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Effects of Physiological Status and Season on Blood Biochemical and Mineral Profile of Holstein Friesian Cross Bred Cattle

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ABSTRACT

The present study was conducted to elucidate the plasma mineral profile and serum biochemical parameters in Holstein Friesian cross bred cattle in different physiological stages and seasons. In each season and each physiological group 8 normal healthy animals randomly selected for blood sampling once in a month for minerals Ca, P, Cu, Zn and Mn; biochemical parameters glucose, BUN, and NEFA analysis. Blood plasma minerals and biochemical parameters were significantly ($P<0.05$) influenced by animal physiological conditions and season of the year. Whereas, season of the year did not influences the serum cholesterol level. Blood Plasma minerals estimated were within the normal range. However, mineral concentration were significantly ($P<0.05$) lower during winter. Similarly, glucose content was lower and the BUN and NEFA content were higher during winter compared to other season. Results indicate that the herd health status on metabolic and mineral profiles need to be evaluated regularly in different seasons to diagnose deficiencies and imbalances to achieve set targets in terms of production performance by adjusting feeding and mineral supplementation.

Introduction

The mineral level and biochemical parameters in animal body depends on large number of factors such as species, breed, age, sex, nutritional and health status, nutrients supplementation, seasonal and physiological variations (Khan *et al.*, 2003; O'Brien *et al.*, 2010; Gattani and Sareen, 2011). For maintaining better health and production animals need optimum concentrations of biochemical parameters and both macro and trace minerals. Recently, the essentiality of major and trace elements in animals for the maintenance of normal metabolic and production levels has been recognized (Khan *et al.*, 2003). The responses of animals to environmental stress during different seasons have profound effects on some serum biochemical parameters (Mellau *et al.*, 2007; Ganaie *et al.*, 2013). Information regarding the effect of seasonal variation on this area for cross bred cattle is lacking hence, the present study was undertaken to investigate the possible influences of seasonal variations of serum biochemical (glucose, BUN, cholesterol and NEFA) and mineral status (Ca, P, Zn, Mn and Cu) in cattle and the study has a potential to serve as the model for seasonal specific nutrient supplementation to animals in different seasons of year.

Materials and Methods

Location and experimental animal

Present study was conducted at Livestock Research Center, National Dairy Research Institute, Karnal, India on crossbred calves (Holstein Friesian × Tharparkar). Karnal is located on 29^0 42' N latitude and 72^0 02' E longitudes, with an altitude of 250 meters above the mean sea level in the bed of Indo-Gangetic alluvial plain. Depending on meteorological variables (Table 1) the whole duration of study was divided into four major seasons like rainy (July-September), autumn (October-November) winter (December-March) and summer (April-June). The study was carried out in Karan fries cattle grouped into six physiological classifications wise calf (aged below 3 months); Heifer (18- 22 months age); Heifer pregnant (6-8 months of pregnancy); Lactating cows (within 30 days after calving); High producing (>15kg/day); Low producing (< 15 kg/day); Dry pregnant cows (60 days before calving). In each season, from each group 8 normal healthy animals randomly selected for blood sampling once in a month for blood minerals and biochemical analysis.

Blood sampling, plasma metabolites and mineral estimation

Blood samples from cattle belonging to different physiological states (n=8) and from different seasons were collected by jugular venipuncture in heparinised vials, centrifuged at 3000 rpm for 10 min to harvest the plasma which was then transferred to sterile, acid-washed vials, labelled and stored at -40°C until transport to laboratory where the samples were stored at -20°C for further analysis. Plasma metabolites; glucose (O-Toluidine end point method, Hultman, 1959), urea nitrogen (Rahmatullah and Boyde, 1980), cholesterol (ferric chloride-sulphuric acid method, Zlatkis *et al.*, 1953), NEFA (modified copper soap extraction method, Shipe *et al.*, 1980) were estimated. Plasma minerals Ca, Zn, Cu and Mn were estimated with the help of Atomic Absorption Spectrophotometer (Philips PU 9100X) whereas; Plasma inorganic phosphorus was estimated by standard method given by Fiske and Subbarow (1925).

Statistical analysis

The statistical analysis was done using Sigmaplot version 11.0 (Systat Software Inc., USA). Data sets were first tested for normality by Shapiro-Wilk's normality test and analysed by two way analysis of variance (ANOVA) with general linear model (GLM). All pair-wise differences in mean were compared by Tukey post hoc test. Difference between means was considered statistically significant when $P \leq 0.05$ and plasma metabolites and mineral concentrations were presented as mean ± SE.

Results and Discussion

The overall plasma mineral and biochemical parameters concentrations are shown in (Table 2 and 3). Mineral and biochemical parameters estimation for different physiological group at deferent season revealed significant ($P<0.05$) influence of animal physiological conditions and season in the present study except for plasma cholesterol level which is significantly influence by physiological condition not by season.

The blood Ca level in different groups was within the normal range and above the critical level in the present study. However, near critical level of Ca was observed in the lactating cows which could be attributed to excessive secretion of Ca through milk (Asif *et al.,* 1996 and Sen *et al.,* 1989). The higher concentration of Ca in calf and heifer could be due to more efficient absorption of Ca in growing animals (Ricks, 1991). The observed Ca was significantly (P<0.05) low in winter followed by rainy, autumn and summer. This might be attributed to higher absorption efficiency during summer compared to winter (Khan, 2003). However, in the present study animals in all physiological stages were not in the risk of deficiency irrespective of seasons.

The study revealed adequate Phosphate irrespective of the physiological status and/ or season of the year with significantly (P<0.05) lower concentration in lactating and pregnant cows compared to young animals. The marked increase in phosphate secretion in milk may be the reason (Braithwait, 1983). The higher serum P in young animals because of the growth hormone results increased renal phosphate resorption (Kaneko *et al.,* 1997). According to our result, there was significantly (P<0.05) lower level of P observed during winter followed by rainy, autumn and summer season. This finding was in accordance with the literature of Srivastava *et al.,* (2000) and Sivaraman*et al.,* (2002).

Zn concentration was adequate in all categories. However, pregnant animal showed higher concentration. These observations could be due to increased demand for Zn for fetus growth (Elnageeb and Abdelatif, 2010). The Zn concentration was significantly (P<0.05) influenced by season of the year. Animal shows significantly (P<0.05) lower level during winter compared to other season. In contrast, Khan *et al.,* (2008) reported higher concentration in winter than summer. This discrepancy in result may be due to geographical area and soil of the study area.

Cu concentration observed was within the normal range. Higher Cu concentration was recorded in pregnant animals and calves. This might be due to increased demand and utilization of maternal Cu for development of fetal nervous systems (Elnageeb and Abdelatif, 2010). The observed Cu was significantly (P<0.05) lower in winter followed by rainy, autumn and summer. These observations were in agreement with the findings of other researchers (Pastrana *et al.,* 1991).

The overall Mn level in KF cattle was 0.50±0.01. The higher level was observed in heifer and lower level in calves. Similarly winter season sample shows lower level compared to other season.

The significant (P<0.05) higher level of blood glucose was observed in calves in all the season compare to other groups and the concentration of plasma glucose differs significantly P<0.05) among the four seasons and also did not follow a similar pattern. It might be due to change or variation in the fodder other management effects. The blood glucose level gives an indicator of the energy status of an animal. In comparison to other groups growing heifers are under nourished as evident from glucose value, which could be one of the reasons for late maturity in our herd (24months). However; it has been established beyond doubt that nutritional status plays a major role in determining variations of the circulating glucose concentration levels (Campanile *et al.,* 1997 and Montemurro *et al.,* 1997). In lactating and pregnant cows the glucose values were higher compare to our findings of Sivaraman *et al.,* (2002). Which could be due to increase in protein concentration in the ration of cows, whose protein requirements had already been met, triggers a more intense gluconeogenesis as depicted by higher glucose levels (Westwood *et al.,* 2002). Similarly Dhoble *et al.,* (2004) also reported increased blood glucose at the time of parturition and it could be due to storage of glucose during advanced pregnancy and the level remain maintained at the time of parturition but after calving the lactation starts and the concentration of glucose level declined up to one month in all the cows due to drainage through milk.

In lactating cows glucose levels were in agreement with findings of Rawat *et al.,* (2006). A decrease in plasma glucose concentration towards calving in some dairy cows was found to be associated with low milk production in early lactation (Schwalam and Schuitz, 1976). The glucose concentration increased significantly with approaching parturition and minimum values observed early phase of lactation. These low concentrations could be attributed to the negative energy balance, which is more pronounced during early phase of lactation. In the present study high yielding crossbred cows presumably reflecting greater demands for glucose in the mammary glands. In heavy lactating animals the glucose is excreted out as lactose through milk leading to hypoglycemia, which body tries to compensate through gluconeogenesis leading to deficiency of oxaloacetate (Kaneko, 1997).

The overall mean BUN concentration was high as compared to that reported by Kaneko (1997). The rise in BUN level may denote imbalance of protein and energy levels in the diet. The lower level of BUN during winter season may also be because of non-availability of leguminous fodder. The BUN level was higher in lactating cows than pregnant cattle agreement with the findings of Sivaraman *et al.,* (2002). Shaffer *et al.,* (1981) observed that the BUN level increased with increasing age. The BUN values of pregnant and lactating cows are agreement with findings of Aswal (2009).Increasing plasma BUN towards calving and early lactation is due to insufficient energy intake that would have leads to an increase deamination of amino acids resulting in the increase in urea concentration. A low level of BUN before calving has been reported by Hammond (1983).

Plasma urea concentrations were close to the normal ranges during summer. The significant seasonal variations were obtained on milk urea concentration in dairy cows (Dhali *et al.,* 2006). They also observed that milk urea significantly associated with crude protein content of the forages than the concentrates. Higher BUN in winter indicates deficiency in energy as sufficient quantity of leguminous fodder with high RDP is supplied during this period.

Table.1 Meteorological variable during the study period

Seasons	Meteorological variables (Mean ± SE)			
	Temperature (^oC)	**Relative Humidity (%)**	**Rain fall (mm)**	**Wind speed (km/hr)**
Rainy	29.28±2.27	80.17±5.46	140.90±27.59	4.20±0.26
Autumn	22.57±4.51	67.00±13.31	7.60±6.6	2.25±0.35
Winter	16.48±2.82	71.12±8.21	4.40±1.8	3.32±0.33
Summer	29.02±3.24	54.00±9.53	62.13±42.37	7.23±0.78

Table.2 Effect of physiological status and seasonal variation on blood plasma minerals in Holstein Friesian cross bred cattle

Parameter	Physiological Status	Season				Overall
		Rainy	Autumn	Winter	Summer	
Ca (mg/dl)	Calf	12.78^{aE}±0.09	12.88^{aC}±0.12	11.7^{cD}±0.08	12.59^{bD}±0.09	12.39^{d}±0.07
	Heifer	11.28^{bD}±0.10	11.33^{bC}±0.13	11.09^{cC}±0.09	11.66^{aC}±0.11	11.32^{c}±0.06
	Heifer pregnant	9.30^{bC}±0.27	9.29^{bB}±0.03	9.16^{cB}±0.02	9.54^{aB}±0.03	9.31^{b}±0.02
	Lactating high yielder	8.48^{cA}±0.05	8.46^{cA}±0.06	8.56^{bA}±0.04	8.67^{aA}±0.05	8.55^{a}±0.03
	Lactating low yielder	8.63^{bB}±0.05	8.43^{dA}±0.06	8.54^{cA}±0.04	8.78^{aA}±0.05	8.60^{a}±0.03
	Dry pregnant cows	9.33^{bC}±0.04	9.28^{bB}±0.04	9.18^{cB}±0.03	9.47^{aB}±0.04	9.31^{b}±0.02
	Overall	9.97^{B}±0.13	9.95^{B}±0.17	9.70^{A}±0.09	10.12^{C}±0.13	9.91±0.06
P(mg/dl)	Calf	6.62^{aD}±0.09	6.67^{aC}±0.11	4.21^{cA}±0.08	6.27^{bC}±0.09	5.74^{c}±0.12
	Heifer	5.52^{bC}±0.09	5.28^{cB}±0.11	4.81^{dB}±0.08	5.65^{aB}±0.09	5.28^{b}±0.06
	Heifer pregnant	4.36^{bA}±0.16	4.45^{bA}±0.20	4.85^{aB}±0.14	5.07^{aA}±0.16	5.07^{a}±0.19
	Lactating high yielder	4.61^{bB}±0.07	4.64^{bA}±0.08	4.67^{bB}±0.06	4.90^{aA}±0.07	4.71^{a}±0.03
	Lactating low yielder	4.67^{cB}±0.06	4.67^{cA}±0.08	4.79^{bB}±0.05	4.97^{aA}±0.06	4.79^{a}±0.03
	Dry pregnant cows	4.67^{bB}±0.04	4.68^{bA}±0.05	4.73^{abB}±0.04	4.75^{aA}±0.04	4.72^{a}±0.02
	Overall	5.08^{B}±0.07	5.06^{B}±0.09	4.68^{A}±0.04	5.27^{C}±0.06	4.99±0.03
Zn(ppm)	Calf	1.52^{aC}±0.02	1.50^{aB}±0.02	1.17^{cC}±0.02	1.41^{bB}±0.02	1.37^{b}±0.02
	Heifer	1.47^{bB}±0.02	1.47^{bB}±0.02	1.24^{cD}±0.02	1.51^{aC}±0.02	1.40^{b}±0.01
	Heifer pregnant	1.81^{aD}±0.01	1.81^{aC}±0.02	1.24^{bB}±0.01	1.80^{aD}±0.01	1.80^{c}±0.01
	Lactating high yielder	1.08^{aA}±0.02	1.10^{aA}±0.02	0.94^{bA}±0.01	1.08^{aA}±0.02	1.03^{a}±0.01

	Lactating low yielder	1.05^{bA}±0.01	1.09^{aA}±0.02	0.95^{cA}±0.02	1.09^{aA}±0.01	1.04^{a}±0.01
	Dry pregnant cows	1.81^{aD}±0.02	1.81^{aC}±0.01	1.13^{bB}±0.02	1.82^{aD}±0.02	1.59^{c}±0.03
	Overall	1.46^{B}±0.31	1.46^{B}±0.03	1.09^{A}±0.01	1.45^{B}±0.03	1.34±0.01
Cu(ppm)	Calf	1.10^{bC}±0.02	1.25^{aD}±0.03	0.80^{cD}±0.02	1.25^{aD}±0.02	1.07^{e}±0.02
	Heifer	0.77^{bA}±0.01	0.79^{aA}±0.01	0.68^{cC}±0.01	0.79^{aA}±0.01	0.75^{a}±0.01
	Heifer pregnant	1.43^{aD}±0.01	1.43^{aE}±0.02	0.60^{cB}±0.01	1.12^{bE}±0.01	1.15+±0.04
	Lactating high yielder	0.93^{cB}±0.01	0.92^{cB}±0.02	1.03^{aE}±0.01	0.96^{bB}±0.01	0.97^{c}±0.01
	Lactating low yielder	1.04^{bC}±0.02	1.03^{bC}±0.02	0.70^{cC}±0.02	1.08^{aC}±0.02	0.93^{b}±0.02
	Dry pregnant cows	1.40^{aD}±0.02	1.41^{aE}±0.03	0.57^{bA}±0.02	1.39^{aE}±0.02	1.00^{d}±0.01
	Overall	1.11^{B}±0.02	1.14^{C}±0.03	0.73^{A}±0.01	1.15^{C}±0.02	1.00±0.01
Mn(ppm)	Calf	0.44^{bA}±0.01	0.47^{aA}±0.01	0.16^{cA}±0.01	0.44^{bA}±0.01	0.35^{a}±0.01
	Heifer	0.71^{bD}±0.01	0.76^{aD}±0.01	0.41^{cE}±0.01	0.71^{bE}±0.01	0.62^{d}±0.02
	Heifer pregnant	0.68^{aC}±0.01	0.67^{aC}±0.01	0.28^{bC}±0.01	0.68^{aCD}±0.01	0.54^{c}±0.02
	Lactating high yielder	0.54^{bB}±0.01	0.54^{bB}±0.01	0.35^{cD}±0.01	0.56^{aB}±0.01	0.56^{c}±0.01
	Lactating low yielder	0.56^{aB}±0.01	0.52^{bB}±0.02	0.34^{cD}±0.01	0.55^{aB}±0.01	0.48^{b}±0.01
	Dry pregnant cows	0.69^{aCD}±0.01	0.69^{aC}±0.01	0.26^{bB}±0.01	0.69^{aC}±0.01	0.55^{c}±0.01
	Overall	0.60^{B}±0.01	0.61^{B}±0.01	0.30^{A}±0.01	0.61^{B}±0.01	0.50±0.01

Means with different superscript in a row (a, b, c, d) and column (A, B, C, D, E) differ significantly ($P<0.05$)

Table.3 Effect of physiological status and seasonal variation on blood biochemical parameters in Holstein Friesian cross bred cattle

Parameter	Physiological Status	Season				Overall
		Rainy	Autumn	Winter	Summer	
Glucose(mg/dl)	Calf	86.54^{aE}±1.02	88.79^{aC}±1.25	78.85^{dE}±0.89	83.59^{cD}±1.02	83.61^{d}±0.63
	Heifer	66.34^{aC}±1.45	56.72^{bA}±1.81	55.04^{bC}±1.28	65.70^{aB}±1.48	60.81^{b}±0.90
	Heifer pregnant	70.03^{bD}±0.74	69.17^{bB}±0.90	67.03^{cD}±0.64	72.45^{aC}±0.74	65.23^{c}±0.08
	Lactating high yielder	61.98^{aB}±0.78	56.90^{bA}±0.96	48.25^{cA}±0.68	57.12^{bA}±0.78	65.29^{c}±0.001
	Lactating low yielder	56.58^{bA}±0.46	52.90^{cA}±0.57	53.51^{cB}±0.40	58.32^{aA}±0.46	65.29^{c}±0.001
	Dry pregnant cows	73.06^{aD}±0.99	65.58^{bB}±1.22	66.16^{bD}±0.86	74.19^{aC}±0.99	56.33^{a}±0.46
	Overall	68.15^{C}±0.81	65.71^{B}±1.63	63.88^{A}±0.64	67.25^{C}±0.74	66.09±0.41
BUN(mg/dl)	Calf	30.70^{aC}±0.35	30.95^{aB}±0.42	28.85^{aA}±0.30	23.15^{bB}±0.35	28.24^{a}±0.36
	Heifer	28.85^{bA}±0.90	23.47^{cA}±1.10	30.83^{aC}±0.78	23.46^{cB}±0.90	27.27^{a}±0.56
	Heifer pregnant	31.82^{aC}±0.82	31.38^{aB}±1.01	30.15^{bAB}±0.07	18.32^{cA}±0.82	27.81^{a}±0.70
	Lactating high yielder	42.07^{bD}±0.42	43.04^{aC}±0.52	42.21^{bD}±0.36	27.10^{cC}±0.42	38.54^{b}±0.71
	Lactating low yielder	42.06^{aD}±0.45	41.70^{aC}±0.55	42.13^{aD}±0.39	26.11^{bC}±0.45	38.03^{b}±0.74
	Dry pregnant cows	31.33^{aC}±0.79	29.87^{bB}±0.96	29.65^{bAB}±0.68	18.75^{cA}±0.79	27.38^{a}±0.65
	Overall	34.47±0.54^{C}	33.40±0.79^{B}	33.97^{BC}±0.49	22.82^{A}±0.32	31.21±0.33
Cholesterol(mg/dl)	Calf	104.76^{aB}±2.34	100.68^{bA}±2.87	92.81^{cA}±2.03	104.59^{aA}±2.34	98.88^{a}±1.29
	Heifer	88.26^{dA}±5.08	120.35^{bB}±6.21	140.02^{aC}±4.40	112.18^{cA}±5.08	116.84^{b}±3.22

	Heifer pregnant	131.70^{aC}±2.01	117.14^{dB}±0.46	121.87^{cB}±1.74	127.68^{bB}±2.01	124.99^{c}±1.12
	Lactating high yielder	208.88^{aD}±1.19	202.53^{cD}±1.45	206.75^{bD}±1.03	206.52^{bC}±1.19	206.25^{d}±0.65
	Lactating low yielder	207.14^{aD}±1.31	204.67^{bD}±1.61	206.65^{abD}±1.14	205.90^{abC}±1.32	205.90^{d}±1.83
	Dry pregnant cows	133.02^{aC}±2.13	128.48^{bC}±2.60	122.95^{cB}±1.84	126.19^{cB}±2.13	127.20^{c}±1.12
	Overall	145.63±3.97	145.64±4.40	148.43±3.31	147.18±3.81	146.95±1.90
NEFA(μmol/l)	Calf	195.15^{bA}±11.77	160.27^{dA}±14.41	178.38^{cA}±10.19	250.61^{aD}±11.77	222.61^{a}±9.61
	Heifer	259.23^{cB}±9.47	205.14^{dB}±11.60	334.31^{aC}±8.20	275.37^{bB}±9.47	279.28^{b}±6.56
	Heifer pregnant	291.27^{aC}±3.51	272.38^{bC}±4.30	295.36^{aB}±3.04	254.02^{cA}±3.51	280.17^{b}±2.45
	Lactating high yielder	382.90^{bD}±3.97	384.57^{abD}±4.86	389.84^{aD}±3.44	355.39^{cD}±3.97	378.87^{c}±2.67
	Lactating low yielder	390.81^{bD}±3.67	387.28^{bD}±4.49	404.62^{aD}±3.18	338.87^{cC}±3.67	381.84^{c}±3.20
	Dry pregnant cows	275.78^{aBC}±3.18	277.66^{aC}±3.90	279.28^{aB}±2.75	248.28^{bA}±3.18	270.38^{b}±2.04
	Overall	299.19^{B}±6.70	281.22^{A}±8.95	313.63^{C}±6.12	303.76^{B}±4.11	302.15±3.23

Means with different superscript in a row (a, b, c, d) and column (A, B, C, D, E) differ significantly ($P<0.05$)

Season of year did not influence the blood cholesterol level. The positive correlation of cholesterol with age of the cow is very important and the cholesterol level increased only up to 4 years of age and then decreased due to acclimatization to stress from lactation and gestation (Roussel *et al.*, 1982). This continued increase of cholesterol with increasing age in present study is also reflected to get acclaimed to the stress of lactation and gestation.

The cholesterol level was higher in lactating stage than pregnant cows was in agreement with findings of Sivaraman *et al.,* (2002) and dynamics of serum cholesterol values was similar to Singh *et al.,* (2009) and Dhoble *et al.,* (2004). In the present study heifers in autumn and winter season showed higher cholesterol level this could be due to negative energy balance as ad lib. Berseem was supplied during these periods.

In calf and heifer significant ($P<0.05$) difference was observed in all the seasons of the year. The NEFA levels in present experiment were found to increase significantly after calving. The results was in accordance with studies undertaken by Kokkonen *et al.,* (2005) which reported that cows with higher BCS and greater BCS loss had increased concentration of NEFA and were particularly sensitive to oxidative stress.

The NEFA level of lactating and pregnant cows of present study was comparable to Aswal (2009). In lactating animal groups in our study with very high negative energy balance may be the reason for high incidence of production diseases in our herd. Drackley (2001) reported that normal values of NEFA for cows in positive energy balance were less than 200 μM. High yielding dairy cows remain in a state of negative energy balance during postpartum because the amount of energy required for maintenance of body tissue functions and milk production exceeds the amount of energy cows could consume.

In conclusion we found significant seasonal changes in mineral and biochemical changes in healthy animals. Our results proposes the metabolic and mineral profiles need to be evaluated regularly in different seasons to diagnose deficiencies and imbalances to achieve set targets in terms of production performance by adjusting feeding and mineral supplementation.

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Conflict of interest

None

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