Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.6567

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **6 (4):** 11-21 (2018)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Blood Biochemical Profile in Cyclic and Non Cyclic Local Buffaloes

Doijad N. S., Yeotikar P. V.*, Deshpande S. D. and Markandeya N. M.

Department of Veterinary Biochemistry, College of Veterinary and Animal Sciences, Parbhani (M.S.) 431402

Maharashtra Animal and Fishery Sciences University, Nagpur-06

*Corresponding Author E-mail: yeopv@rediffmail.com

Received: 3.06.2018 | Revised: 11.07.2018 | Accepted: 19.07.2018

ABSTRACT

Biochemical studies reflect nutritive and reproductive status of farm animals. Hence, a study was undertaken to record blood biochemical profile in cyclic and non cyclic local buffaloes. The animals carrying various reproductive stages were selected from local area of Parbhani district of Maharashtra State and animals belonging to local or non descript breeds of buffaloes were grouped as cyclic and non cyclic category with each group consisting of twenty buffaloes. The serum samples were collected and analysed with clinical analyser by adopting standard methodology. The average values of biochemical constituents viz, Serum Calcium, Phosphorus, Total serum protein, Albumin, Globulin, A: G Ratio, Glucose and Cholesterol were 9.775 mg/dl, 6.380 mg/dl,7.165 g/dl, 4.342 g/dl, 2.795 g/dl, 1.705, 55.962 mg/dl and 107.565 mg/dl in cyclic buffaloes and the same were 9.676 mg/dl, 5.775mg/dl, 6.771g/dl, 3.035g/dl, 3.770 g/dl, 0.875, 49.195 mg/dl and 94.021mg/dl in non cyclic local buffaloes, respectively. Amongst the blood biochemical constituents, Serum Calcium, Serum phosphorus and Total serum protein did not differ non significantly in cyclic and non cyclic buffaloes, whereas Serum albumin (P<0.01), Serum globulin(P<0.05), A: G Ratio (P<0.01), Serum glucose (P<0.05) and Serum cholesterol(P<0.05) differed significantly in cyclic and non cyclic buffaloes. The lower serum albumin level among the non cyclic buffaloes might be due to difference in feeding practices. The infection of reproductive tract like that of metritis in non cyclic local buffaloes was related with higher serum globulin. The triggered immunoglobulin causes higher concentration of globulin in non cyclic buffaloes. The higher A: G Ratio values among the cyclic local buffaloes might be due to higher values of serum albumin. The continued cyclicity in cyclic local buffaloes was evidenced by high concentration of glucose. The anoestrous condition in non cyclic local buffaloes was apparent as serum cholesterol levels were on lower side. Thus, it was concluded that blood biochemical analysis interprets reproductive status and indicates chances of fertility in local buffaloes.

Key words: *Blood Biochemistry, Cyclic and Non Cyclic, Local Buffalo.*

Cite this article: Doijad, N.S., Yeotikar, P.V., Deshpande, S.D. and Markandeya, N.M., Blood Biochemical Profile in Cyclic and Non Cyclic Local Buffaloes, *Int. J. Pure App. Biosci.* **6(4):** 11-21 (2018). doi: http://dx.doi.org/10.18782/2320-7051.6567

INTRODUCTION

The serum profile is a potential aid in characterizing the reproductive performance of animal. There are certain biochemical constituents, which directly influences the reproductive performances of an animal either through stimulating hormone synthesis, hormone action or response of target tissues, or by acting as precursor for hormone synthesis. Buffalo has recently gained a lot of attention due to its high milk yield, fat percentage, tolerances to hot and humid climate, lean meat, draught ability and a reasonable growth rate on roughage feeding. Buffaloes are preferred for their high milk fat content and their adaptability to Indian agricultural conditions. Government of India is making efforts to increase the productivity of milch animals and thus increase the per capita availability of milk. Buffalo is an integral part of agriculture in Asia having pivotal role in Indian livestock industry contributing towards production of milk, meat and draft power. India possesses the best and diverse buffalo genetic resources comprising 12 registered breeds and several local populations adapted to different ecological niches. Buffalo accounts for 30 per cent of the total 4.9 million tons of meat production in the country and the buffalo meat production has increased by 21 per cent during the last five years that shows the recent increasing interest of buffalo industry in meat production. High environmental stress together with under- nutrition might therefore be responsible for the long periods of seasonal anoestrous in buffaloes[35]. Biochemical studies are important in diagnosis of healthy and diseased conditions of the animals. The blood picture may vary in normal cycling and anoestrous animals. By keeping this in view the present study was planned to investigate the blood biochemical profile in cyclic and non cyclic local buffaloes.

MATERIAL AND METHODS

Selection of animals

The experiment was conducted on 40 adult she buffaloes reared in and around Parbhani. The screened animals in the present study were categorized into two groups as follows:

Group I: A total of 20 cyclic buffaloes selected on the basis of gyaneco-clinical examination by per rectal examination having corpus luteum on one of the ovaries were used in the experiment.

Group II: A total of 20 non cyclic buffaloes selected after gyaneco- clinical examination by per rectal examination having inactive ovaries and those buffaloes failed to come in heat within the 21 days were used in the experiment.

Collection of blood sample

Blood samples from selected animals were collected by jugular venipuncture on the day of gyaneco-clinical examination under necessary aseptic precautions in the morning before let loose for grazing. Each animal of both the groups (Group I and Group II) was observed for signs of heat up to 21 days. Aliquot of blood sample (about 10 ml) was collected for obtaining serum. All blood samples were kept in ice immediately after collection and carried to laboratory for further processing. The serum samples were stored at -20^0 C until used for biochemical analysis. Serum samples were analysed for various constituents within 24-48 hours of collection.

Analytical methods

Serum Calcium, Serum Phosphorus, Total Serum Protein, Serum Albumin, Serum Glucose, Serum Cholesterol were estimated by reagent kits (Ambica Diagnostic Pvt. Ltd.) on semi-automatic clinical analyzer of Systronics make, (Model- 635) was used to read the transmittance of coloured solutions and Serum Globulin, A: G Ratio were determined by mathematical calculation.

Serum calcium was estimated by O-CPC method[15, 7]. Serum phosphorous was estimated by UV Molybdate method [28, 30, 39].Total Serum Protein was estimated by Biuret Method[42, 20]. Serum albumin was estimated by Bromo cresol Green (BCG) Method[13, 34]. Serum globulin concentration was calculated as the difference between Total Serum Protein and Albumin and the value was expressed as gm/dl. Serum albumin: globulin ratio was calculated as the albumin value divided by globulin value. Serum glucose was estimated by glucose oxidase / peroxidase (GOD/POD) method [6, 39]. Serum cholesterol was estimated by CHOD/POD method[4].

RESULTS AND DISCUSSIONS

Serum Calcium

The mean values of Serum calcium in cyclic and non cyclic buffaloes are presented in table 1 and depicted in Fig.1.Comparison of the mean values of serum calcium between two groups indicated non significant difference. But, the mean value of Group I was higher than the mean value of Group II . The mean values were found to be higher non significantly in cyclic buffaloes when compared to non cyclic buffaloes. However, the non cyclic buffaloes showed non-significant numerical reduction in mean values than the mean values of cyclic buffaloes (Table 1). Similar findings of higher values of serum calcium in cyclic buffaloes as compared to non cyclic buffaloes had been reported by Hedaoo *et al.* [17] and Chaurasia *et al.* [11]. The mean values of serum calcium in both groups were higher than the values reported by Chaurasia *et al.*[11], Kumar *et al.* [24] and Yotov *et al.* [44] and lower than Shrivastava and Karache [37], Dutta *et al.* [14] and Umesh *et al.* [41].

Calcium (Ca) is a mineral plays an important role in maintaining homeostasis of vertebrate animals including hormone secretion. Thus, a constant concentration of calcium is maintained by hormone like estrogens[9]. The calcium absorption and its circulating level in blood depend upon parathyroid hormone. Calcium has no direct relation with reproduction. It might be due to indirect effect of Phosphorous. The raised level of calcium in cyclic buffalo might be due to normal level of phosphorous levels.

Serum Phosphorous:

The mean values of Serum phosphorous in cyclic and non cyclic local buffalo are presented in table 1 and depicted in Fig.2. Comparison of the mean values of Serum phosphorous between two groups indicated non-significant difference. But, the mean value of Group I was higher than the mean value of Group II. The mean values were found to be higher non significantly in cyclic buffaloes when compared to non cyclic buffaloes. However, the non cyclic buffaloes showed non-significant numerical reduction in mean values than the mean values of cyclic buffaloes (Table 1). Similar findings of higher values of serum Phosphorous in cyclic buffaloes as compared to non cyclic buffaloes had been reported by Umesh *et al.* [41], Newer *et al.*[31], Yadav *et al.* [43], Chaurasia *et al.*[11], Kumar *et al.* [24], Ali and Shukla[3] and Yotov *et al.* [44]. The mean values of serum phosphorus in both groups were higher than the values reported by Dutta *et al.* [14], Ali *et al.*[2], Umesh *et al.* [41], Yadav *et al.* [43], Hedaoo *et al.* [17], Bohara and Devkota[8], Chaurasia *et al.* [11], Kumar *et al.* [24], Ali and Shukla[3] and Yotov *et al.*[44].The absorption of Phosphorous is a correlated phenomena with calcium which depends upon the level of phosphorous and calcium giving closer ratio of 1: 2. The involvement of Phosphorous, phospholipids and cyclic AMP synthesis may be key factor of its effect on reproduction[44]. A close correlation between the reproductive hormones and inorganic phosphorus exists marginal phosphorus deficiency may lead to anoestrus conditions[35]. The low levels of inorganic phosphorus in non cyclic buffaloes might be due intake of phosphorus deficient feed. It might also be due to phosphorus deficient rations in non cyclic buffalo. The symptoms of oestrous were suppressed similarly reported that marginal deficiency of phosphorus is sufficient.

Total serum Protein

The mean values of Total serum protein in cyclic and non cyclic local buffalo are presented in table 1 and depicted in Fig. 3.

Comparison of the mean values of Total serum protein between two groups indicated non-significant difference. But, the mean value of Group I was higher than the mean value of Group II. The mean values in cyclic buffaloes were found to be higher when compared to non cyclic buffaloes. However, the non cyclic buffaloes showed non-significant numerical reduction in the mean values than the mean values of cyclic buffaloes (Table 1). Similar findings of higher values of total serum protein in cyclic buffaloes as compared to non cyclic buffaloes had been reported by Dutta *et al.*[14], Ali *et al.*[2], Umesh *et al.* [41], Kabir *et al.*[21], Yadav *et al.* [43], Bohara and Devkota[8], Akhtar *et al.*[1], Kumar *et*

al. [24]. The mean values of total serum protein in both groups were higher than the values reported by Umesh *et al.*[41] and Bohara and Devkota[8] and lower than Chandolia and Verma[10], Gandotra *et al.*[25], Kabir *et al.*[21], Khasatiya *et al.*[23], Hedaoo *et al.*[17], Akhtar *et al.*[1] and Kumar *et al.* [24].

The protein is second most important nutrient after energy for reproduction. The quality and circulating level of protein are important for growth, reproduction and regular expression of cyclicity. If it decreases, the animal shows irregular cycle. The lower protein level observed in non cyclic buffalo hinders the growth of sex organs and then affects the reproductive performances[18].

Serum albumin:

The mean values of Serum Albumin in cyclic and non cyclic local buffalo are presented in table 1 and depicted in Fig.4.

Comparison of the mean values of Serum Albumin between two groups indicated highly significant (P<0.01) hike in Group I than the Group II. The mean values in cyclic buffaloes were found to be higher when compared to non cyclic buffaloes. However, the non cyclic buffaloes showed significant reduction in the mean values than the mean values of cyclic buffaloes (Table1). Similar findings of higher values of serum albumin in cyclic buffaloes as compared to non cyclic buffaloes had been reported by Singh and Singh[38] and Kumar *et al.*[24]. The mean values of serum albumin in both groups were higher than the values reported by Singh and Singh[38] and lower than Kumar *et al.*[24].

Albumin is a major storage reservoir of protein and has important function of providing osmotic activity and transport of protein of plasma[22]. The significant lower values of serum albumin in non cyclic buffalo as compared to cyclic one could have been due to low protein diet. As well as, it might be due to difference in the feeding and managemental conditions, since the animals belonged to different localities in contrast to the above speculation.

Serum globulin:

The mean values of Serum Globulin in cyclic and non cyclic local buffalo are presented in table 1 and depicted in Fig.5.

Comparison of the mean values of Serum globulin between two groups indicated significant (P<0.05) hike in Group II than Group I. The mean values in cyclic buffaloes were found to be lower when compared to non cyclic buffaloes. However, the cyclic buffaloes showed significant reduction in the mean values than the mean values in non cyclic buffaloes (Table 1). Similar findings of higher values of serum globulin in non cyclic buffaloes as compared to cyclic buffaloes had been reported by Latif *et al.*[25] and Singh and Singh[38].

Serum globulin in the present investigation was estimated by taking difference between total serum protein and albumin in the studied animals. In the non cyclic local buffalo, due to infection like that of metritis, production of immunoglobulins is triggered which might have causes higher concentrations of globulins in non cyclic buffaloes in present work. In the support of this observation, increases in serum globulin in non cyclic buffaloes have been reported[38].

A: G Ratio

The mean values of A: G Ratio in cyclic and non cyclic buffaloes are presented in table 1 and depicted in Fig. 6.

Comparison of the mean values of A: G Ratio between two groups indicated highly significant (P<0.01) rise in Group I than Group II . The mean values in cyclic buffaloes were found to be higher when compared to non cyclic buffalo. However, the non cyclic buffalo showed significant reduction in the mean values than the mean values in cyclic buffalo (Table 1). Similar findings of higher values of A: G ratio in cyclic buffalo as compared to non cyclic buffalo had been reported by Singh and Singh[38]. In the present work, the mean value of A: G ratio in cyclic buffaloes was higher than non cyclic buffaloes, irrespective of significantly higher values of serum globulin in non cyclic buffaloes. This was due to higher values of the numerator i.e. serum albumin in cyclic buffaloes. In the support of this observation, increase in A: G ratio in cyclic buffaloes has been reported[38].

Serum glucose

The mean values of serum glucose in cyclic and non cyclic local buffalo are presented in table 1 and depicted in Fig.7.

Comparison of the mean values of Serum glucose between two groups noted significant (P<0.05) hike in Group I than Group II. The mean values in cyclic buffaloes were found to be higher when compared to non cyclic buffaloes. However, the non cyclic buffaloes showed significant numerical reduction in the mean values than the mean values of cyclic buffaloes (Table 1). Similar findings of higher values of serum glucose in cyclic buffaloes as compared to non cyclic buffaloes had been reported by Shrivastava and Kharche[37], Umesh *et al.* [41], Sharma *et al.* [36], Kabir *et al.* [21], Khasatiya *et al.* [23], Singh and Singh[38], Bohara and Devkota[8] and Akhtar *et al.*[1]. The mean values of serum glucose in both groups were higher than the values reported by Umesh *et al.* [41] and lower than Shrivastava and Kharche[37], Sharma *et al.* [36], Kabir *et al.*[21], Khasatiya *et al.* [23], Bohara and Devkota[8], Akhtar *et al.*[1], Kumar *et al.*[24] and Ali and Shukla[3].

The values of serum glucose level in cyclic buffaloes had significantly risen than non cyclic buffaloes which were indicative of continued cyclicity. The high concentration of glucose shows signs of oestrous with high fertility rate. More the concentration of glucose, high will be the energy status which is directly proportional to more progesterone secretion which increases fertility rate. Several workers have supported this view that, the concentration of glucose replaces the energy states and reproductive activity of the animals[26, 29, 32]. The variation in blood glucose was clearly linked to cyclicity and fertility[26]. Hence, feeding of glucose or energy rich feed to buffaloes primarily required for continuous cyclicity, otherwise glucose deficiency or relative hypoglycaemia might possibly effect the ovarian activity for release of gonadotrophin from hypothalamus[19]. The concentration of glucose and insulin in blood were associated with nutritional anoestrous[32]. The Pituitary function appears to be adversely influenced by lower glucose level[5]. Since FSH hormone is glycoprotein, glucose is essential for biological activity of hormone[16]. The hypoglycaemic state in buffaloes leads to reduced hypoglycaemic ovarian axis signal transmission leading to anoestrous condition[36].

Serum cholesterol

The mean values of Cholesterol in cyclic and non cyclic local buffalo are presented in table 1 and depicted in Fig.8.

Comparison of the mean values of Serum Cholesterol between two groups indicated significant (P<0.05) hike in Group I than Group II. The mean values in cyclic buffaloes were found to be higher when compared to non cyclic buffaloes. However, the non cyclic buffaloes showed significant reduction in the mean values than the mean values of cyclic buffaloes (Table1). Similar findings of higher values of serum cholesterol in cyclic buffaloes as compared to non cyclic buffaloes had been reported by Dutta *et al.*[14], Umesh *et al.*[41], Sharma *et al.* [36], Kabir *et al.*[21], Khasatiya *et al.* [23], Bohara and Devkota[8] and Akhtar *et al.*[1], Ali and Shukla[3] and Yotov[44]. The mean values of serum cholesterol in both groups were higher than the values reported by Shrivastava and Kharche[37], Umesh *et al.*[41], Sharma *et al.*[36], Yadav *et al.*[43] and lower than Dutta *et al.*[14], Kabir *et al.*[21], Khasatiya *et al.*[23], Bohara and Devkota[8] and Akhtar *et al.*[1].

Serum cholesterol being a precursor for steroid hormone synthesis, its concentration varies during different reproduction phases such as pregnancy, parturition and lactation[27]. There is direct relationship between cholesterol level in blood and reproductive performance[12]. Hence, the deficiency of cholesterol could be responsible for anoestrous condition in buffaloes. The lower cholesterol level in anoestrous buffaloes may be responsible for the cessation or decrease in gonadal activity affected by the established relationship between the gonadal steroid and blood cholesterol level[33].

Table 1. Range, mean, standard errors and't' value of biochemical profile in cyclic and Non-cyclic buffaloes

Sr.No.	Parameter	Cyclic buffaloes (Group - I)			Non-cyclic buffaloes (Group-II)			't' value
		No.	Range	Mean ± SE	No.	Range	Mean ± SE	
1	Serum Calcium (mg/dl)	20	08.20 -11.95	9.775±0.235	20	07.20 – 12.70	9.676±0.304	0.266 NS
2	Phosphorus (mg/dl)	20	04.10 -08.40	6.380±0.345	20	04.10-07.70	5.775±0.242	1.815 NS
3	Total Serum Protein (g/dl)	20	06.13-08.51	7.165 ± 0.214	20	06.11-088.22	6.771 ± 0.171	1.311 NS
4	Serum Albumin(g/dl)	20	02.89-05.99	4.342 ± 1.264	20	02.10-04.12	3.035 ± 0.120	4.964 **S
5	Serum Globulin (g/dl)	20	02.11 – 04.33	2.795 ± 0.407	20	02.93 – 05.31	3.770 ± 0.432	2.615 *S
6	A:G Ratio	20	00.68 – 02.71	1.705 ± 0.407	20	0.50 – 1.52	0.875 ± 0.061	5.413 **S
7	Serum Glucose (mg/dl)	20	39.39-65-30	55.962 ± 1.698	20	39.20-62.20	49.195 ± 1.802	2.677 *S
8	Serum Cholesterol (mg/dl)	20	76.52-142.0	107.565 ± 4.592	20	69.12-126.0	94.021 ± 3.705	2.733 *S

* Significant at 0.05 per cent level.
** Significant at 0.01 per cent level.
NS : Non-Significant.

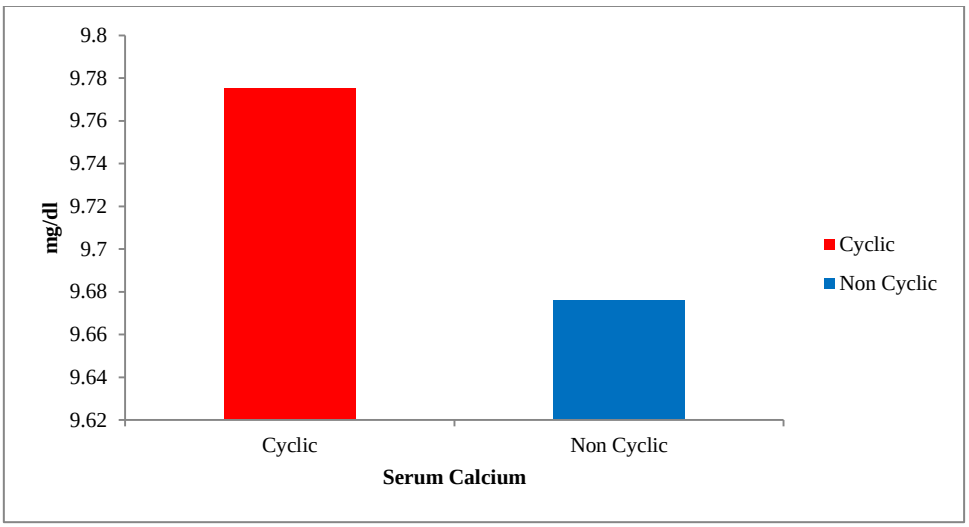

Fig. 1: Serum Calcium of cyclic and non-cyclic local buffaloes

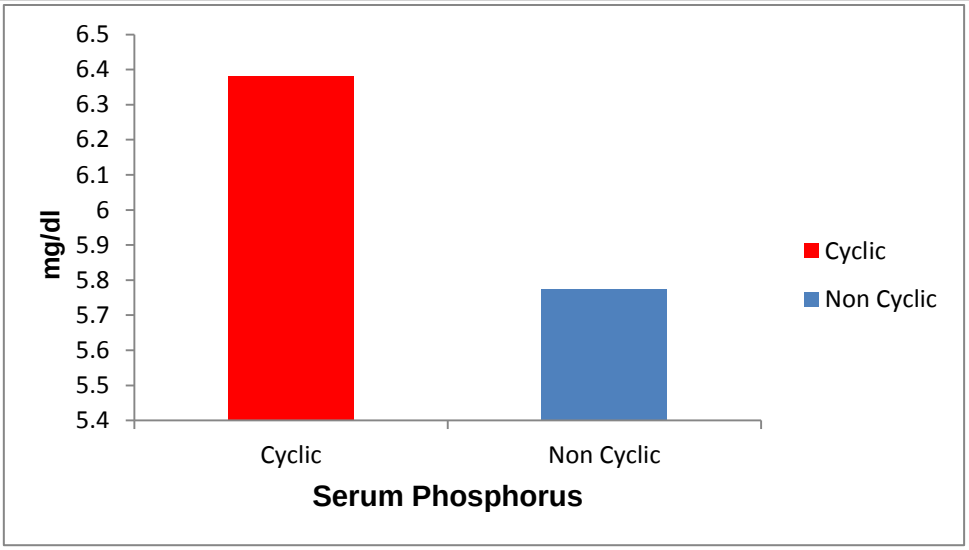

Fig. 2: Serum Phosphorus of cyclic and non-cyclic local buffaloes

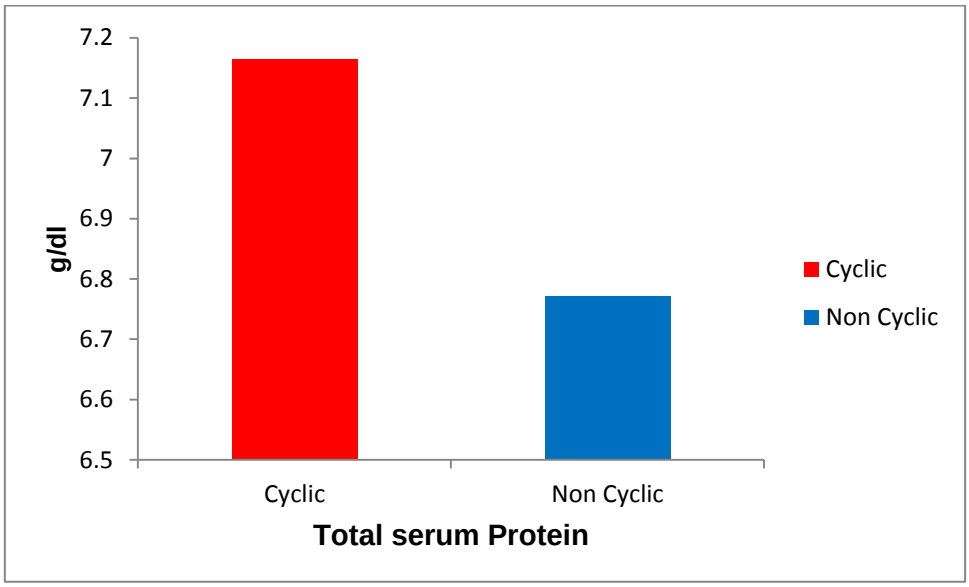

Fig. 3: Total Serum Protein of cyclic and non-cyclic local buffaloes

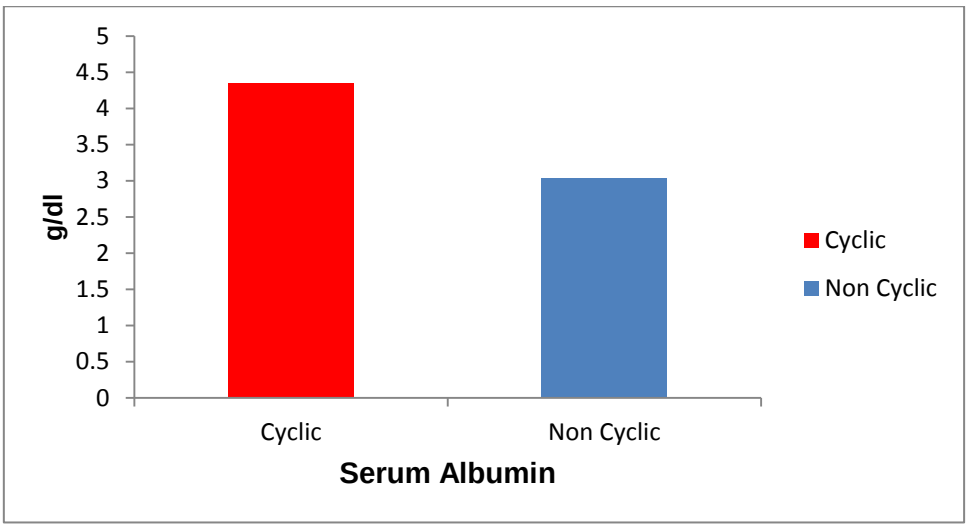

Fig. 4: Serum Albumin of cyclic and non-cyclic local buffaloes

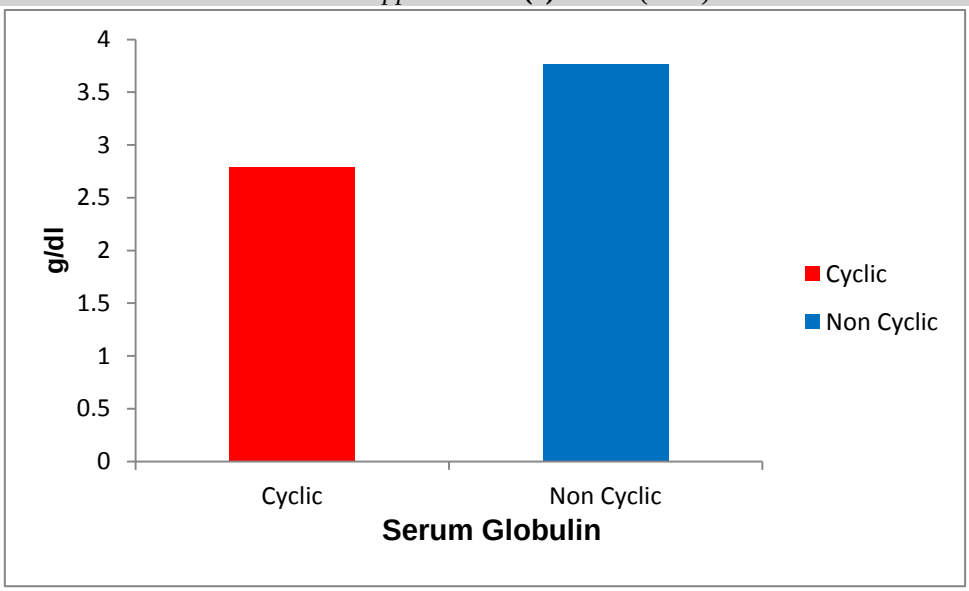

Fig. 5: Serum Globulin of cyclic and non-cyclic local buffaloes

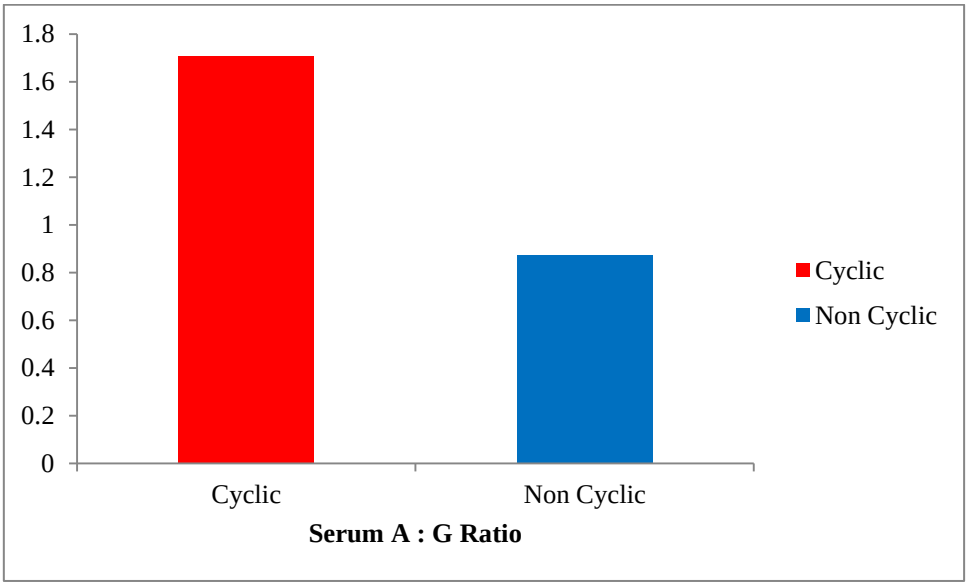

Fig. 6: Serum A: G Ratio of cyclic and non-cyclic local buffaloes

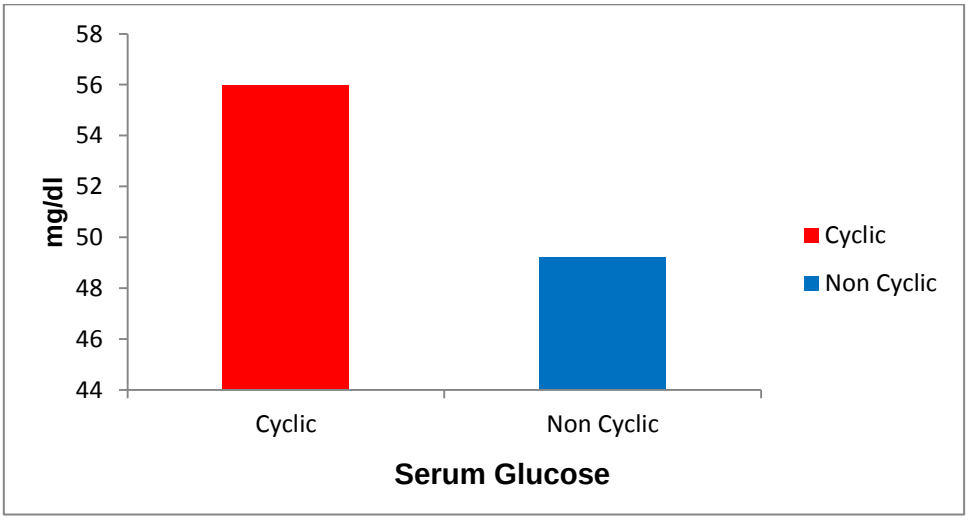

Fig. 7: Serum Glucose of cyclic and non-cyclic local buffaloes

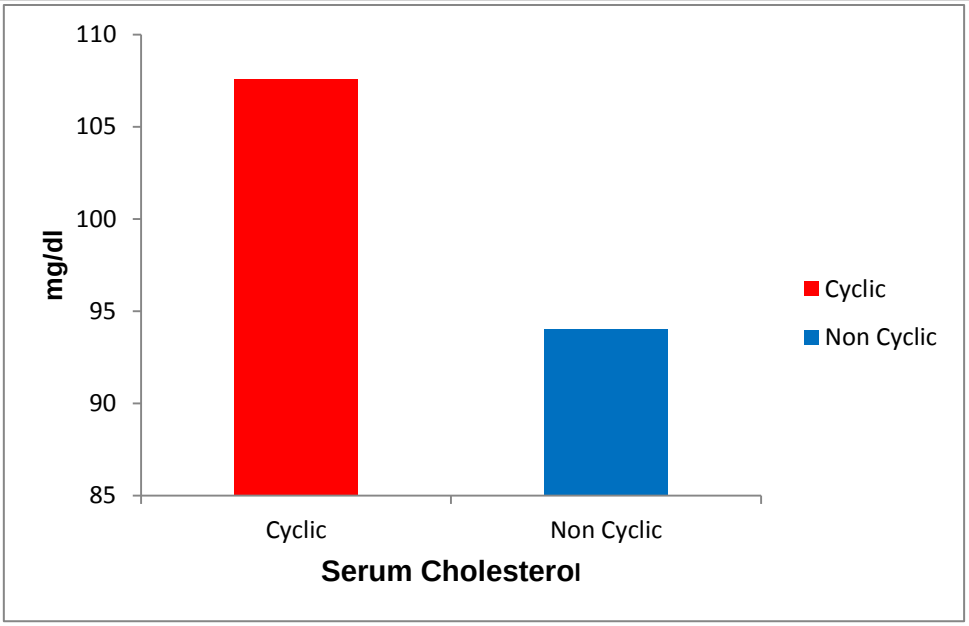

Fig. 8: Serum Cholesterol of cyclic and non-cyclic local buffaloes

Acknowledgement

The authors are highly thankful to the Associate Dean, College of Veterinary and Animal Sciences, MAFSU, Parbhani for providing infrastructure and facilities to conduct this study.

REFERENCES

1. Akhtar, M.S., Farooq, A.A. and Mustaq, M., Biochemical and hormonal profile in anestrous Nili Ravi buffaloes, *Indian Vet journal*, June 2010; **87:** 603-604 (2010).
2. Ali, M.D.M., Kanjilal, B.E., Bandopadhyay, S.K., Royochoudhary, R. and Ghosh, B.B., Serum calcium and inorganic phosphorus and serum calcium-phosphorus in anoestrus rural cross-bred higher. *Indian J. Anim. Reprod*, **12(1):** 32-35 (1991).
3. Ali, R. and Shukla, S.P., Haemato – Biochemical changes in post – partum anesrus buffaloes during low breeding season. *Researcher*, **4 (9):** 55-58 (2012).
4. Allain et al., Clin. Chem., **20:** 471 (1974).
5. Arthur, G.H., Veterinary Reproduction and Obstetrics. The English Language Book Society and Bailliere, Tindali (1975).
6. Bergmayer, H.V., Method of Enzymatic Analysis (1974).
7. Berthelot, E.S., Clin. Chem. Acta, **46:** 46 (1973).
8. Bohara, T.P. and Deokata, B., Assessment of serum biochemical profile and ovarian status of cyclic and non-cyclic anestrous buffaloes of Shivnagar VDC and IAAS livestock farm Chitwan, *Nepal J. Inst. Agric. Sci*., **30 :** 199-205 (2009).
9. Capen, C.C. and Rosol, T.J., Hormonal control of mineral metabolism. In disease mechanisms in small animal surgery. 12th Edn. Dukes physiology of domestic animals edited by William O Reece Kaneko, J.J. Harvey, J.W. and M.L. 1997 clinical biochemistry of domestic animals. *5th Edn. Academic Press, New York* (1993).
10. Chandolia, R.K. and Verma, S.K., Studies on biochemical profiles in anestrous buffalo heifers: *Indian Vet. J*., **64:** 482-484 (1987).
11. Chaurasia, R., Kushwaha, H.S., Chaurasia, D., Gendley, M.K. and A.K. Santra, Comparative studies of certain macro minerals during various reproductive states in buffaloes. Buffalo Bulletin, 29 **(4):** 291 – 298 (2010).
12. Coles, E.H., Veterinary clinical pathology, 4th Ed., W.B. Saunder's Company, London, U.K. (1986).

13. Dumas, B.T. Watson, W.A. and Biggs, H.G., Clin Chem. Acta, **31:** 87 (1971).
14. Dutta, J.C., Buruah, R.N. Leena, D. and Talukdar, S.C., Blood biochemical studies in anestrus and normal cyclic cattle. Indian Vet. J., **65:** 239-241 (1988).
15. Gitelman, H.J., Annal. Biochem., 18: 521 (1967).
16. Hafez, E.S.E., Studies on blood glucose levels in different physiological status in buffaloes. *Livestock Advisor*, **9 (6):** 43-48 (1969).
17. Hedaoo, M.K., Khllare, K.P., Meshram, M.D., Sahatpure, S.K. and Patil, M.G., Comparative studies of certain Bio-chemical constituents of normal cyclic and anoestrus surti buffaloes. Veterinary World, **1(4):** 105 -106 (2008).
18. Herick, J.B., External factors affecting reproduction in dairy cattle. Some papers contributed to the first all India symposium on animal reproduction, *Punjab Agril. Univ., Ludhiana*: 1-9 (1977).
19. Howland, B.E., Klarkaptrick, P.L., Pope, A.O. and Casida, L.H., *J. Anim. Sci*., **25:** 716 (1996).
20. Josephson, B. and Gyllenswar, C., Scand. J. Clin. *Lab. Invest*., **9:** 29 (1957).
21. Kabir, K.K., Varshney, J.P., Rawal, C.V. Sand, M.R. Ansari, Indian Vet. J. **78:** 1116-1118 (2001).
22. Kaneko, J.J., Clinical biochemistry of domestic animals. 3rd Edn., *Academic press, New York, USA* (1980).
23. Khasatiya, C.T., Dhamni, A.J. and Kavani, F.S., Reproductive traits and metabolic profile in Postpartum ferile and Infertile Surti buffaloes: *Indian Vet .J.,* **82 :** 637-641 (2005).
24. Kumar, S., Saxena, A. and Ramsagar, Comparative studies on metabolic profile of anestrous and normal cyclic Murrah buffaloes. Buffalo Bulletin, **29 (1):** 7 – 11 (2010).
25. Latif, A., Siddiqui, G.M. and Vadodaria, V.P., Biochemical Studies on Postpartum anestrous in Mehsani buffaloes: IJAR, **14(1):** 64 (1993).
26. McClure, T.J., A nutritional cause of low non return rates in dairy h herds. Aust., Vet. J., **41:** 199 (1965).
27. McDonald, L.E., Veterinary Endocrinology and Reproduction. 3rd Indian Edn. Lea and Febiger, Philadelphia (1980).
28. Morin, L.G., Clin. Chem. Acta, **46:** 113. (1973).
29. Morrow, D.A., Phosphorus deficiency and infertility in dairy heifers. JAVAMA J Am Vet Med Assoc., 154-761 (1969).
30. Munoz, M.A., Clin. Chem., **29(2):** 372 (1981).
31. Newer, B. Bauruah, K.K., Anubha Baruah, Bhuvan, D. and Kalita, D.J. Studies on certain micro mineral studies in anestrous and cyclic Postpartum swamp buffaloes: *Indian Vet Journa*l., **76:** 671-672 (1999).
32. Richards, M.W., Wteeman, R.P., Scoeneman and Welty, S.D., Association between anestrus and blood glucose and insulin in Hereford cows. Animal Science Research Report, Agricultural Experiment Station, Oklahoma State University, M.P.-199. 75-80. (Anim. Breed. Abst. **57:** 1598) (1987).
33. Robinson, T.J., Pregnancy. The Progress in Physiology of Farm Animals. Vild. 3 pp 793. John Hammand Butterworth Publication, London (1977).
34. Rodkey, F.L., Clin. Chem., **10:** 606 (1964).
35. Shah, S.N.H., Willemse, A.H. and Van de Wiel, D.F.M. Influence of season and parity on several reproductive parameters of Nili-Ravi buffaloes in Pakistan. Animal Reprod. Sci., **21:** 177-190 (1989).
36. Sharma, K.B., Nayyar, S., Malik, V.S. and S.P.S. Sodhi, Biochemical studies in cyclic, anestrus and subestrus buffalo heifer. *Ind. J. Anim. Sci.,* **68 (5):** 469–470 (1998).
37. Shrivastava, H.K. and Kharche, K.G., Studies on some blood constituents in normal and abnormalcycling buffaloes: IJAR **7:1:** 62-65 (1986).
38. Singh, A.P., Shah, R.S., Singh, R.B., Akhtar, M.H., Roy, G.P, Singh, C. and

Kunj, V., Response of mineral mixture, Prajana and GnRH on serum biochemical constituents and conception rate in anoestrus buffaloes. Indian J. Anim. Reprod., **27(1):** 51-54 (2006).

39. Tinder, P., Annals. Clin. *Bio Chem.* **6:** 24 (1969).
40. Tindler, E.M., Clin. Chem., **15:** 807 (1969).
41. Umesh, K.R., Sudhir, V., Reddy Chandra, Seshgiri Rao A., Reddy Eswar. C., Reddy Suresh V. and G.V. Narasa Reddy, Studies on certain blood constituents of rural buffaloes during cyclic and postpartum anestrous periods: *Indian Vet Journal.*, **72:** 469-471 (1995).
42. Weichselbaum, T.E., Amer, J. Clin. Path., **16:** 40-48 (1946).
43. Yadav, K.V.S., Ansari, M.R. and Kumaresan, A. Profile of macro, microelement, total protein and cholesterol in serum of cyclic and acyclic murrah buffaloes: *Indian J. Vet. Res.* **15 (2):** 10-13 (2006).
44. Yotov, S.A., Atanasov, A.S. and Llivea, Y.A., Relationship of some blood serum parameters with reproductive parameters with reproductive performance of Bulgerian Murrah Buffaloes after hormonal treatment during the early postpartum (Preliminary study), *J Vet Adv*., **3 (5):** 160-164 (2013).