Research Article

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Efficacy of Herbal Powder in Reducing Oxidative Stress during Periparturient Period in Crossbred Dairy Cows

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Abstract

Background: *Periparturient period is very important and critical period in dairy cows because during this period dairy cows are at increased risk of developing various metabolic and production diseases such as ketosis, milk fever, mastitis, retained placenta etc. due to the increased stress imposed upon the animal by the developing fetus and also due to the initiation of lactation after parturition. Now a days, use of herbal medicine as an alternative medicine is under constant debate due to the associated toxicity and side effects of allopathic medicines.*

Objectives: *A therapeutic trial was conducted to check the efficacy of Herbal product "Stress check" on various metabolic and oxidative stress parameters during periparturient period.*

Methodology: *A total of 16 crossbred cows divided into two groups, Group I was kept as control and Group II given herbal powder stress check @ 15 grams per day starting 15 days of expected parturition upto 15 days after parturition.*

Results: *A significant decrease was recorded in the lipid peroxidation (LPO) levels during the fresh period in comparison to control cows, and a non-significant increase was also noticed in both the SOD and GSH levels during the early lactation period, suggesting its role in reducing stress and improving metabolism during early lactation period. Also a non-significant increase was noticed in plasma glucose levels and lower protein, urea, levels during the fresh period, whereas, no effect was observed on other metabolic parameters after the feeding of Stress Check powder.*

Conclusion: *Herbal powder "Stress Check" was effective in controlling the oxidative stress in supplemented cows in comparison to control cows as evidenced by the lower LPO levels and higher SOD and GSH levels.*

Keywords: Herbal powder, Copper, Zinc, Beta hydroxyl butyric acid, Metabolic parameters, Lipid peroxidation, Super oxide dismutase, Reduced Glutathione.

Introduction

During the periparturient period, dairy cows are in a state of negative energy balance due to the sudden increase in the energy demand leading to many metabolically and endocrinological changes in the body causing an increase in the production of the reactive oxygen metabolites (ROMs) at the cellular level [1-3]. This ROMs production may lead to development of oxidative stress which is found responsible for the production of various metabolic and reproductive diseases [4,5].

Veterinary herbal medicine is the use of plant-based medicines for their therapeutic, prophylactic, and diagnostic application in animal health care. These herbal medicines are both used at the small and large scale. In the rural areas, these herbal medicines are

used for the curing of the livestock, as they are unable to spend on quality health of their livestock, mainly due to non-affordability whereas, at the high-end these herbal medicines are used as an alternative medicine due to the side effects of the allopathic drugs such as the development of antibiotic resistance and other deleterious effects [6-9].

India has one of the richest traditions in the herbal drugs in the world, and more than 800 plants have been used in indigenous systems of medicine [9,10]. The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy uses several plant species to treat different ailments however the use of herbal medicine is increasing day by day due to the associated toxicity and side effects of allopathic medicines, due to which there is sudden increase in the number of herbal drug manufactures [11,12].

As the use of herbal drugs in veterinary medicine is increasing day by day, but very less systemic studies have been carried out on the use of herbal drugs in veterinary medicine. So, keeping all these facts in mind a drug trial was conducted to check the efficacy of Herbal product "Stress check" on various metabolic and oxidative stress parameters in dairy cows during the periparturient period.

Methodology

Sample size and Selection

A total of 16 healthy crossbred cows in their last trimester of pregnancy were selected for the study. The cows were divided into two groups with eight cows in each group. Group-I was selected as control and the cows in Group-II were fed herbal powder 'Stress Check' (Indian Herbs, Saharanpur (Uttar Pradesh, India) @ 15 gm per day starting from 15 days of expected parturition upto 15 days after parturition.

From each animal, blood samples were collected thrice during different stages of periparturient period, viz. (i) Far off dry - > 10 days following dry off and not < 30 days prior to calving. (ii) Close up dry- Between 3 and 21 days prior to calving and (iii) Fresh- 3 to 30 days in milk.

Analysis

A prophylactic trial was conducted in crossbred cows to evaluate the efficacy of herbal powder 'Stress Check' (Indian Herbs Saharanpur Uttar Pradesh) on the various metabolic viz. haemoglobin (Hb), Packed cell volume (PCV), total erythrocyte count (TEC) and Total leucocyte count (TLC); biochemical constituents (total plasma proteins (TPP), albumin, plasma urea nitrogen (PUN), creatinine, glucose, Beta hydroxy butyric acid (BHBA) and Non esterified fatty acid (NEFA) and plasma minerals concentrations viz. calcium (Ca), magnesium (Mg), plasma inorganic phosphorus (Pi), sodium (Na), potassium (K), copper (Cu), Iron (Fe) and Zinc (Zn) as well as on oxidative stress parameters viz. lipid peroxidation (LPO), superoxide dismutase (SOD) and reduced glutathione (GSH).

Hematological parameters analysis

All the haematological parameters were estimated on Seimens Advia 2120 Hematology Analyzer, USA.

Biochemical parameters analysis

Various biochemical parameters (TPP, albumin, PUN, creatinine and glucose) were accessed on semi-automatic Biochemistry Analyzer (DT Analyzer) using kits provided by Ortho Clinical Diagnostics, UK. (DT Analyzer) using kits provided by Ortho Clinical Diagnostics, UK. Both plasma BHBA and NEFA levels were estimated in the ELISA plates with the help of kits provided by Diasys Diagnostic's systems, Germany.

Copper, iron and zinc estimation

The plasma samples were digested as per the procedure described by Kolmer[13] and the digested samples were estimated by Atomic Absorption Spectrophotometer (AAS) (Perkin Elmer Analyst 700, USA).

Calcium, magnesium, sodium and potassium estimation

The Ca, Mg, Na and K concentrations in plasma were estimated on the AAS (Perkin Elmer Analyst 700, USA) directly from the plasma samples as described in Perkin Elmer [14].

Plasma inorganic phosphorus estimation

Plasma Pi was determined by using method given by Tausky and Shorr and the readings were taken using spectrophotometer (Perkin Elmer Lamda 25 UV/VIS Spectrometer, USA) [15].

Oxidative stress parameters estimation

Lipid peroxidation (LPO) was assayed by the methods of Placer et al. SOD in haemolysate was measured by Nishikimi et al. and reduced glutathione was estimated by the method of Hafeman et al. [16-18].

Data analysis

Data was analysed by one way anova and independent sample t-test by using SPSS software (version 16.0; Microsoft).

Results and Discussion

Effect of Stress Check powder on Haematological Parameters

The overall mean Hb, PCV and TEC levels showed decrease in both the groups from FOD to fresh period (significant in Group-I for Hb and significant in Group-II for PCV), and a significantly lower value was observed in Group-II for mean PCV during fresh period in comparison to Group-I. However, mean TLC levels did not show any significant difference and a non-significant increase was noted in both groups (Table 1).

Parameters	Period	Group-I (n=8)	Group-II (n=8)
Hb (g/dl)	FOD	11.81 ± 0.54^{awx}	10.66 ± 0.50^{ax}
	CUD	10.97 ± 0.43^{axy}	10.25 ± 0.51^{ax}
	Fresh	10.36 ± 0.47^{azy}	9.38 ± 0.34^{ax}
PCV (%)	FOD	35.12 ± 1.71^{ax}	34.30 ± 1.60^{axw}
	CUD	34.58 ± 0.95^{ax}	31.50 ± 1.26^{axy}
	Fresh	33.01 ± 2.54^{ax}	29.02 ± 1.41^{byz}
TEC (x10^6/µl)	FOD	6.21 ± 0.23^{ax}	5.72 ± 0.25^{ax}
	CUD	5.92 ± 0.28^{ax}	6.06 ± 0.26^{ax}
	Fresh	5.69 ± 0.35^{ax}	5.40 ± 0.22^{ax}

TLC (x10^3/µl)	FOD	8.72 ± 0.68^{ax}	9.83 ± 0.57^{ax}
	CUD	9.11 ± 0.47^{ax}	10.11 ± 0.59^{ax}
	Fresh	9.05 ± 0.65^{ax}	10.18 ± 0.44^{ax}

Table 1: Effect of Stress Check herbal powder on haematological parameters (Mean ± S.E.). Values bearing different superscripts (a, b) in a row differ significantly. Values bearing different superscripts (w, x, y, z) in a column differ significantly.

Effect of Stress Check powder Plasma Biochemical Parameters

Total Plasma Proteins: The mean TPP levels observed in both the groups is shown in Table 2. Significant ($p<0.05$) differences was observed in both the groups during FOD period, while within a group, a non-significant decrease was recorded in Group-I, whereas, a non-significant increase in Group-II was observed from FOD upto fresh period.

Albumin: Non-significant differences were recorded in both the groups from FOD to fresh period (Table 2).

Parameters	Period	Group-I (n=8)	Group-II (n=8)
TPP (g/dl)	FOD	8.18 ± 0.31^{ax}	6.88 ± 0.22^{bx}
	CUD	7.27 ± 0.25^{ax}	6.91 ± 0.30^{ax}
	Fresh	7.32 ± 0.39^{ax}	7.10 ± 0.24^{ax}
Albumin (g/dl)	FOD	2.95 ± 0.21^{ax}	2.52 ± 0.12^{ax}
	CUD	2.75 ± 0.11^{ax}	2.66 ± 0.09^{ax}
	Fresh	2.85 ± 0.10^{ax}	2.63 ± 0.12^{ax}
PUN (mg/dl)	FOD	8.37 ± 0.99^{ax}	7.27 ± 0.18^{bx}
	CUD	11.75 ± 0.55^{ax}	8.87 ± 0.22^{by}
	Fresh	13.25 ± 1.44^{ax}	5.87 ± 0.39^{bz}
Creatinine (mg/dl)	FOD	1.15 ± 0.07^{ax}	1.10 ± 0.07^{ax}
	CUD	1.40 ± 0.2^{1ax}	1.11 ± 0.11^{ax}
	Fresh	1.48 ± 0.18^{ax}	1.01 ± 0.10^{ax}
Glucose (mg/dl)	FOD	76.12 ± 3.29^{aw}	55.37 ± 5.67^{bx}
	CUD	62.25 ± 4.89^{axy}	51.62 ± 2.69^{ax}
	Fresh	56.75 ± 4.67^{axz}	56.00 ± 4.07^{ax}
BHBA (mmol/L)	FOD	0.42 ± 0.04^{awy}	0.36 ± 0.01^{ax}
	CUD	0.52 ± 0.05^{axy}	0.35 ± 0.04^{bx}
	Fresh	0.69 ± 0.06^{az}	0.47 ± 0.08^{ax}
NEFA (mmol/L)	FOD	0.16 ± 0.02^{awx}	0.10 ± 0.02^{ax}
	CUD	0.22 ± 0.02^{axy}	0.23 ± 0.05^{ax}
	Fresh	0.23 ± 0.03^{azy}	0.27 ± 0.05^{ax}

Table 2: Effect of Stress Check herbal powder on biochemical profile (Mean ± S.E.). Values bearing different superscripts (a, b) in a row differ significantly. Values bearing different superscripts (w, x, y, z) in a column differ significantly.

Plasma urea nitrogen: The mean PUN values showed significant difference between both the groups from FOD upto the fresh period. A non-significant increase was noticed in Group I, while a significant ($p<0.05$) decrease was observed in Group II from FOD to CUD to fresh period (Table 2), indicating that the herbal powder "Stress Check" has some role in decreasing the protein catabolism in cows as evidenced from the lower TPP and PUN levels in supplemented cows in comparison to control cows.

As the urea is synthesized in the liver from the ammonia absorbed from the rumen or the gut, so a higher urea concentration in the blood is positively correlated with the higher concentration of ammonia [19-21]. Similarly in our study higher PUN levels were seen in control cows, whereas, significant decrease was seen in supplemented cows from the FOD to fresh period indicating the role of herbal powder decreasing protein catabolism in the body.

Creatinine: The mean plasma creatinine levels observed in both the groups were within the normal physiological range, though a non-significant increase and a non-significant decrease was observed in Group-I and Group II from FOD upto fresh period (Table 2).

Glucose: A significant ($p<0.05$) difference was observed in the glucose level in between both the group during FOD period, and a significant ($p<0.05$) decrease was noticed in Group-I from FOD upto the fresh period, while a non-significant increase was observed in Group-II from FOD upto the fresh period. As during the periparturient period, there is massive need for glucose in the animal body for the development of foetus and also for the milk production, [22]. Similarly in our study a non-significant increase was noted in Group II, indicating the role of the herbal powder in providing energy to the body as evidenced by the increase in the glucose levels in supplemented cows (Table 2).

Beta Hydroxy Butyric Acid: A significant increase was noticed in the BHBA values in Group I from FOD to fresh period, where as a non-significant increase was observed in Group II cows (Table 2).

Non Esterified Fatty Acid: A significant increase was noticed in Group I cows in between the FOD and fresh period, where as a non-significant increase was recorded in Group II cows from FOD to fresh period (Table 2).

Both BHBA and NEFA are the indicators of energy balance during the periparturient period, excessive increase in their levels in dairy cows during the periparturient periods are the indicator of negative energy balance. [23]. As there is lipolysis during the transition period to provide energy to the animal body, resulting in production of NEFA in the body [24].

As in our study though the BHBA and NEFA levels, were within the normal range in both the groups, but a significant increase was seen in Group I, where as a non-significant increase was seen in Group II, suggesting the role of herbal powder in providing the energy to the body as evidenced from the lower levels of BHBA, NEFA and higher glucose levels in supplemented cows in comparison to control cows.

Effect of Stress Check powder on Plasma Mineral concentrations

Calcium: A non-significant decrease was recorded in both the groups from the FOD upto the fresh period (Table 3).

Parameters	Period	Group-I (n=8)	Group-II (n=8)
Ca (mg/dl)	FOD	13.15 ± 1.11^{ax}	11.57 ± 0.34ax
	CUD	11.89 ± 0.66^{ax}	11.15 ± 0.28ax
	Fresh	10.85 ± 0.64^{ax}	10.93 ± 0.62ax
Mg (mg/dl)	FOD	4.56 ± 0.51^{ax}	5.08 ± 0.54awy
	CUD	4.44 ± 0.50^{ax}	4.13 ± 0.14axy
	Fresh	3.90 ± 0.33^{ax}	3.97 ± 0.16axz
Pi (mg/dl)	FOD	5.03 ± 0.17^{ax}	4.95 ± 0.15aw
	CUD	4.94 ± 0.20^{ax}	5.76 ± 0.19byx
	Fresh	5.06 ± 0.26^{ax}	5.72 ± 0.15b^{zx}
Na (ppm)	FOD	121.73 ± 3.27^{ax}	126.28 ± 3.04^{ayx}
	CUD	123 ± 2.12^{axw}	114.90 ± 4.10^{azw}
	Fresh	114.56 ± 1.48^{awz}	118.86 ± 1.85^{axw}
K (ppm)	FOD	5.64 ± 0.15^{azy}	5.50 ± 0.22^{bxy}
	CUD	6.04 ± 0.30^{ay}	5.16 ± 0.13^{bzy}
	Fresh	5.01 ± 0.31^{az}	4.96 ± 0.15^{az}
Cu (ppm)	FOD	0.68 ± 0.06^{ax}	0.70 ± 0.06^{ax}
	CUD	0.65 ± 0.06^{ax}	0.58 ± 0.07^{ax}
	Fresh	0.81 ± 0.08^{ax}	0.45 ± 0.11^{bx}
Fe (ppm)	FOD	2.16 ± 0.44^{ax}	1.82 ± 0.31^{ax}
	CUD	2.28 ± 0.38^{ax}	1.87 ± 0.32^{ax}
	Fresh	2.85 ± 0.71^{ax}	2.06 ± 0.50^{ax}
Zn (ppm)	FOD	0.93 ± 0.15^{ax}	0.79 ± 0.17^{ax}
	CUD	0.99 ± 0.16^{ax}	1.23 ± 0.40^{ax}
	Fresh	0.69 ± 0.11^{ax}	1.51 ± 0.12^{bx}

Table 3: Effect of Stress Check herbal powder on plasma minerals concentrations (Mean ± S.E.). Values bearing different superscripts (a, b) in a row differ significantly. Values bearing different superscripts (w, x, y, z) in a column differ significantly.

Magnesium: A significant decrease was noticed in the mean plasma magnesium levels in Group-II from FOD upto the fresh period (Table 3).

Plasma Inorganic Phosphorus: A significant difference was observed between both groups during CUD and fresh period, while within a group, significant increase was recorded in Group II between FOD and fresh period (Table 3).

Sodium: A significant ($p<0.05$) difference was noted in between FOD and fresh period in Group I, while in between FOD and CUD period in Group II respectively (Table 3).

Potassium: A significant decrease was observed in the mean K levels between the FOD and fresh period in both the groups (Table 3).

Copper: The mean copper levels observed in both the groups from FOD to fresh period is presented in Table 3 Within a group, a non-significant increase in Group-I, while a non-significant decrease was observed in Group-II from FOD upto fresh period and significantly lower levels were observed in group II in comparison to group I during the fresh period.

Iron: The mean plasma Fe levels observed in both the groups were above the normal range, and non-significant increase in both groups was observed from FOD upto fresh period (Table 3).

Zinc: Significantly higher level was observed in Group II as compared to Group I in the fresh period. While a non-significant decrease was noticed in Group-I and a non-significant increase in the mean plasma Zn levels in Group-II from FOD up to the fresh period (Table 3).

No marked effect was seen of Stress Check powder on most of the mineral concentrations, however significantly higher levels were seen for Pi and Zn in supplemented cows in comparison to control cows during the fresh period, the possible reason for this increase might be due to the increased antioxidant effect provided by the herbal powder. As it is well known that SOD is a Cu-Zn dependent enzyme, so any increase in the antioxidant activity will result in increase in the SOD levels resulting in the conservation of these minerals [25].

Effect of Stress Check Powder on Oxidative stress parameters

Lipid Peroxidation: The mean erythrocytic LPO levels observed in both the groups is presented in Table. 4. Significantly ($p<0.05$) lower levels were observed in Group II as compared to Group I during the fresh period, where as a significant increase was noticed in Group I from FOD to fresh period and a significant decrease was noticed in Group II from the CUD period upto the fresh period. (Table 4).

Parameters	Period	Group-I (n=8)	Group-II (n=8)
LPO (n mol/g Hb)	FOD	177.38 ± 16.50^{ax}	239.49 ± 21.11^{bwx}
	CUD	268.03 ± 11.88^{ay}	264.21 ± 14.60^{ayx}
	Fresh	325.68 ± 14.37^{az}	173.85 ± 10.82^{bz}
SOD (U/ mg Hb)	FOD	80.55 ± 3.04^{ax}	68.15 ± 8.58^{ax}
	CUD	60.73 ± 7.66^{ax}	62.65 ± 7.25^{ax}
	Fresh	55.21 ± 12.29^{ax}	73.61 ± 2.38^{ax}
GSH (mM)	FOD	1.70 ± 0.15^{ax}	1.22 ± 0.22^{ax}
	CUD	1.75 ± 0.21^{ax}	1.23 ± 0.09^{ax}
	Fresh	1.69 ± 0.16^{ax}	1.43 ± 0.10^{ax}

Table 4: Effect of Stress Check herbal powder on oxidative stress and antioxidant status in cows (Mean ± S.E.). Values bearing different superscripts (a, b) in a row differ significantly. Values bearing different superscripts (w, x, y, z) in a column differ significantly.

Super oxide dismutase: The mean SOD levels measured from FOD to CUD to fresh period in Group I and Group II is presented in (Table 4). Within a group, non-significant decrease in Group-I, whereas a non-significant increase in Group-II was measured from FOD upto the fresh period (Table 4).

Reduced Glutathione: The mean GSH levels recorded in two groups did not show any significant difference during different periods. While within a group, a non-significant decrease in Group-I, and a non-significant increase was noticed in Group-II

from FOD period to CUD to fresh period respectively (Table 4).

Lipid peroxidation is a non-enzymatic chain reaction based on oxidation of mainly unsaturated fatty acids and is associated with the presence of reactive oxygen species (ROS).As lipids are most susceptible to peroxidative damage due to the presence of unsaturated bonds. The significant increase in the lipid peroxidation observed in Group I could be due to the increased metabolic demands imposed on the cow by colostrum production and the onset of lactation that far exceeded the demands of the fetus, [26, 27, and 28]. Whereas, significantly lower LPO levels observed in Group II and a non-significant increase in the SOD and GSH levels in Group II suggest the role of herbal powder in decreasing the oxidative stress in crossbred cows during the periparturient period.

Conclusion

Herbal powder 'Stress check' was effective in reducing the LPO levels during early lactation period as evident from the significantly lower LPO levels during the fresh period in comparison to control cows, and a non-significant increase was also noticed in both the SOD and GSH levels during the early lactation period, suggesting its role in decreasing stress during early lactation period. Also a non-significant increase in plasma glucose levels and lower protein, urea, BHBA and NEFA levels during the fresh period was noticed in supplemented cows, suggesting the role of Herbal powder "Stress Check" in providing energy to the cows during the peiparturient period.

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References

1. Goff JP, Horst RL (1997) Physiological changes at parturition and their relationship to metabolic disorders. Journal of Dairy Science 80: 1260-1268.
2. Ingvarsten KL, Andersen JB (2000) Itegration of metabolism and intake regulation: A review focusing on periparturient animals. Journal of Dairy Science 83: 1573-1597.
3. Bernabucci U, Ronchi B, Lacetera N, Nardone A (2005). Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. Journal of Dairy Science 88: 2017-2026.
4. Castillo CJ, Hernandez A, Bravo M, Lopez-Alonso V, Pereira, Benedito JL (2005) Oxidative status during late pregnancy and early lactation in dairy cows. The Veterinary Journal 169: 286-292.
5. Gaal TP, Ribiczeyne-Szabo, Stadler K, Jakus J, Reiczigel J, Pal Kover M, Mezes L, Sumeghy (2006) Free radicals, lipid peroxidation and the antioxidant system in the blood of cows and newborn calves around calving. Comparative Biochemistry and Physiology 143: 391-396.
6. Galav P, Jain A, Katewa SS (2013) Traditional Veterinary Medicines used by livestock owners of Rajasthan, India. International Journal of Traditional Knowledge 12: 47-55.
7. Phondani PC, Maikhuri RK, Kal CP (2010) Ethno-veterinary uses of medicinal plants among traditional herbal healers in Alaknanda catchment of Uttarakhand, India. African Journal of Traditional Complement and Alternate Medicine 7: 195-206.
8. Chattopadhyay MK (1996) Herbal medicines. Current Science 71: 5.
9. Kamboj VP (2000) Herbal Medicine. Current Science 78: 35-39.
10. Chopra RN, Nayar SL, Chopra IC (1956) In Glossary of Indian medicinal plants Council of Scientific and Industrial Research, New Delhi. 1: 197.
11. Rabe T, Staden JV (1997) Antibacterial activity of South African plants used for medicinal purposes. Journal of Ethno-pharmacology 56: 81-87.
12. Agarwal A (2005) Critical issues in quality control of Herbal products Pharmacology Times 37: 9-11.
13. Kolmer JA, Spanbling EH, Robinson HW (1951) Approved Laboratory Technique. Appleton Century Crofts, New York.
14. Perkin Elmer (2000) Atomic Spectroscopy A guide to selecting the appropriate technique and system. PerkinElmer, Inc. 940 Winter Street, Waltham, 02451 USA.
15. Tausky HH, Shorr E (1953) A micro colorimetric method for determination of inorganic phosphorus. Journal of Biological Chemistry 202: 675-685.
16. Placer ZA, Cushman LL, Johnson BC (1966) Estimation of product of lipid peroxidation in biochemical systems. Anal Biochemistry 16: 359-364.
17. Nishikimi MN, Rao A, Yagi KA (1972) The occurrence of super oxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochemical Biophysics Research Communication 46: 849-854.
18. Hafeman DG, Sunde RA, Hoekster WG (1974) Effect of dietary selenium on erythrocyte and liver glutathione in the rat. Journal of Nutrition 104: 580-587.
19. Lewis D (1957) Blood-urea concentration in relation to protein utilization in the ruminant. Journal of Agricultural Science 48: 438-446.
20. Petit HV, Flipot PM (1992) Feed utilization of beef steers fed grass as hay or silage with or without nitrogen supplementation Journal of Animal Science 70: 876-883.
21. Davidson S, Hopkins BA, Diaz DE, Bolt SM, Brownie C, Fellner V, Whitlow LW (2003) Effects of amounts and degradability of dietary protein on lactation, nitrogen utilization, and excretion in early lactation Holstein cows. Journal of Dairy Science 86: 1681-1689.
22. Tabrizi AB, Safi S, Asri Rezaee S, Hassanpour A, Mousavi G (2007) Evaluation of beta-hydroxy butyrate and glucose in subclinical ketosis in industrial herds of Holstein cows. Proceedings of 13th International congress in Animal Hygiene Tartu, Estonia 434-439
23. Bell AW (1995) Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. Journal of Animal Science 73: 2804-2819.
24. Herdt TH (1997) Gastro-intestinal physiology and metabolism. Textbook of Veterinary Physiology (2nd Edn.). WB Sanders

Company.
25. Kaneko JJ, Harvey JW, Bruss ML (2008). Clinical Biochemistry of Domestic Animals. 6th ed. Elsevier/ Academic Press, Amsterdam.
26. Castillo C, Hernandez J, Lopez-alonso M, Miranda M, Benedito JL (2003) Values of plasma lipid hydroperoxides and total antioxidant status in healthy dairy cows: preliminary observations. Archiv Teizucht 46: 227-233.
27. Saleh M, Salam A, Mel IMH (2007). Oxidative antioxidant status during transition from late pregnancy to early lactation in native and cross bred cows in the Egyptian oasis. Assiut Veterinary Medical Journal 53: 113-19.
28. Singh G, Randhawa SNS, Nayyar S and Randhawa CS (2014) Evaluation of oxidative stress during periparturient period in crossbred dairy cows Intas Polivet 15: 188-191.