HAEMATO-BIOCHEMICAL ALTERATIONS DURING DIFFERENT STAGES OF LACTATION IN MEHSHANI BUFFALOES

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ABSTRACT

The present study was carried out to investigate the haemato-biochemical profile of Mehshani buffalo, a native breed of Gujarat, India with the purpose of analyzing the physiological variations under the influence of different lactation stages in terms of determining possible biomarkers to monitor the energetic balance and the metabolic adequacy during lactation. Eighteen clinically healthy lactating buffaloes were categorized into three groups based on the length of their lactation: group I (early stage), group II (mid stage) and group III (late stage). Non significant variations were observed in case of the hematological parameters amongst the three groups of animals. The packed cell volume (PCV), RBC count and haemoglobin (Hb) concentration was lowest in the buffaloes of the early stage of lactation. Other haematological parameters viz. total leucocyte count (TLC), differential leucocyte count (DLC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and platelet count were recorded to be within the normal limits. Similarly, the values of the blood biochemical analytes varied apparently, but the differences were statistically non significant amongst the groups studied. The glucose level was recorded to be the lowest in the early stage of lactation; whereas, the protein and creatinine concentrations were slightly higher in this stage. No significant alteration in the concentration of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) was noticed amongst the three groups of buffaloes under the current study. Data generated during the current study may be useful as reference values for the scientific community as this is the first study of its kind in case of Mehshani buffalo.

Keywords: haemato-biochemical, lactation stage, mehshani buffalo

INTRODUCTION

Blood biochemical parameters vary during different physiological stages of animals (Ahmad *et al*., 2003). Pregnancy and lactation are two most important stages in the life of dairy animals, which affect metabolism resulting in the alteration of the haemato-biochemical profile (Krajnicakova *et al*., 2003; Iriadam, 2007). There are numerous reports on the effects of different phases of the reproductive cycle and pregnancy on haemato-biochemical indices in domestic animal species including

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buffalo (Jain *et al*., 2009). However, no such study could be traced investigating the blood picture during different stages of lactation in Mehshani buffalo, a unique milch breed of Gujarat, India. It is well established that milk and milk components are directly and indirectly synthesized from blood. The rate at which blood flows to the mammary gland is one of the key-factors in determining milk synthesis.

Approximately, 400 to 500 liters of blood circulate through mammary gland to produce one liter of milk (Fernandez and Hoeffler, 1998). There is a 2 to 6 folds increase in blood flow in the mammary gland starting 2 to 3 days prepartum. During lactation, the mammary gland secretory cells utilize 80% of the blood metabolites for milk synthesis depending on the infiltration of precursors of milk components like amino acids, glucose and fatty acids (Piccione *et al*., 2009). Hence, blood biochemical parameters including total protein, triglycerides, free fatty acids and urea are important indicators of the metabolic activity in lactating animals (Karapehlivan *et al*., 2007). Since the milk yield and composition varies across the length of lactation stage, it is, therefore, imperative, to study haematolo-biochemical constituents during different stages i.e. early, mid and late stage of lactation. Accordingly, the present study was undertaken to investigate the variations in haemato-biochemical profile during different stages of lactation in Mehshani buffalo.

MATERIALS AND METHODS

Experimental animals

Eighteen (18) clinically healthy lactating Mehshani buffaloes were selected from the herd maintained at Livestock Research Station, Sardarkrushinagar Dantiwada Agricultural University, Sadarkrushinagar, Gujarat, India. The buffaloes were in various stages of lactation and based on the length of their lactation, the animals were identified as in early (7 to 105 days), mid (106 to 210 days) and late (211 to 315 days) lactational stage. Accordingly, they were categorized into three different groups of six animals each viz. group I (early lactation), group II (mid lactation) and group III (late lactation).

Collection of blood samples

Blood samples were collected aseptically from each animal of all the three groups by jugular vein puncture into collection tubes containing anticoagulants viz. K_3EDTA and Lithium heparin for hematological and biochemical analysis, respectively.

Haematological analysis

Collected blood samples were analyzed for different hematological parameters including packed cell volume (PCV), haemeoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), total erythrocyte count (TEC), total leucocyte count (TLC), differential leucocyte count (DLC) and platelet count (PLT) using Automated Haematology Analyzer (Cell-Dyn 3700, Abbott Diagnostics, USA).

Biochemical analysis

Blood samples were analyzed for different biochemical analytes viz. Glucose, Total protein, Blood Urea Nitrogen (BUN), Calcium (Ca), Creatinine, Total Bilirubin, Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Asparate aminotransferas (AST) by employing Dry Chemistry discs/cartridges in Piccolo Xpress

Chemistry Analyzer (Abaxis, USA).

Data analysis

The results were statistically analyzed using one-way ANOVA as per the method of Snedecor and Cochran (1994). $P<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

The results (mean±SE) of the biochemical and haematological analysis have been presented in Table 1 and Table 2, respectively.

Table 1 reveals no significant difference ($P>0.05$) in the concentrations of various biochemical constituents amongst the three groups of lactating Mehshani buffaloes. This finding corroborates the report of Hagawane *et al*. (2012). Present study further indicated that the mean concentration of blood glucose was lowest (40.67±2.04 $mgdl^{-1}$) in early stage and increased subsequently as the lactation advances. The observed values of blood glucose for mid and late stage of lactation were 42.5±4.57 $mgdl^{-1}$ and 46.37±4.31 $mgdl^{-1}$, respectively. Current trend of variations are consistent with earlier report in lactating ewes (Roubies *et al*., 2006) and in lactating mares (Heidler *et al*., 2002). In contrast, glucose levels were reported to be the same throughout the three stages of lactation by Peterson and Waldern (1981); whereas, Doornenbal *et al*. (1988) reported somewhat higher ($P<0.05$) glucose concentration at parturition that declined during lactation period. The lower level of blood glucose recorded during early stage of lactation may be ascribed to the utilization of large amount of blood glucose by mammary gland for the synthesis of lactose (Schultz, 1968). It is reported that lactose synthesis and milk yield show a linear positive correlation with glucose uptake and thus the lactose synthesis potential is accompanied by greater glucose uptake by lactating mammary gland (Afshar and Fathi, 2012). The total protein level (8.45 gdl^{-1}) was found to be slightly higher in group I as compared to group II and group III animals. This observation is on the contrary to the finding of Yaylak *et al*. (2009), who recorded lower protein values in dry and early stages of lactation in case of Holstein cows. Krajnicakova *et al*. (2003) also observed an increasing trend of total protein level of serum with the progress of lactation in lactating goats and concluded that this is due to the catabolism of protein for milk synthesis. The variation may be attributed to the differences in species, nutrition, husbandry, environment and methods of assay (Beaunoyer, 1992; Osman and Al-Busadah, 2003). However, Hagawane *et al*. (2012) reported highest protein value in the early stage of lactation, which is comparable to current findings. The possible explanation for this phenomenon may be the haemoconcntration and water losses due to parturition. Further, earlier investigations have clearly shown that the expression of major milk proteins increases dramatically and in a concerted way during the onset of lactation (Bionaz and Loor, 2011).

Similarly, the mean value of blood urea nitrogen (BUN) was also recorded to be higher in initial stage of lactation and decreased as the lactation progresses. This may hold good in relation to observed apparently increased level of total protein. The BUN values observed in the present study at different stages of lactation were higher than those reported in earlier investigation (Hagwane *et al*., 2012). Reinartz and Hofmann (1989) also found that serum urea concentration was significantly influenced by the lactation stage.

Table 1. Mean±S.E. values of biochemical analytes at different stages of lactation.

Parameters	Early lactation	Mid lactation	Late lactation
Glucose (mg/dl)	40.67±2.04	42.5±4.57	46.37±4.31
Total protein (g/dl)	8.45±0.13	8.12±0.22	8.01±0.29
Blood Urea Nitrogen (mg/dl)	22.67±1.3	20.5±1.58	18.89±2.25
Calcium (mg/dl)	7.0±0.93	8.1±0.54	8.19±0.23
Creatinine (mg/dl)	1.33±0.08	1.2±0.07	1.15±0.16
Total Bilirubin (mg/dl)	0.23±0.03	0.22±0.04	0.24±0.04
Alanine aminotransferase (ALT) (U/L)	66.67 ±5.35	72.67±6.37	67.5±5.07
Aspartate aminotransferase (AST) (U/L)	154.0±4.46	148.5±17.97	159.5±7.87
Alkaline phosphatase (ALP) (U/L)	178.67±81.50	168.67±47.03	165.56±45.50

(P<0.05; statistically non significant)

Table 2. Mean±S.E. values of hematological indices at different stages of lactation.

Parameters	Early lactation	Mid lactation	Late lactation
WBC (K/µl)	9.63±0.65	8.24±0.79	9.55± 0.98
NEU (%)	30.83±2.19	28.28±1.58	29.12±2.01
LYM (%)	50.17±2.02	48.35±1.69	50.15±2.29
MONO (%)	7.17±0.40	7.18±1.03	8.95±1.96
EOS (%)	5.83±1.49	6.44±0.97	5.36±0.57
BASO (%)	0.841±0.15	0.746±0.16	0.644±0.17
RBC (M/µl)	5.67±0.98	6.38±0.35	7.49±0.41
HGB (g/dl)	12.35±4.48	14.1±4.17	13.73±2.84
PCV (%)	30.87±0.97	32.72±1.87	31.33±1.35
MCV (fL)	48.07±2.021	45.22±2.54	41.93±0.87
MCH (pg)	16.22±0.69	15.48±0.80	15.17±0.58
PLT (K/µl)	326.5±33.63	617.17±104.86	507.17±51.62

(P< 0.05; statistically non significant)

It is recorded that the efficiency for utilization of metabolisable protein for milk production (0.68) is less than that of maintenance (1.00) (McDonald *et al*., 1995). So, as the milk production increases, the overall protein utilization efficiency decreases, which consequently leads to more drainage of nitrogen in terms of urea through urine and milk (Roy *et al.*, 2003). An increase in urea value was further observed in the first 8 weeks of lactation (Ndibualonji and Godeau, 1993) and found to be peak at 12 weeks postpartum, which decreased slowly thereafter (Rajcevic *et al*., 1993). However, other researchers found a different trend of variation in case of BUN. During the first month of lactation lower milk urea (MU) concentration was recorded by Carlsson *et al*. (1995). Likewise, Whitaker *et al.* (1995) also reported that cows in early lactation often have much lower MU level. In contrast, no relation was reported between urea concentration in milk and stage of lactation by Erbersdobler *et al*. (1990) and values were relatively constant between 200 to 300 mg/l. Similarly, Coustumier (1996) also found no correlation between lactation stage and urea levels except just after calving. Similar to our study, Schepers and Meijer (1998) also observed that stage of lactation had no significant influence on BUN and thus on MU concentration. Hence, in the light of varying observations of different researchers, a systemic and critical investigation may be established in this aspect.

In this study, the drop in calcium (Ca) level (7.0±0.93 $mgdl^{-1}$) was more pronounced during early stage of lactation as compared to mid and late stage. This may be due to excessive drainage of blood calcium pool through colostrum and milk during this stage. As the stage of lactation progresses, the blood calcium level is increased, which is in agreement with the findings of Rowlands *et al*, (1975) and Nale (2003). It may be hypothesized that the buffaloes gradually recover from the stress of parturition and excess demand of calcium for initiation of lactation. On the contrary, Ramakrishna (1991) recorded higher values (9.77±0.33 $mgdl^{-1}$) of calcium in lactating buffaloes. Further, Yokus *et al*. (2004) also concluded that the levels of Ca decreased slightly in early pregnancy to late pregnancy and then increased during lactation period in sheep.

Present study also indicated that the blood creatinine level was higher in group I as compared to group II and group III buffaloes. The apparent increase in creatinine level at the early stage of lactation may be ascribed to uterine involution and myometrial protein degradation (Bell *et al*., 2000). Nonetheless, Peterson and Waldern (1981) found no differences in creatinine concentrations amongst the various stages of lactation, but observed that creatinine levels rose in dry cows with increasing days of pregnancy. Kronfeld (1982), working with 21 Holstein herds, reported the highest serum creatinine levels during the peak of lactation. Total bilirubin concentration recorded in this study was found to be consistent throughout the lactation period indicating that its concentration remains unaffected with the stage of lactation. Similarly, no significant alteration in the concentration of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) was recorded amongst the three groups of buffaloes under the current study. The concentration of AST was found to be highest (72.67±6.37 U/L) in the mid lactation stage. Ling *et al*. (2003) observed that the blood concentration of AST increases between day 117 and 151 of lactation (mid stage) in Holstein mares, which is in accordance with the present findings. Conversely, Yaylak *et al*. (2009) reported that the stage of lactation affects AST and ALT activities

significantly. An increase in ALT, AST and GGT (γ-glutamyl transferase) activity in the blood of ewes during lactation is indicative of increase in hepatic metabolism (Antunovic *et al*., 2004, 2011). Further, AST and ALP are considered to be effective biomarkers to detect the energetic and mineral imbalance in Saanen dairy goats (Mundim *et al*., 2007). Changes in activities of these enzymes may also be related to reduced dry matter intake around parturition, which lead to hepatic lipidosis and alter the normal function of the liver (Greenfield *et al*., 2000). However, no indications were found in the literature to explain the relationship of the recorded trends of variations in the concentrations of these enzymes with different stages of lactation.

Table 2 indicated that although the observed values of the haematological parameters varied apparently, the differences were statistically non significant. Similar types of observations were also recorded by Hagawane *et al*. (2012). The packed cell volume (PCV), RBC count and haemoglobin (Hb) concentration was found to be lowest in Mehshani buffaloes in early stage of lactation corroborating with those of Esievo and Moore (1979), who concluded that the concentrations of PCV RBC, Hb along with serum iron (SI), iron binding capacity (IBC) and serum albumin decreased in early lactation and rose to pre-lactation levels by mid-lactation. Decline in the number of RBC in the blood of ewes in the early lactation was also reported by Antunovic *et al*. (2011). Other haematological indices such as the TLC, DLC, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and platelet count were found to be within the limits of normal values laid for the buffaloes. Similar to current investigation, non significant differences in various haematological indices were also reported by Flores *et al.* (1990) during early and late stage of lactation.

It may be concluded that stage of lactation does not play significant role in alteration of haemato-biochemical profile in lactating Mehshani buffaloes. The limited sensitivity of these blood parameters to stage of lactation in clinically normal dairy animals is not surprising because, most of these parameters are under the homeostatic control systems (Cozzi *et al*., 2011**)**. Nonetheless, data generated during the current study may be useful as reference values for the scientific community as this is the first study of its kind in case of Mehshani buffalo. Further, blood profile has traditionally been used to assess the metabolic health status of the animals; hence the present investigation may also be helpful in this regard. In addition, this study may also assist the nutritionists to formulate ration for optimum productivity of the Mehshani buffaloes since blood-biochemical analytes are being widely considered to identify dietary causes of diseases leading to low productivity.

REFERENCES

Afshar, M. and H. Fathi. 2012. Lactose in ruminants feeding: A review. *Annals of Biological Research*, **3**(1): 645-650.

Ahmad, I., A. Gohar, N. Ahmad and M. Ahmad. 2003. Haematological profile in cyclic, non Cyclic and Endometritic Cross-Bred cattle. *International Journal of Agriculture Biological*, **5**(3): 332-334.

Antunovic, Z., J. Novoselect, H. Sauerwein, M. Speranda1, M. Vegeraand and V. Pavic. 2011. Blood metabolic profile and some of hormone concentration in ewes during different physiological status. *Bulg. J. Agric. Sci.*, **17**(5): 687-695.

Antunovic, Z., M. Speranda and Z. Steiner. 2004. The influence of age and the reproductive status to the blood indicators of the ewes. *Arch. Tierz. Dummerstorf.*, **47**(3): 265-273.

Beaunoyer, D.E. 1992. Changes in Serum Enzyme Activities after maximal exercise, p. 331-333. *In Proceedings of First International Camel Conference,* 2nd-6th ed. Publications. New market Ltd.

Bell, A.W., W.S. Burhans and T.R. Overton. 2000. Protein nutrition in late pregnancy, maternal protein reserves and lactation performance in dairy cows. *The Proceedings of the Nutrition Society*, **59**: 119-126.

Bionaz, M. and J.J. Loor. 2011. Gene networks driving bovine mammary protein synthesis during the lactation cycle. *Bioinform. Biol. Insights*, **5**: 83-98.

Carlsson, J., J. Bergstrom and B. Pehrson. 1995. Variations and breed, age, season, yield, stage of lactation and herd in the concentration of urea in bulk milk and individual cow's milk. *Acta Vet. Scand.*, **36**: 245-254.

Coustumier, J.L.E. 1996. The balance of ration is not the whole story. *Production Laitiere Moderne*, **261**: 96-97.

Cozzi, G., L.F. Gottardo, A.L. Stefani, B. Contiero, L. Moro, M. Brscic and P. Dalvit. 2011. Reference values for blood parameters in Holsteindairy cows. Effects of parity, stage of lactation, and season of production. *J. Dairy Sci*., **94**: 3895-3901.

Doornenbal, H., A.K.W. Tong and N.L. Murray. 1988. Reference values of blood parameters in beef cattle of different ages and stages of lactation. *Can. J. Vet. Res.*, **52**: 99-105.

Erbersdobler, H.F., S. Braasch and E.A. Trautwein. 1990. Concentrations of taurine, urea and free amino acids in milk as influenced by stage of lactation and breed of the cows. *J. Anim. Physiol. An. N.*, **63**: 1-7.

Esievo, K.A. and W.E. Moore. 1979. Effects of dietary protein and stage of lactation on the haematology and erythrocyte enzymes activities of high-producing dairy cattle. *Res. Vet. Sci.*, **26**(1): 53-58.

Fernandez, J.M. and J.P. Hoeffler. 1998. Transgenic Expression, p.410. *In Gene Expression Systems: Using Nature for the Art of Expression*, Academic Press, U.S.A.

Flores, A., R. Althaus, J.C. Toibero, O.J. Garnero and L. Perren. 1990. Metabolic profile of dairy cow before and after parturition. *Revista Argentia de Production Animal*, **10**(4): 289-293.

Greenfield, R.B., M.J. Cecava, T.R. Johnson and S.S. Donkin. 2000. Impact of dietary protein amount and rumen undegradability on intake, peripartum liver triglyceride, plsama metabolites and milk production in transition dairy cattle. *J. Dairy Sci.*, **83**: 703-710.

Hagawane, S.D., S.B. Shinde and D.N. Rajguru. 2012. Haematological and blood biochemical profile in lactating Buffaloes in and around Parbhani city. *Veterinary World*, **2**(12): 467-469.

Heidler, B., H. Sauerwein, U. Heintges, J. Aurich, W. Pohl and C. Aurich. 2002. Metabolic profiles and plasma leptin concentrations in lactating and non-lactating mares. *Theriogenology*, **58**: 557-561.

Iriadam, M. 2007. Variation in certain haematological and biochemical parameters during the pre-partum period in Kilis does. *Small Ruminant Res.*, **73**: 54-57.

Jain, R.K., D.D. Nimkar, C.M. Saksule and R.K. Dhakad. 2009. Haemato-biochemical

profile of haemoglobinuric buffaloes. *Buffalo Bull*., **28**(4): 184-187.

Karapehlivan, M., E. Atakisi, M. Citil, O. Kankavi and O. Atakisi. 2007. Serum sialic acid levels in calves with pneumonia. *Vet. Res. Commun.*, **31**: 37-41.

Krajnicakova, M.E., V. Hendrichovscky and I. Maracek. 1993. Concentrations of total lipid, cholesterol and progesterone during oestrus synchronization and pregnancy in sheep. *Vet. Med-us.*, **38**: 349-357.

Krajnicakova, M.E., E. Bekeova, G. Kovac, M. kostecky, I. valocky, I. Maracek, I. Sutiakova and L. Lenhardt. 2003. Selected clinical-biochemical parameters in the puerperal period of goats. *B. Vet. I. Pulawy*, **47**: 177-182.

Kronfeld, D.S., S. Donoghue, R.L. Copp, F.M. Stearns and R.H. Engle. 1982. Nutritional status of dairy cows indicated by analysis of blood. *J. Dairy Sci*., **65**: 1925-1933.

Ling, K., H. Jaakson, J. Samarutel and A. Leesmae. 2003. Metabolic status and body condition score of Estonian Holstein cows and their relation to some fertility parameters. *Veterinariaja Ir. Zootechnika. T*., **24**: 94-100.

McDonald, P., R.A. Edwards, J.F.D. Greenhalgh and C.A. Morgan. 1995. *Animal Nutrition*, 5th ed. Addison Wesley Lonman, Efinburgh Gate, Harlow, Essex CM202JE, United Kingdom.

Mundim, A.V., A.S. Costa, S.A.P. Mundim, E.C. Guimaraes and F.S. Espindola. 2007. Influence of parity and stage of lactation on the blood biochemical profile of Saanen goats. *Arq. Bras. Med. Vet. Zoo.*, **59**(2): 306-312.

Nale, R.A. 2003. *Metabolic profiling in buffaloes before and after parturition*. M.V. Sc. thesis submitted to MAFSU, Nagpur, India. p. 29-34.

Ndibualonji, B.B. and J.M. Godeau. 1993. Changes in plasma amino acids, urea and glucose in relation to the end of gestation and the onset of lactation in low yielding dairy cows. *Mededelingen Vande Faculteit Land bouwwetenschappen Universitiet Gent*, **58**: 1713-1717.

Osman, T.E.A. and K.A. Al-Busadah. 2003. Normal Concentration of Twenty Serum Biochemical Parameters of She-Camels, Cows and Ewes in Saudi Arabia. *Pakistan Journal of Biological Sciences*, **6**(14): 1253-1256.

Peterson, R.G. and D.E. Waldern. 1981. Repeatabilities of serum constituents in Holstein-Friesians affected by feeding, age, lactation and pregnancy. *J. Dairy Sci*., **64**: 822-831.

Piccione G, G. Caola, C. Giannetto, F. Grasso, S.C. Runzo, A. Zumbo and P. Pennisi. 2009 Selected biochemical serum parameters in ewes during pregnancy, post-parturition, lactation and dry period. *Anim. Sci. Pap. Rep*., **27**: 321-330.

Rajcevic, M., I. Jazbec and M. Ponikvas. 1993. Urea in milk and blood as indicator of nutritive matter supply in high-yielding dairy cows. *Zbornik Biotehniske Fakultete Univerze Edvasda Kardelja V Ljubljani, Kmetijstvo*, **62**:179-184.

Ramakrishna, K.V. 1991. Comparative studies on certain biochemical constituents of lactating and dry Murrha buffaloes. *Livestock Advisor*, **14**: 16-19.

Reinartz, M. and W. Hofmann. 1989. Serum urea

estimation in dairy herds. *Prakt. Tierarzt*, **70**: 22-28.

Roubies, N., N. Panousis, A. Fytianou, P.D. Katsoulos, N. Giadinis and H. Karatzias. 2006. Effects of age and reproductive stage on certain serum biochemical parameters of chios sheep under greek rearing conditions. *J. Vet. Med. A.*, **53**: 277-281.

Rowlands, G.J., R. Manston, M. Rita, Pocock and M.D. Sally. 1975. Relationships between stage of lactation and pregnancy and blood composition in a herd of dairy cows and the influences of seasonal changes in management on these relationships. *J. Dairy Res.*, **42**: 349-362.

Roy, B.R., K. Mehla and S.K. Sirohi. 2003. Influence of Milk Yield, Parity, Stage of Lactation and Body Weight on Urea and Protein Concentration in Milk of Murrah Buffaloes. *Asian Austral. J. Anim.,* **16**: 1285-1290.

Schepers, A.J. and R.G.M. Meijer. 1998. Evaluation of the utilization of dietary nitrogen by dairy cows based on urea concentration in milk. *J. Dairy Sci*., **81**: 579-584.

Schultz, L.H. 1968. Ketosis in dairy cows. *J. Dairy Sci*., **51**: 1133-1140.

Snedecor, G.W. and W.G. Cochran. 1989. *Statistical Method*, 8th ed. Oxford and IBH Publishing Co., New Delhi, India.

Whitaker, D.A., J.M. Kelly and H.F. Eayros. 1995. Assessing dairy cow diets through milk urea tests. *Vet. Rec*., **136**: 179-180.

Yaylak, E., C. Yenisey and K. Seyrek. 2009. Effects of Lameness, Stage of lactation and body conditions score on some blood parameters in Holstein cows. *Asian J. Anim. Vet. Adv.*, **4**(5): 245-251.

Yokus, B., D.U. Cakmr and D. Kurt. 2004. Effects of seasonal and physiological variations on the serum major and trace element levels in sheep. *Biol. Trace. Elem. Res.*, **101**: 241-255.