the external signs of oestrus even after 6 months of parturition. For this 8 anoestrus buffalo cows and 8 anoestrus buffalo heifers were taken and each divided into two groups and allotted to the following treatment regimens-

- a) Bypass fat alone: bypass fat (Fatomax marketed by Intas Pharmaceutical Ltd. Matoda, Ahmedabad. India) 100 gram per day per animal for 45 days.
- b) Bypass fat + vitamins+ minerals: bypass fat 100 gram per day per animal for 45 days + intramuscular injection of inorganic phosphorus 2 gm (Tonophosphan VET marketed by Intervet India Pvt. Ltd. Briahnagar, Pune India) at alternate days for 3 days + intramuscular injection of vitamin A 2,50,000 IU (Intavita-H marketed by Intas Pharmaceutical LTD. Matoda, Ahmedabad. India) at alternate days for 3 days + 30 gram mineral mixture (Minfa Gold marketed by Intas Pharmaceutical Ltd. Matoda, Ahmedabad. India) orally daily for 45 days.



Schedule of Examination

Rectal palpation of the genital organs was done in each animal on 0, 15, 30 and 60 days of treatment. On each day of examination the vulva, cervix, uterus and ovaries of the animals were examined to record the changes of the genital organs and the percentage frequencies were worked out. 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. Vulva was considered as oedematous when the vulvar wrinkles were less prominent on visual examination. Vaginal mucus was recorded as free flowing, scanty and absent. Patency of the cervix as open or closed. Uterine tone as good or moderate. Ovaries for presence of graffian follicle or corpus luteum.

Study of Blood Biochemical Constituents

Blood sample was collected from each animal on 0, 15, 30 and 60 days of treatment and analysed for leptin, ghrelin, calcium, phosphorus and cholesterol using standard methods.

Collection of Blood

Blood was collected from jugular vein with a sterile 18 gauze needle. For obtaining serum, 5 ml of the collected whole blood was taken in a sterilized vacutainer containing clot activator and was kept in slanting position. The clotted blood sample was centrifuged for separation of serum. Serum was collected by sterilized pipette and kept in a clean dry sterilized plastic vial at -20 degree centigrade.

Estimation of Blood Biochemical Constituents

The biochemical constituents of serum samples were estimated by using quality reagent kits of calcium (Greiner Diagnostic GmbH- Unter Gereuth 10-D-79353 Bahlingen- Germany), phosphorus (Greiner Diagnostic GmbH-Unter Gereuth 10-D-79353 Bahlingen- Germany) and cholesterol (DIATEK Healthcare Pvt. Ltd. Amar Bangla Complex, Kolkata). ELISA techniques were employed to estimate the serum ghrelin (Cloud-Clone Corp. marketed by 1304 Langham Creek Dr, Suite 226, Houston, TX 77084, USA) and leptin (Diagnostics Biochem Canada Inc. marketed by 41 Byron Avenue, Dorchester, Ontario, Canada) in the collected samples.

Statistical Analysis

The statistical analysis of the data was done using software IBM-SPSS-20 (<http://www.spss.co.in>) and Micro Soft Excel-2010 (<http://office.microsoft.com>).

Results and Discussion



Response to Treatment with Bypass Fat

In the present study 50.00 per cent anoestrus Swamp buffalo cows and 25.00 per cent anoestrus heifers responded to the treatment with bypass fat alone against 75 per cent oestrus response rate in each of buffalo cows and heifers treated with bypass fat fortified with minerals and vitamins. The average post treatment oestrus interval in cows and heifers was 45 days and 60 days respectively in bypass fat treated animals and 40 and 50 days respectively in bypass fat + minerals and vitamins treated animals. This result indicated that bypass fat fortified with minerals and vitamins enhance the oestrus response rate and shortened the post treatment oestrus interval. Sahoo *et al.* (2016) reported that bypass fat and mineral supplementation led to 71.42 per cent oestrus response rate in cows. Supplementation of bypass fat in periparturient period reduced post-partum oestrus interval as reported by Ramteke *et al.* (2014) in buffaloes and Singh *et al.* (2016) in cows. Devkota *et al.* (2013) reported that vitamin and mineral supplementation was effective for the treatment of anoestrus in buffaloes but needed a longer period of treatment *i.e.* more than 2 months. Roberts (1971) mentioned that supplementation of nutrition increased secretion of gonadotropin hormone from pituitary and increased the blood metabolites. Fats in the diet can influence reproduction positively by altering both ovarian follicle and CL function via improved energy status and by increasing precursors (insulin and IGF-I) for the synthesis of reproductive hormones such as steroids and prostaglandins (Rahbar *et al.*, 2014). The fatty acid supplementation increased the blood glucose concentration which had a positive effect on preovulatory follicles (Lammoglia *et al.*, 1997), with formation of larger corpora lutea having optimum to high progesterone concentration.

Table 1: Percent responded and post treatment interval in anoestrous Swamp buffalo cows and heifers treated with different treatment regimens

Treatment Regimens	No. of animals Treated	No of animal responding to Treatment	Per cent responded (%)	Average Post treatment oestrus interval (days)
Cows				
Bypass fat	4	2	50	45
Bypass fat + Minerals + Vitamins	4	3	75	40
Heifers				
Bypass fat	4	1	25	60
Bypass fat + Minerals + Vitamins	4	3	75	50

Genital Changes

Frequency of occurrence of different changes observed in vulva, cervix, uterus and ovaries of the anoestrus buffalo cows in day 0, 15, 35 and 60 days of treatment are presented in Table 2. Frequency of occurrence of different changes observed in vulva, cervix, uterus and ovaries of the anoestrus buffalo heifers in day 0, 15, 35 and 60 days of treatment are presented in Table 3.

Table 2: Frequency of occurrence (%) of genital changes at different days of treatment in anoestrous Swamp buffalo cows treated with different treatment regimens

Genital Changes	Bypass Fat (n=4)				Bypass Fat + Minerals + Vitamins (n=4)			
	Day 0	Day 15	Day 30	Day 60	Day 0	Day 15	Day 30	Day 60
Congested vulva	0 (0)	0 (0)	25.00 (1)	25.00 (1)	0 (0)	0 (0)	50.00 (2)	25.00 (1)
Oedematous vulva	0 (0)	0 (0)	25.00 (1)	25.00 (1)	0 (0)	0 (0)	50.00 (2)	25.00 (1)
Patent cervix	0 (0)	0 (0)	25.00 (1)	25.00 (1)	0 (0)	0 (0)	50.00 (2)	25.00 (1)
Tonous uterus	0 (0)	0 (0)	25.00 (1)	25.00 (1)	0 (0)	0 (0)	50.00 (2)	25.00 (1)
Palpable follicle	0 (0)	0 (0)	50.00 (2)	25.00 (1)	0 (0)	25.00 (1)	75.00 (3)	50.00 (1)
Palpable corpus luteum	50.00 (2)	25.00 (1)	75.00 (3)	75.00 (3)	75.00 (3)	50.00 (2)	100.00 (4)	100.00 (4)

Figures in the parentheses indicate number of observations

Table 3: Frequency of occurrence (%) of genital changes at different days of treatment in anoestrous Swamp buffalo heifers treated with different treatment regimens

Genital Changes	Bypass Fat (n=4)				Bypass Fat+ Minerals+ Vitamins (n=4)			
	Day 0	Day 15	Day 30	Day 60	Day 0	Day 15	Day 30	Day 60
Congested vulva	0 (0)	0 (0)	0 (0)	25.00 (1)	0 (0)	0 (0)	25.00 (1)	50.00 (2)
Oedematous vulva	0 (0)	0 (0)	0 (0)	25.00 (1)	0 (0)	0 (0)	25.00 (1)	50.00 (2)
Patent cervix	0 (0)	0 (0)	0 (0)	25.00 (0)	0 (0)	0 (0)	25.00 (1)	50.00 (2)
Tonous uterus	0 (0)	0 (0)	0 (0)	25.00 (1)	0 (0)	0 (0)	25.00 (1)	50.00 (2)
Palpable follicle	0 (0)	0 (0)	25.00 (1)	50.00 (2)	0 (0)	0 (0)	50.00 (2)	50.00 (2)
Palpable corpus luteum	0 (0)	25.00 (1)	25.00 (1)	75.00 (3)	25.00 (1)	75.00 (3)	50.00 (2)	75.00 (3)

Figures in the parentheses indicate number of observation

As regards to genital changes in anoestrous buffalo cows and heifers treated with bypass fat with or without minerals and vitamins supplementation in the present study it was observed that greater proportion of animals treated with bypass fat fortified with minerals and vitamins showed genital changes from 30 days in cows and 60 days in heifers of treatment. In case of bypass fat treatment genital changes were not apparent in greater proportion of animals even at 60 days of treatment in both cows and heifers. This clearly indicated that bypass fat fortified with minerals and vitamins was more effective than bypass fat alone for treatment of anoestrus in Swamp buffaloes. Minerals and vitamins supplementation might have evoked better genital stimulation resulting in better manifestation of genital changes. Roberts (1971) also stated that oestrogen was known to produce clinical manifestation of genital changes. Minerals and vitamins known to enhance the reproductive efficiency greatly in postpartum anoestrous cows as they function as co-enzymes of various metabolic functions particularly in production of steroid hormones. Moreover, dietary fats may have considerable effect on reproduction because fatty acids and cholesterol are substrates for steroid hormone synthesis. So, feeding high fat diets to postpartum anoestrous cows increased follicular growth with more progesterone production and lengthened the lifespan of the corpus luteum. Further, nutritional supplement and hormonal treatment might have improved resumption of ovarian activity and subsequent conception rates as they maintained the energy balance and body reserve which helped in enhancing reproductive performance (Butler, 2000).



Blood Biochemical Constituents

The mean levels of serum leptin, ghrelin, calcium, phosphorus and cholesterol in anoestrous Swamp buffalo cows at 0, 15, 30 and 60 days of treatment with bypass fat and bypass fat fortified with minerals and vitamins are presented in Table 4 and in heifers presented in Table 5. Among the different serum biochemical profile in Swamp buffalo cows all the constituents varied significantly ($P < 0.05$) between treatment regimens as well as between days of treatment except in ghrelin mean serum level varied significantly ($P < 0.05$) between days of treatment but not in between treatment regimens. In heifers all the blood biochemical constituents varied significantly ($P < 0.01$) between days of treatment but serum phosphorus level varied significantly ($P < 0.01$) between treatment regimens and highly significant between days of treatment.

However, the rate of increase in the level of serum leptin, calcium phosphorus and cholesterol and rate of decrease in the level of serum ghrelin in Swamp buffalo cows and heifers with days was similar in both the treatment groups which clearly indicated that addition of minerals and vitamins to bypass fat did not add beneficial effect in the treatment of anoestrus in buffalo cows and heifers. In the present study the mean serum leptin level was lower than the normal leptin level in cyclic animals as reported by as reported by Tazik and Nazifi (2011) in buffaloes (7.30 ± 0.19 ng/ml) and Guzel and Tanriverdi (2014) in cattle (5.12 ± 0.43 mg/dl). In dairy cattle, decreased leptin production due to negative energy balance during lactation hampered fertility (Jayaprakash *et al.* 2016). Higher level of serum leptin and lower level of serum ghrelin indicated positive energy balance (Nowroozi-Asl *et al.* 2016). Leptin, as peripheral signal for energy abundance, serves an important role in reproduction and threshold leptin levels (as indicator of sufficient energy stores) are needed for proper activation and function of the reproductive axis (Casanueva and Dieguez 1999). Conversely, ghrelin, as peripheral signal for energy insufficiency, might play an opposite role. Systemic ghrelin may indeed participate in the control of reproduction, as ghrelin inhibits LH secretion in different experimental models and reduces circulating prolactin levels in pubertal rats (Fernandez-Fernandez *et al.* 2004). In the present study mean serum calcium and phosphorus level was lower than the normal calcium (8.73-11.753 mg/dl) and phosphorus (5.56 to 6.98 mg/dl) level in cyclic buffaloes as reported by Jayachandran *et al.* (2013) and Kumar *et al.* (2016). Devasena *et al.* (2010) and Kajabhai (2013) reported significant increase in the mean serum calcium and phosphorus level with days of treatment in anoestrus animals following supplementation with minerals. Ullah *et al.* (2010) in Nili-Ravi buffaloes and Soni *et al.* (2015) in Surti buffaloes however observed a non-significant increase in the mean serum calcium and phosphorus level in anoestrus cows and heifers after treatment with minerals and vitamins. The mean level of serum cholesterol in anoestrus buffaloes under both the treatment regimens in the present study was lower than the normal cholesterol level in cyclic buffaloes as reported by Butani *et al.* (2011), Jayachandran *et al.* (2013) and Ghani *et al.* (2017) which ranged from 142.83 to 183.09 mg/dl.

Table 4: Mean levels of serum leptin (ng/ml), ghrelin (pg/ml), calcium (mg/dl), phosphorus (mg/dl) and cholesterol (mg/dl) at different days of treatment in anoestrus Swamp buffalo cows treated with bypass fat and bypass fat fortified with minerals and vitamins

Days of treatment	Leptin (Mean $\pm$ SE)		Ghrelin (Mean $\pm$ SE)		Calcium (Mean $\pm$ SE)		Phosphorus (Mean $\pm$ SE)		Cholesterol (Mean $\pm$ SE)	
	Bypass fat	Bypass fat + minerals + vitamins	Bypass fat	Bypass fat + minerals + vitamins	Bypass fat	Bypass fat + minerals + vitamins	Bypass fat	Bypass fat + minerals + vitamins	Bypass fat	Bypass fat + minerals + vitamins
0 th day	1.28 ^a _A $\pm$ 0.06	3.25 ^{ab} _B $\pm$ 1.38	330.00 ^a $\pm$ 41.23	332.50 ^a $\pm$ 40.29	7.51 ^a _A $\pm$ 0.14	7.68 ^{ab} _B $\pm$ 0.05	4.22 ^a _A $\pm$ 0.14	4.29 ^{ab} _B $\pm$ 0.16	128.54 ^a _A $\pm$ 6.58	142.85 ^{ab} _B $\pm$ 6.85
15 th day	2.29 ^{ab} _A $\pm$ 0.13	3.82 ^b _B $\pm$ 1.16	250.00 ^{ab} $\pm$ 31.09	235.00 ^{bc} $\pm$ 30.96	7.85 ^{abc} _A $\pm$ 0.13	8.01 ^{bcd} _B $\pm$ 0.08	4.33 ^{ab} _A $\pm$ 0.13	4.56 ^{cd} _B $\pm$ 0.10	132.95 ^{ab} _A $\pm$ 7.91	153.77 ^{bc} _B $\pm$ 5.96
30 th day	3.19 ^{bc} _A $\pm$ 0.48	4.38 ^{bc} _B $\pm$ 1.15	225.00 ^{bc} $\pm$ 36.63	187.50 ^{bc} $\pm$ 11.09	8.18 ^{cd} _A $\pm$ 0.14	8.39 ^{cd} _B $\pm$ 0.07	4.41 ^{abc} _A $\pm$ 0.17	4.68 ^{de} _B $\pm$ 0.13	145.35 ^{bc} _A $\pm$ 11.5	165.80 ^c _B $\pm$ 4.01
60 th day	4.16 ^{cd} _A $\pm$ 0.51	5.25 ^c _B $\pm$ 1.24	152.50 ^c $\pm$ 21.36	167.50 ^{bc} $\pm$ 11.09	8.63 ^{ef} _A $\pm$ 0.26	8.87 ^f _B $\pm$ 0.10	4.78 ^d _A $\pm$ 0.18	4.97 ^f _B $\pm$ 0.11	151.93 ^c _A $\pm$ 8.05	183.56 ^d _B $\pm$ 3.99

Means bearing similar superscript (a, b, c, d, e and f) in a column do not differ significantly; Means bearing similar subscript (A and B) in a row within a parameter do not differ significantly.

Table 5: Mean levels of serum leptin (ng/ml), ghrelin (pg/ml), calcium (mg/dl), phosphorus (mg/dl) and cholesterol (mg/dl) at different days of treatment in anoestrus Swamp buffalo heifers treated with bypass fat and bypass fat fortified with minerals and vitamins

Days of treatment	Leptin (Mean $\pm$ SE)		Ghrelin (Mean $\pm$ SE)		Calcium (Mean $\pm$ SE)		Phosphorus (Mean $\pm$ SE)		Cholesterol (Mean $\pm$ SE)	
	Bypass fat	Bypass fat + minerals + vitamins	Bypass fat	Bypass fat + minerals + vitamins	Bypass fat	Bypass fat + minerals + vitamins	Bypass fat	Bypass fat + minerals + vitamins	Bypass fat	Bypass fat + minerals + vitamins
0 th day	2.06 ^{ab} $\pm$ 0.82	2.14 ^{ab} $\pm$ 0.69	417.50 ^{ab} $\pm$ 55.73	372.50 ^{ab} $\pm$ 63.43	7.34 ^{ab} $\pm$ 0.16	7.47 ^{ab} $\pm$ 0.21	4.25 ^a _A $\pm$ 0.14	4.35 ^a _B $\pm$ 0.13	134.39 ^{ab} $\pm$ 11.06	127.03 ^a $\pm$ 7.00
15 th day	2.41 ^{abc} $\pm$ 0.77	2.41 ^{abc} $\pm$ 0.78	297.50 ^{abc} $\pm$ 34.73	277.50 ^{bc} $\pm$ 49.73	7.65 ^{bc} $\pm$ 0.13	7.89 ^{cd} $\pm$ 0.24	4.35 ^a _A $\pm$ 0.09	4.37 ^a _B $\pm$ 0.12	143.37 ^{abc} $\pm$ 9.42	140.45 ^{abc} $\pm$ 6.79
30 th day	2.76 ^{abcd} $\pm$ 0.68	2.72 ^{abcd} $\pm$ 0.78	232.50 ^c $\pm$ 26.26	252.50 ^c $\pm$ 52.66	8.08 ^{cd} $\pm$ 0.09	8.20 ^d $\pm$ 0.26	4.63 ^b _A $\pm$ 0.16	4.72 ^b _B $\pm$ 0.13	154.21 ^{cde} $\pm$ 9.47	148.87 ^{bcd} $\pm$ 10.21
60 th day	3.41 ^d $\pm$ 0.72	3.08 ^d $\pm$ 0.85	187.50 ^c $\pm$ 24.28	165.00 ^c $\pm$ 15.55	8.59 ^e $\pm$ 0.13	8.66 ^e $\pm$ 0.16	4.96 ^c _A $\pm$ 0.13	5.04 ^c _B $\pm$ 0.12	166.35 ^f $\pm$ 7.32	161.71 ^{de} $\pm$ 6.16

Means bearing similar superscript (a, b, c, d, e and f) in a column do not differ significantly; Means bearing similar subscript (A and B) in a row within a parameter do not differ significantly.



Conclusion

Present study concluded that bypass fat with minerals and vitamins was more effective than bypass fat alone for the treatment of anoestrus in swamp buffalo cows and heifers.

Acknowledgments

Author are thankful to Dr. B.C. Deka Professor and Head, Department of ARGO for his guidance and support to carry out this study.

References

1. Bindari, Y. R., Shrestha, S., Shrestha, N., & Gaire T.N. (2013). Effects of nutrition on reproduction- A review. *Advances in Applied Science Research*, 4(1), 421-429.
2. Butani, M.G., Dhami, A.J., & Kumar, R. (2011). Comparative blood profile of progesterone metabolites and minerals in anoestrus, suboestrus, repeat breeding and normal cyclic buffaloes. *Indian Journal of Field Veterinarian*, 7(2), 20-24.
3. Butler, W.R. (2000). Nutritional interactions with reproductive performance in dairy cattle. *Animal of Reproduction Science*, 60-61, 449-457.
4. Casanueva, F. F., & Dieguez, C. (1999). Neuroendocrine regulation and actions of leptin. *Frontiers Neuroendocrinology*, 20, 317-363.
5. Devasena, B., Reddy, I.J., Ramana, J.V., Prasad, P.E., & Prasad, J.R. (2010). Effect of supplementation of area specific mineral mixture on reproductive performance of crossbred cattle: A field study. *Indian Journal of Animal Nutrition*, 27(3), 265- 270.
6. Devkota, B., Nakao, T., Kobayashi, K., Sato, H., Sah, S.K., Singh, D.K., Dhakal, I.P., & Yamagishi, N. (2013). Effects of treatment for anestrus in water buffaloes with PGF 2α and GnRH in comparison with vitamin-mineral supplement, and some factors influencing treatment effects. *Journal of Veterinary Medical Science*, 75(12), 1623-1627.
7. Fernandez-Fernandez, R., Tena-Sempere, M., Aguilar, E., & Pinilla, L. (2004). Ghrelin effects on gonadotropin secretion in male and female rats. *Neuroscience Letters*, 362, 103-107.
8. Ghani, M.U., Ahmad, I., Ahmad, N., Ijaz, N., & Mehfooz, A. (2017). Hematology, serum total cholesterol and thyroid hormone concentrations in cyclic and acyclic Nili-Ravi buffaloes. *Pakistan Veterinary Journal* bari, 37(1), 31-34.
9. Guzel, S., & Tanriverdi, M. (2014). Comparison of serum leptin, glucose, total cholesterol and total protein levels in fertile and repeat breeder cows. *Revista Brasileira De Zootecnia*, 43(12), 643-647.
10. Jayachandran, S., Nanjappan, K., Muralidharan, J., Selvaraj, P., & Manoharan, A. (2013). Blood biochemical and mineral status in cyclic and postpartum anestrus buffaloes. *International Journal of Food, Agriculture and Veterinary Science*, 3 (1), 93-97.
11. Jayapraksh, G., Sathiyabarathi, M., Robert, M.A., Tamilmani, T. & Azhaguraja, M. (2016).Effect of leptin in dairy cattle. *International Journal of Science, Environment and Technology*, 5(3), 1127-1130.
12. Kajabhai, C. R. (2013). Supplementation of appropriate mineral mixture to anestrus and repeat breeder buffaloes in tribal areas of Vadodara district. (M.V.Sc. Thesis). Anand Agricultural University.
13. Kumar, R., Ghosh, M., Rawat, S.S., Kumar, P., & Kumar, S. (2016). Plasma mineral profile of normal cyclic and postpartum anoestrous Murrah buffaloes in organised farms. *Animal Science Reporter*, 10(2), 43-47.
14. Lammoglia, M. A., Willard, S. T., Hallford, D. M., & Randel, R. D. (1997). Effects of dietary fat on follicular development and circulating concentrations of lipids and insulin on follicular development and circulating concentrations of lipids, insulin, progesterone, estradiol 17 β ,13,14-dihydro-15-keto-prostaglandin F 2α and growth hormone in estrous cyclic Brahman cows. *Journal of Animal Science*, 75, 1591-1600.



15. Nowroozi-Asl, A., Aarabi, N., & Rowshan-Ghasrodashti, A. (2016). Ghrelin and its correlation with leptin, energy related metabolites and thyroidal hormones in dairy cows in transitional period. *Polish Journal of Veterinary Science*, 19(1), 197-204.
16. Rahbar, B., Amir, H., Asgari, S., & Nasroallah, M. K. (2014). Mechanisms through which fat supplementation could enhance reproduction in farm animal. *Europeans. Journal of Experimental Biology*, 4(1), 340-348.
17. Ramteke, P.V., Patel, D.C., Parnerkar, S., Shankhpal, S.S., Patel, G.R., & Pandey, A. (2014). Effect of bypass fat supplementation during prepartum and postpartum on reproductive performance in buffaloes. *Livestock Research International*, 2(3), 54-58.
18. Roberts, S.J. (1971). *Veterinary Obstetrics and Genital disease*. 2nd Edition. Scientific Book Agency, Calcutta.
19. Sahoo, J.K., Das, S.K., Sethy, K., Mishra, S.K., Swain, R.K., Mishra, P.C., & Satapathy, D. (2016). Effect of supplementation of mineral mixture and bypass fat on performance of crossbred cattle. *Journal of Animal Research*, 6 (4), 611-618.
20. Singh, M., Yadav, G., Roy, A.K., & Thakur, S. (2016). Productive performance and metabolic hormonal profile in cows supplemented with prilled fat. *Indian Journal of Traditional Knowledge*, 15(2), 292-296.
21. Soni, D.K., Khasatiya, C.T., Rede, A.D., Patel, M.D., & Tyagi, K. K. (2015). Fertility parameters and Ca: P ratio of postpartum Surti buffaloes having inactive ovaries. *Veterinary Science Research Journal*, 6 (2), 80-84.
22. Staples, C.R., Burke, J.M. and Thatcher, W.W. 1998. Influence of supplemental fats on reproductive tissues and performance of lactating cows. *Journal of Dairy Science*, 81, 856-871.
23. Tajik, J., & Nazifi, S. (2011). A study of correlation of serum leptin with trace elements in water buffalo (*Bubalus bubalis*). *Pakistan Veterinary Journal*, 31(3), 231-234.
24. Ullah, N., Anwar, M., Andrabi, S.M.H., Ansari, M.S., Murtaza, S., Ali, Q., & Asif, M. (2010). Effect of mineral supplementation on post-partum ovarian activity in Nili-Ravi buffaloes (*Bubalus bubalis*). *Pakistan Journal of Zoology*, 43(2), 195-200.

