



Evaluation of Serum Biochemical Profile in Heifer and Adult Sahiwal Cows Reared in Maharashtra

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ABSTRACT

Background: The Sahiwal cattle, one of the best dairy breeds of Zebu cattle in India and Pakistan, originated from the Montgomery district of Pakistan. The performance of this breed in the tropical environment is also better than other cattle breeds. Now a day Sahiwal is choice of farmers in Maharashtra as it is a milch type breed mostly preferred for Deshi cow milk production and for organic farming. Blood profile of an individual is an important indicator of an individual's well-being. Normal blood levels of various biochemical components are essential for normal function of various systems in the body including reproductive system. Hence, the current study was aimed to evaluate of serum biochemical profile of Sahiwal cows reared in Pune district of Maharashtra. Pune district of Maharashtra comes under western Maharashtra plain zone with Grayish black soils. The annual rainfall ranges from 150 to 700 mm. Soils formed from trap rock are grayish black in colour and varying texture. The natural vegetation is of shrub. The area mostly grows paddy, sugarcane, pearl millet, sorghum and groundnut.

Methods: Serum biochemical profile of Sahiwal heifers and cows were evaluated at different stages *i.e.* below 1 year heifer, above 1 year heifer, early lactating cow, mid lactating cow, late lactating cow, dry pregnant cow and dry non pregnant cows. Total seventy (70) animals was selected *i.e.* ten (10) animals from each group stage. All animals were maintained under standard feeding and management conditions. The blood samples of selected cows were collected by puncturing in jugular vein in heparinized vacutainer tubes. Analysis of serum profile of 70 blood samples were carried out by using automatic blood analyzer machine (Miura 200 I.S.E. group, Italy).

Result: The concentrations of serum cholesterol, glucose, triglyceride, uric acid, calcium, phosphorus, copper, zinc, total protein, globulin, A: G ratio ALP, ALT and AST were significant in different stages of Sahiwal cows. The non-significant difference was observed in different stages of Sahiwal cows in regards to serum concentration of amylase, bilirubin direct, bilirubin total, creatinine, urea, albumin, magnesium, iron and GGT.

Key words: Biochemical, Sahiwal, Serum.

INTRODUCTION

Biochemical profile is important bio indicator of the physiological state of cattle. These parameters may also be used as diagnostic tool for analyzing reproductive and productivity disorders (Virmani *et al.*, 2011). Therefore, optimum blood biochemical components are essential for the normal function of the reproductive system, as well as other systems of the body. The blood profile of an individual provides an opportunity to clinically investigate the presence of different metabolites and other constituents in the body that plays a vital role in the assessment of physiological and nutritional status of an individual. The knowledge of biochemical indices is useful in diagnosing various metabolic disorders, which can adversely affect the productive and reproductive performance of livestock resulting in great economic losses to dairy farmers (Aggarwal *et al.*, 2016).

Normal blood levels of various biochemical components are essential for normal function of various systems in the body including reproductive system. Changes in several biochemical components have been blamed for reproductive failures. In animals, minerals function as structural components of organs and tissues, as activators in enzyme and hormones systems. Therefore, study was conducted to generate comprehensive serum profiling of Sahiwal cows

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in Maharashtra which might be helpful in characterizing production, reproduction and management problems.

MATERIALS AND METHODS

Selection of Animals

Present study was conducted in apparently healthy, growing, adult non-lactating and lactating Sahiwal cows reared in Pune district of Maharashtra. Experimental animals were divided into 7 groups (ten (10) animals from each) according to their physiological state *i.e.* below 1 year heifer (T_1), above 1 year heifer (T_2), early lactating cow (T_3), mid lactating cow

(T_4), Late lactating cow (T_5), Dry pregnant cow (T_6) and Dry non pregnant cows (T_7).

Collection of blood samples

The blood samples of selected cows were collected by puncturing in jugular vein in heparinized vacutainer tubes. The tubes were transferred into ice bucket and carried to the laboratory. The serum was separated by centrifugal separator at 3000 rpm for 20 minutes and stored at -20°C for further analysis.

Analysis of blood

Analysis of serum profile of 70 blood samples were carried out by using automatic blood analyzer machine (Miura 200 I.S.E. group, Italy). Blood serum samples were analyzed for estimation of Metabolites (Amylase, Bilirubin Direct, Bilirubin Total, Cholesterol, Creatinine, Glucose, Triglycerides, Urea and Uric Acid), Protein Profile (Total Protein, Albumin, Globulin and A:G ratio), Minerals (Calcium, Magnesium, Phosphorous, Copper, Zinc and Iron) and Enzymes (ALT, AST, ALP and GGT).

Statistical analysis

The data was analysed by Completely Randomized Design as per Snedecor and Corchran (1995).

RESULTS AND DISCUSSION

Metabolic profile

Serum concentrations of amylase, bilirubin direct, bilirubin total, cholesterol, creatinine, glucose, triglycerides, urea and uric acid are given in Table 1.

Serum amylase (U/L) concentration levels found in below 1 year heifer (T_1), above 1 year heifer (T_2), early lactating cow (T_3), mid lactating cow (T_4), late lactating cow (T_5), dry pregnant cow (T_6) and dry non pregnant cow (T_7) were 19.10 ± 0.97 , 22.30 ± 2.19 , 21.90 ± 1.99 , 20.80 ± 1.8 , 22.80 ± 2.28 , 19.40 ± 1.19 and 23.70 ± 1.49 , respectively. In present study there was no significant difference observed in amylase concentration. However, amylase concentration was higher in T_7 while lowest amylase concentration was observed in T_1 . Similar finding was reported by Tajik *et al.*, (2011) who reported no significant difference in amylase concentration (U/L) between early lactation and mid lactation cows. Amylase plays an important role in rumen as it hydrolyzes dietary starch. Rumen amylase activity could relate to the low milk fat syndrome of dairy cows.

Serum bilirubin direct (mg/dl) concentration levels found in below 1 year heifer (T_1), above 1 year heifer (T_2), early lactating cow (T_3), mid lactating cow (T_4), late lactating cow (T_5), dry pregnant cow (T_6) and dry non pregnant cow (T_7) were 0.28 ± 0.09 , 0.34 ± 0.08 , 0.48 ± 0.13 , 0.32 ± 0.10 , 0.21 ± 0.02 , 0.22 ± 0.04 and 0.15 ± 0.04 , respectively. In present study there was no significant difference observed in bilirubin direct concentration. However, Bilirubin concentration was higher in T_3 while lowest bilirubin direct concentration was observed in T_7 . Similar finding was reported by kumar *et al.*, (2017) who reported that Bilirubin direct level did not differ

Table 1: Serum metabolite concentrations (mg/dl) in different stages of sahiwal cows.

Animal parameters	Below 1 year (T_1)	Above 1 year (T_2)	Early lactating (T_3)	Mid lactating (T_4)	Late lactating (T_5)	Dry pregnant (T_6)	Dry non-pregnant (T_7)	SEm ($\pm$)	CDat 5%	CV (%)
Amylase (U/L)	19.10 ± 0.97	22.30 ± 2.19	21.90 ± 1.99	20.80 ± 1.8	22.80 ± 2.28	19.40 ± 1.19	23.70 ± 1.49	1.797529	NS	26.52667
Bilirubin direct	0.28 ± 0.09	0.34 ± 0.08	0.48 ± 0.13	0.32 ± 0.10	0.21 ± 0.02	0.22 ± 0.04	0.15 ± 0.04	0.079962	NS	88.06173
Bilirubin total	0.44 ± 0.12	0.48 ± 0.11	0.66 ± 0.18	0.43 ± 0.14	0.33 ± 0.05	0.33 ± 0.07	0.20 ± 0.05	0.111497	NS	86.35764
Cholesterol	161.45 ± 16.03^{bc}	113.91 ± 6.19^{ab}	206.36 ± 30.72^{cd}	241.33 ± 16.20^d	173.48 ± 18.7^c	102.82 ± 5.05^a	106.74 ± 9.95^a	16.8266	47.55333	33.6749
Creatinine	1.56 ± 0.07	1.59 ± 0.08	1.68 ± 0.14	1.65 ± 0.11	1.75 ± 0.08	1.93 ± 0.15	1.94 ± 0.08	0.105627	NS	19.32515
Glucose	84.12 ± 3.68^b	75.82 ± 3.50^b	52.67 ± 5.51^a	55.21 ± 5.21^a	55.16 ± 5.25^a	52.21 ± 3.12^a	57.67 ± 2.86^a	4.288552	12.11979	21.93195
Triglyceride	23.76 ± 2.92^b	27.11 ± 3.87^b	19.74 ± 3.82^{ab}	13.18 ± 0.89^a	21.14 ± 2.79^{ab}	24.07 ± 1.95^b	36.79 ± 3.34^c	2.967609	8.3867	39.62482
Urea	11.21 ± 1.14	19.37 ± 2.48	14.10 ± 1.32	16.74 ± 3.60	15.27 ± 1.99	10.31 ± 1.32	15.93 ± 2.86	2.268701	NS	48.78744
Uric acid	0.82 ± 0.05^a	0.78 ± 0.07^a	1.32 ± 0.12^b	1.22 ± 0.10^b	1.18 ± 0.11^b	1.14 ± 0.07^b	1.18 ± 0.11^b	0.093248	0.263527	27.04013

significantly in different age wise groups. The high levels of bilirubin at birth are likely due to the trauma of the birth process, which often results in varying amounts of extravasation of blood.

Serum bilirubin total (mg/dl) concentration levels found in below 1 year heifer (T_1), above 1 year heifer (T_2), early lactating cow (T_3), mid lactating cow (T_4), late lactating cow (T_5), dry pregnant cow (T_6) and dry non pregnant cow (T_7) were 0.44 ± 0.12 , 0.48 ± 0.11 , 0.66 ± 0.18 , 0.43 ± 0.14 , 0.33 ± 0.05 and 0.20 ± 0.05 , respectively. In present study there was no significant difference observed in Bilirubin Total concentration. However, Bilirubin total concentration was higher in T_3 while lowest bilirubin total concentration was observed in T_7 . Similar finding was reported by Mohamed (2014) who reported significant increase in bilirubin total level in early lactation than mid lactation in dairy cows. Bilirubin is formed by the breakdown of red blood cells in the body. The liver helps to excrete it. High level of bilirubin may indicate liver damage or disease.

Serum cholesterol (mg/dl) concentration levels found in below 1 year heifer (T_1), above 1 year heifer (T_2), early lactating cow (T_3), mid lactating cow (T_4), late lactating cow (T_5), dry pregnant cow (T_6) and dry non pregnant cow (T_7) were 161.45 ± 16.03 , 113.91 ± 6.19 , 206.36 ± 30.72 , 241.33 ± 16.20 , 173.48 ± 18.7 , 102.82 ± 5.05 and 106.74 ± 9.95 , respectively. In present study cholesterol concentration was significantly higher in T_4 and at par with T_3 while lowest cholesterol was observed in T_6 and T_7 but at par with T_2 . Also T_1 and T_2 were at par with each other. Similar finding was reported by Otto *et al.*, (2000) who reported highest cholesterol level in lactating cows as compare to non-pregnant and pregnant cows. Also, Naser *et al.*, (2014) reported a significant increase in serum cholesterol during mid-lactation as compared to early lactation in dairy cows. Tusimovae *et al.*, (2015) reported that cholesterol concentration was significantly increased in mid lactation as compared to beginning of lactation in dairy cow. Nehra priyanka (2016) reported that cholesterol increased significantly with the advancement of lactation period showing a definite increasing pattern.

Serum creatinine (mg/dl) concentration levels found in below 1 year heifer (T_1), above 1 year heifer (T_2), early lactating cow (T_3), mid lactating cow (T_4), late lactating cow (T_5), dry pregnant cow (T_6) and dry non pregnant cow (T_7) were 1.56 ± 0.07 , 1.59 ± 0.08 , 1.68 ± 0.14 , 1.65 ± 0.11 , 1.75 ± 0.08 , 1.93 ± 0.15 and 1.94 ± 0.08 , respectively. In present study there was no significant difference observed in creatinine concentration. However, creatinine concentration was higher in T_7 while lowest creatinine concentration was observed in T_1 . Similar finding was reported by Suratani *et al.*, (2013) who reported a non-significant difference in serum creatinine levels between Sahiwal heifers and Sahiwal cows. Also, Shripad *et al.*, (2016) reported no significant difference in creatinine level of animals grouped according to the age *i.e.* 0-3 years, 3-6 years and 6-9 years. Creatinine is important indicator of

kidney health because it is an easily measured by product of muscle metabolism that is excreted unchanged by the kidney. Creatinine level in blood varies depending on age and body size and can rise temporarily from strenuous exercise or by using certain medication.

Serum glucose (mg/dl) concentration levels found in below 1 year heifer (T_1), above 1 year heifer (T_2), early lactating cow (T_3), mid lactating cow (T_4), late lactating cow (T_5), dry pregnant cow (T_6) and dry non pregnant cow (T_7) were 84.12 ± 3.68 , 75.82 ± 3.50 , 52.67 ± 5.51 , 55.21 ± 5.21 , 55.16 ± 5.25 , 52.21 ± 3.12 and 57.67 ± 2.86 , respectively. In present study glucose concentration was significantly higher in T_1 and at par with T_2 while lowest glucose was observed in T_6 and at par with T_3 , T_4 , T_5 and T_7 . Similar finding was reported by Naser *et al.*, (2014) who observed that concentration of glucose was significantly increased in mid lactation as compared with the early lactation stage in dairy cows. Whereas, Nehra priyanka (2016) reported that glucose was non-significant in lactation stages of Sahiwal cattle. Kumar *et al.*, (2017) reported significantly higher level glucose in animals of 0-1 year age group. The lowered blood glucose concentration in early stage of lactation might be due to large amount of blood glucose withdrawal by the mammary gland for synthesis of milk lactose (Naser *et al.*, 2014).

Serum triglycerides (mg/dl) concentration levels found in below 1 year heifer (T_1), above 1 year heifer (T_2), early lactating cow (T_3), mid lactating cow (T_4), late lactating cow (T_5), dry pregnant cow (T_6) and dry non pregnant cow (T_7) were 23.76 ± 2.92 , 27.11 ± 3.87 , 19.74 ± 3.82 , 13.18 ± 0.89 , 21.14 ± 2.79 , 24.07 ± 1.95 and 36.79 ± 3.34 , respectively. In present study triglyceride concentration was significantly higher in T_7 while lowest triglyceride was observed in T_4 but at par with T_3 and T_5 . Also T_1 , T_2 , T_3 , T_5 and T_6 were at par with each other. Similar finding was observed by Piccione *et al.*, (2012) reported significant decrease in triglyceride level in early and mid-lactation than dry period in dairy cows. The decreasing pattern of serum triglycerides in early lactation was reported which showed the lowest values of these compounds at the onset of lactation for their growing requirement for energy (Marcos *et al.*, 1990 Piccone *et al.*, 2009).

Serum urea (mg/dl) concentration levels found in below 1 year heifer (T_1), above 1 year heifer (T_2), early lactating cow (T_3), mid lactating cow (T_4), late lactating cow (T_5), dry pregnant cow (T_6) and dry non pregnant cow (T_7) were 11.21 ± 1.14 , 19.37 ± 2.48 , 14.10 ± 1.32 , 16.74 ± 3.60 , 15.27 ± 1.99 , 10.31 ± 1.32 and 15.93 ± 2.86 , respectively. In present study there was no significant difference observed in Urea concentration. However, urea concentration was higher in T_2 while lowest Urea concentration was observed in T_6 . There is increase in concentration of urea in T_3 as compared to T_6 . Also there is increase in urea concentration in T_4 as compared with T_3 . Similar finding was reported by Shripad *et al.*, (2016) who reported no significant difference in urea biochemical parameters of animals grouped according to the age *i.e.* 0-3 years, 3-6 years and 6-9 years. Deepak *et al.*, (2017) reported that urea was not statistically

different in various age groups viz. 0-1 year, 1-2 year, 2-3 year and >3 year. Whereas Naser *et al.*, (2014) reported a highly significant increase in urea concentration in mid lactation as compared with early lactation in dairy cow. Increased urea levels in early and mid-lactation period could be either due to increased deamination or increased protein intake. Urea server an important role in the metabolism of nitrogen containing compounds by animal and is the main nitrogen containing substance in the urine. The animal body uses it in many process most notable nitrogen excretions. Increased concentration indicates renal dysfunction.

Serum uric acid (mg/dl) concentration levels found in below 1 year heifer (T_1), above 1 year heifer (T_2), early lactating cow (T_3), mid lactating cow (T_4), late lactating cow (T_5), dry pregnant cow (T_6) and dry non pregnant cow (T_7) were 0.82 ± 0.05 , 0.78 ± 0.07 , 1.32 ± 0.12 , 1.22 ± 0.10 , 1.18 ± 0.11 , 1.14 ± 0.07 and 1.14 ± 0.07 , respectively. In present study uric acid concentration was significantly higher in T_3 and at par with T_4 , T_5 , T_6 and T_7 while lowest uric acid was observed in T_2 but at par with T_1 . Uric acid concentration decreases as lactation progresses. Similar finding was reported by Carcangiu *et al.*, (2015) who recorded a significant increase in concentration of uric acid in early lactation as compared to dry period in Sarda sheep.

During lactation due to metabolic stress there is increase in secretion of cortisol which affects the breakdown of protein to cope up with increased demand of energy. Cortisol causes catabolism in protein and leading to increased uric acid.

Protein profile

Serum values of total protein, albumin, globulin and A:G ratio are given in Table 2.

Serum total protein (g/dl) concentration levels found in below 1 year heifer (T_1), above 1 year heifer (T_2), early lactating cow (T_3), mid lactating cow (T_4), late lactating cow (T_5), dry pregnant cow (T_6) and dry non pregnant cow (T_7) were 6.03 ± 0.14 , 6.66 ± 0.12 , 7.80 ± 0.27 , 7.76 ± 0.18 , 8.18 ± 0.15 , 8.22 ± 0.17 and 8.31 ± 0.24 , respectively. In present study total protein concentration was significantly higher in T_7 and at par with T_3 , T_4 , T_5 and T_6 while lowest total protein was observed in T_1 . Also T_3 , T_4 , T_5 and T_6 were at par with each other. Similar finding was reported by Mohamed (2014) who reported highly significant increase in total protein level in early lactation than mid lactation in dairy cows. Also Adedibu *et al.*, (2013) reported that total protein was significantly higher in non-lactation than lactation period in Friesian $\times$ bunaji cows. Total serum proteins levels were affected by the physiological period and increased during lactation. The variations reflect the maternal requirements of proteins need for milking and providing Immunoglobulin (Bell *et al.*, 2000; Mohri *et al.*, 2007). Increase in total protein level of serum with the progress of lactation might be due to the catabolism of protein for milk synthesis (Krajnicakova *et al.*, 2003; Darwesh *et al.*, 2013).

Table 2: Serum protein profile concentrations (g/dl) in different stages of sahiwal cows.

Parameters	Stages of animal							SEM ($\pm$)	CD at 5%	CV (%)
	Below 1 year (T_1)	Above 1 year (T_2)	Early lactating (T_3)	Mid lactating (T_4)	Late lactating (T_5)	Dry pregnant (T_6)	Dry non pregnant (T_7)			
Total protein	6.03 ± 0.14^a	6.66 ± 0.12^b	7.80 ± 0.27^{cd}	7.76 ± 0.18^{cd}	8.18 ± 0.15^{cd}	8.22 ± 0.17^{cd}	8.31 ± 0.24^d	0.187025	0.528547	7.816718
Albumin	3.43 ± 0.07	3.44 ± 0.10	3.34 ± 0.12	3.36 ± 0.10	3.51 ± 0.08	3.38 ± 0.07	3.47 ± 0.06	0.087992	NS	8.140516
Globulin	2.60 ± 0.14^a	3.22 ± 0.13^b	4.46 ± 0.19^c	4.41 ± 0.14^c	4.67 ± 0.12^c	4.85 ± 0.18^c	4.84 ± 0.20^c	0.16145	0.456272	12.30837
A : G ratio	1.36 ± 0.09^c	1.09 ± 0.06^b	0.76 ± 0.03^a	0.77 ± 0.03^a	0.76 ± 0.03^a	0.71 ± 0.04^a	0.73 ± 0.03^a	0.050416	0.142478	18.0975

Serum albumin (g/dl) concentration levels found in below 1 year heifer (T_1), above 1 year heifer (T_2), early lactating cow (T_3), mid lactating cow (T_4), late lactating cow (T_5), dry pregnant cow (T_6) and dry non pregnant cow (T_7) were 3.43 ± 0.07 , 3.44 ± 0.10 , 3.34 ± 0.12 , 3.36 ± 0.10 , 3.51 ± 0.08 , 3.38 ± 0.07 and 3.47 ± 0.06 , respectively. In present study there was no significant difference observed in albumin concentration. However, Albumin concentration was higher in T_5 while lowest albumin concentration was observed in T_3 . Similar finding was reported by Mohamed (2014) who reported no significant difference in albumin level in early lactation and mid lactation in dairy cows. However, Ghanem *et al.*, (2012) observed significant decrease in albumin level during early lactation than control group in dairy cows. Lower albumin level is due to malnutrition and can have liver disease or an inflammatory disease. Highest albumin level may be caused by acute infection, burns and stress.

Serum globulin (g/dl) concentration levels found in below 1 year heifer (T_1), above 1 year heifer (T_2), early lactating cow (T_3), mid lactating cow (T_4), late lactating cow (T_5), dry pregnant cow (T_6) and dry non pregnant cow (T_7) were 2.60 ± 0.14 , 3.22 ± 0.13 , 4.46 ± 0.19 , 4.41 ± 0.14 , 4.67 ± 0.12 , 4.85 ± 0.18 and 4.84 ± 0.20 , respectively. In present study globulin concentration was significantly higher in T_6 and at par with T_3 , T_4 , T_5 and T_7 while lowest globulin was observed in T_1 . Similar finding was reported by Mohamed (2014) who reported highly significant increase in globulin level in early lactation than mid lactation in dairy cows. An opposite pattern was observed by Ghanem *et al.*, (2012) who reported a significant decrease in globulin concentration in early lactation than in heifers.

Serum A:G ratio (g/dl) concentration levels found in below 1 year heifer (T_1), above 1 year heifer (T_2), early lactating cow (T_3), mid lactating cow (T_4), late lactating cow (T_5), dry pregnant cow (T_6) and dry non pregnant cow (T_7) were 1.36 ± 0.09 , 1.09 ± 0.06 , 0.76 ± 0.03 , 0.77 ± 0.03 , 0.76 ± 0.03 , 0.71 ± 0.04 and 0.73 ± 0.03 , respectively. In present study A: G ratio concentration was significantly higher in T_1 while lowest A:G ratio was observed in T_6 but at par with T_3 , T_4 , T_5 and T_7 . Similar finding was reported by Mohamed (2014) who reported a non-significant increase in A: G ratio in mid lactation than early lactation in dairy cows. Adedibu *et al.* (2013) reported non-significant increase in A: G ratio in non-lactating as compared with lactation in Friesian $\times$ Bunaji cows. The serum levels of proteins are indicators of hepatic functionality and decrease in their concentration may reflect fat infiltration in animals with high lip mobilization.

Mineral profile

Serum values of calcium, magnesium, phosphorous, copper, zinc and iron are given in Table 3.

Serum Calcium (mg/dl) concentration levels found in below 1 year heifer (T_1), above 1 year heifer (T_2), early lactating cow (T_3), mid lactating cow (T_4), late lactating cow (T_5), dry pregnant cow (T_6) and dry non pregnant cow (T_7) were 8.03 ± 0.12 , 8.01 ± 0.13 , 7.48 ± 0.16 , 7.71 ± 0.10 , 7.60 ± 0.12 , 7.51 ± 0.14 and 7.40 ± 0.16 , respectively. In present

Table 3: Serum mineral concentrations (mg/dl) in different stages of sahiwal cows.

Stages of animal	Below 1 year (T_1)	Above 1 year (T_2)	Early lactating (T_3)	Mid lactating (T_4)	Late lactating (T_5)	Dry pregnant (T_6)	Dry non-pregnant (T_7)	SEm ($\pm$)	CD at 5%	CV (%)
Calcium	8.03 ± 0.12^b	8.01 ± 0.13^b	7.48 ± 0.16^a	7.71 ± 0.10^{ab}	7.60 ± 0.12^a	7.51 ± 0.14^a	7.40 ± 0.16^a	0.133793	0.37811	5.510948
Magnesium	3.43 ± 0.18	2.88 ± 0.26	3.01 ± 0.38	3.73 ± 0.30	3.23 ± 0.24	3.15 ± 0.15	3.61 ± 0.50	0.307264	NS	29.53488
Phosphorus	7.72 ± 0.28^c	6.93 ± 0.26^{bc}	5.81 ± 0.76^{ab}	5.64 ± 0.30^a	5.27 ± 0.30^a	5.24 ± 0.32^a	5.19 ± 0.42^a	0.409288	1.156682	21.68342
Copper (ug/dl)	65.74 ± 3.27^c	60.17 ± 5.09^{bc}	52.87 ± 5.13^{ab}	60.62 ± 3.99^{bc}	55.33 ± 3.84^{abc}	67.03 ± 4.15^c	48.16 ± 3.49^a	4.192458	11.84822	22.63993
Iron (ug/dl)	129.67 ± 8.76	120.32 ± 4.26	108.24 ± 14.38	106.52 ± 7.65	121.46 ± 9.35	137.93 ± 9.61	118.88 ± 7.66	9.249583	NS	24.28759
Zinc (ug/dl)	154.80 ± 3.02^a	192.86 ± 9.03^b	162.46 ± 7.20^a	175.10 ± 13.30^{ab}	159.05 ± 5.55^a	157.86 ± 3.98^a	157.42 ± 5.10^a	7.475499	21.12636	14.27088

study calcium concentration was significantly higher in T_1 and at par with T_2 and T_4 while lowest calcium was observed in T_7 and at par with T_3 , T_4 , T_5 and T_6 . Heifer has slightly higher calcium percentage than lactating cows. Similar finding was reported by Naser *et al.*, (2014) who reported a significant increase in calcium concentration during mid-lactation than early lactation of dairy cows. Also Ghanem *et al.*, (2012) reported a significant decrease in serum calcium during early lactation in cattle than heifer which is also in support of our findings. Nehra (2016) reported that there was a significant decrease in calcium during early lactation which further increased during mid-lactation. Lower percentages of calcium in lactating cows may be due to milk production. In young animal there is involvement of skeletal growth but in older animal there is a decreased need of calcium for this purpose and this is why calcium in blood level of cows were lower than those of calves.

Serum magnesium (mg/dl) concentration levels found in below 1 year heifer (T_1), above 1 year heifer (T_2), early lactating cow (T_3), mid lactating cow (T_4), late lactating cow (T_5), dry pregnant cow (T_6) and dry non pregnant cow (T_7) were 3.43 ± 0.18 , 2.88 ± 0.26 , 3.01 ± 0.38 , 3.73 ± 0.30 , 3.23 ± 0.24 , 3.15 ± 0.15 and 3.61 ± 0.50 , respectively. In present study there was no significant difference observed in magnesium concentration. However, Magnesium concentration was higher in T_4 while lowest magnesium concentration was observed in T_2 . Similar finding was reported by Piccione *et al.*, (2012) who reported that magnesium concentration was non-significantly different in early lactation and dry period in dairy cows. Nehra (2016) reported a non-significant variation in serum Mg concentration during early lactation and mid lactation period. Hagawane *et al.*, (2009) reported that serum magnesium concentration of buffaloes did not differ significantly in various group i.e. early lactation mid lactation, late lactation and dry (pregnant).

Serum phosphorous (mg/dl) concentration levels found in below 1 year heifer (T_1), above 1 year heifer (T_2), early lactating cow (T_3), mid lactating cow (T_4), late lactating cow (T_5), dry pregnant cow (T_6) and dry non pregnant cow (T_7) were 7.72 ± 0.28 , 6.93 ± 0.26 , 5.81 ± 0.76 , 5.64 ± 0.30 , 5.27 ± 0.30 , 5.24 ± 0.32 and 5.19 ± 0.42 , respectively. In present study phosphorus concentration was significantly higher in T_1 and at par with T_2 while lowest phosphorus was observed in T_7 but at par with T_3 , T_4 , T_5 and T_6 . Also T_2 and T_3 were at par with each other. Similar pattern was found by Adebibu *et al.*, (2013) also reported that phosphorus increase significantly in lactation period than non-lactation period in Friesian×Bunaji crosses cows. Nehra (2016) reported that phosphorus increased significantly during early lactation than in mid lactation. Increased phosphorus concentration may be due to increase in milk production as more phosphorus from the ingested amount is transferred to milk and less is excreted with faeces (Valk *et al.*, 2002 Piccione *et al.*, 2012). There is increase in the phosphorus concentration during the early and mid-lactation may be due to the extra mineral supplementation to balanced rationed.

Serum copper (ug/dl) concentration levels found in below 1 year heifer (T_1), above 1 year heifer (T_2), early lactating cow (T_3), mid lactating cow (T_4), late lactating cow (T_5), dry pregnant cow (T_6) and dry non pregnant cow (T_7) were 65.74 ± 3.27 , 60.17 ± 5.09 , 52.87 ± 5.13 , 60.62 ± 3.99 , 55.33 ± 3.84 , 67.03 ± 4.15 and 48.16 ± 3.49 , respectively. In present study copper concentration was significantly higher in T_6 and at par with T_1 , T_2 , T_4 and T_5 while lowest copper was observed in T_7 and but at par with T_2 , T_3 and T_5 . Also T_2 , T_3 , T_4 and T_5 were at par with each other. Similar finding was reported by Muhammad *et al.*, (2014) who reported that repeat breeder buffalo has lower copper concentration than normal cyclic. Whereas opposite pattern was observed by Barui *et al.*, (2015) who observed non-significant ($p > 0.05$) variations of copper level between cyclic and repeat breeder cow. Copper has significant role in maintaining the optimum fertility as it behaves in a regular way as an indicator for FSH, LH and estrogen activity. Copper concentrations of aborting cows were significantly lower than recently calved cows so, it seems that copper deficiency has close relationship with abortion in cattle.

Serum iron (ug/dl) concentration levels found in below 1 year heifer (T_1), above 1 year heifer (T_2), early lactating cow (T_3), mid lactating cow (T_4), late lactating cow (T_5), dry pregnant cow (T_6) and dry non pregnant cow (T_7) were 129.67 ± 8.76 , 120.32 ± 4.26 , 108.24 ± 14.38 , 106.52 ± 7.65 , 121.46 ± 9.35 , 137.93 ± 9.61 and 118.88 ± 7.66 , respectively. In present study there was no significant difference observed in iron concentration. However, iron concentration was higher in T_6 while lowest amylase concentration was observed in T_4 . Similar finding was reported by Muhammad *et al.*, (2014) who reported that repeat breeder buffalo has lower iron concentration than normal cyclic. Iron functions in transport of oxygen to tissues maintenance of oxidative enzyme system and is concerned with ferritin formation. However lower level of serum iron results in anemia, which in turn affects reproduction adversely in the form of repeat breeding, requiring increased number of insemination per conception and occasionally leading to abortion.

Serum zinc (ug/dl) concentration levels found in below 1 year heifer (T_1), above 1 year heifer (T_2), early lactating cow (T_3), mid lactating cow (T_4), late lactating cow (T_5), dry pregnant cow (T_6) and dry non pregnant cow (T_7) were 154.80 ± 3.02 , 192.86 ± 9.03 , 162.46 ± 7.20 , 175.10 ± 13.30 , 159.05 ± 5.55 , 157.86 ± 3.98 and 157.42 ± 5.10 , respectively. In present study zinc concentration was significantly higher in T_2 and at par with T_4 while lowest zinc was observed in T_1 but at par with T_3 , T_4 , T_5 , T_6 and T_7 . Similar finding was reported by Muhammad *et al.*, (2014) who reported that repeat breeder buffalo has lower zinc concentration than normal cyclic. Also Barui *et al.*, (2015) reported highly significant variations of zinc level between cyclic and repeat breeder cow. Optimum level of zinc is essential to maintain the activity of FSH and LH and there by facilitates normal reproductive performance. Zinc deficiency may lead to reduction in GnRH secretion by hypothalamus and

eventually lead to decrease levels of luteinizing hormone and follicular hormone and arrest of ovulation. Zinc deficiencies have been associated with abortion, fetal mummification, lower birth weight and prolonged labor as Zn plays important role in uterine lining.

Enzymetic profile

Serum enzyme activities of ALT, AST, ALP and GGT are given in Table 4.

Serum ALP (U/L) concentration levels found in below 1 year heifer (T_1), above 1 year heifer (T_2), early lactating cow (T_3), mid lactating cow (T_4), late lactating cow (T_5), dry pregnant cow (T_6) and dry non pregnant cow (T_7) were 159.45 ± 8.92 , 156.68 ± 15.90 , 189.56 ± 16.59 , 225.25 ± 22.68 , 232.34 ± 34.38 , 221.37 ± 15.12 and 250.13 ± 24.73 , respectively. In present study ALP concentration was significantly higher in T_7 and at par with T_4 , T_5 and T_6 while lowest ALP was observed in T_1 and T_2 but at par with T_3 . Also T_3 , T_4 , T_5 and T_6 were at par with each other. ALP concentration is increases from early lactation to late lactation as lactation progresses. Similar finding was reported by Mohamed (2014) who reported that ALP value was increased highly significantly in mid lactation than early lactation of Friesian dairy cow. Also Nehra (2016) reported that significant increase for ALP with the progression/ advancement of lactation. Zaidan *et al.*, (2015) observed that ALP activity increased in lactation due to an increase in the production of iso enzyme from bone. From the study it is observed that ALP concentration has shown increase as the lactation progress this might be because of bone metabolism is more active in lactation periods.

Serum ALT (U/L) concentration levels found in below 1 year heifer (T_1), above 1 year heifer (T_2), early lactating cow (T_3), mid lactating cow (T_4), late lactating cow (T_5), dry pregnant cow (T_6) and dry non pregnant cow (T_7) were 23.24 ± 0.92 , 30.69 ± 2.12 , 30.32 ± 2.93 , 37.57 ± 2.90 , 33.47 ± 2.70 , 27.74 ± 1.77 and 31.96 ± 4.20 , respectively. In present study ALT concentration was significantly higher in T_4 and at par with T_2 , T_3 , T_5 and T_7 while lowest ALT was observed in T_1 and at par with T_2 , T_3 and T_6 . Also T_2 , T_3 , T_5 , T_6 and T_7 were at par with each other. Similar finding was reported by Stojevic *et al.*, (2005) who reported significant highest ALT activity during mid-lactation than in early lactation and dry period in Holstein cows. Also, Liu *et al.*, (2012) in Holstein cows reported significant increase in ALT level in mid lactation than the early lactation. The possible reason for this highest ALT activity in mid lactation may be the peak milk yield which creates a negative energy balance during mid-lactation.

Serum AST (U/L) concentration levels found in below 1 year heifer (T_1), above 1 year heifer (T_2), early lactating cow (T_3), mid lactating cow (T_4), late lactating cow (T_5), dry pregnant cow (T_6) and dry non pregnant cow (T_7) were 53.67 ± 2.30 , 57.49 ± 2.75 , 60.07 ± 5.00 , 58.92 ± 2.04 , 59.28 ± 3.81 , 56.31 ± 4.36 and 45.34 ± 2.45 , respectively. In present study AST concentration was significantly higher in

Table 4: Serum enzyme concentrations (U/L) in different stages of sahiwal cows.

Stages of animal Parameters	Below 1 year (T_1)	Above 1 year (T_2)	Early lactating (T_3)	Mid lactating (T_4)	Late lactating (T_5)	Dry pregnant (T_6)	Dry non- pregnant (T_7)	Sem ($\pm$)	CD at 5%	CV (%)
ALP	159.45 ± 8.92^a	156.68 ± 15.90^a	189.56 ± 16.59^{ab}	225.25 ± 22.68^{bc}	232.34 ± 34.38^{bc}	221.37 ± 15.12^{bc}	250.13 ± 24.73^c	21.19436	59.89695	32.69901
ALT	23.24 ± 0.92^a	30.69 ± 2.12^{abc}	30.32 ± 2.93^{abc}	37.57 ± 2.90^c	33.47 ± 2.70^{bc}	27.74 ± 1.77^{ab}	31.96 ± 4.20^{bc}	2.684632	7.586985	27.64207
AST	53.67 ± 2.30^{ab}	57.49 ± 2.75^b	60.07 ± 5.00^b	58.92 ± 2.04^b	59.28 ± 3.81^b	56.31 ± 4.36^b	45.34 ± 2.45^a	3.413286	9.64622	19.32026
GGT	20.66 ± 1.34	18.57 ± 1.63	23.05 ± 2.42	21.64 ± 2.28	22.04 ± 2.15	18.20 ± 1.03	17.08 ± 0.77	1.762811	NS	27.62935

T₃ and at par with T₁, T₂, T₄, T₅ and T₆ while lowest AST was observed in T₇ and at par with T₁. Similar finding was reported by Mohamed (2014) in Friesian dairy cows who observed highly significant increase in early lactation than mid lactation. Similarly, Stojevic *et al.*, (2005) also observed significantly higher value in early lactation than mid lactation in Holstein cows. Higher concentrations of AST in dairy cattle can be possibly associated with fatty liver syndrome and ketosis signs (Stojevic *et al.*, 2005; Ghanem *et al.*, 2012).

Serum GGT (U/L) concentration levels found in below 1 year heifer (T₁), above 1 year heifer (T₂), early lactating cow (T₃), mid lactating cow (T₄), late lactating cow (T₅), dry pregnant cow (T₆) and dry non pregnant cow (T₇) were 20.66±1.34, 18.57±1.63, 23.05±2.42, 21.64±2.28, 22.04±2.15, 18.20±1.03 and 17.08±0.77, respectively. In present study there was no significant difference observed in GGT concentration. However, GGT concentration was higher in T₃ while lowest GGT concentration was observed in T₇. Similar finding was reported by Mohamed (2014) who reported a no significant difference in GGT concentration in early lactation and mid lactation of Friesian dairy cows. However, Stojevic *et al.*, (2005) reported significant increase in early lactation than mid lactation in dairy cows. Nehra (2016) reported that GGT value was significantly increased during early lactation than that of control group. There was overall increase in GGT concentration during lactation than that of non- lactating stages which indicate that this increase might be due to the excessive stress in the animal body regarding milk production. And also might be due to combined effect of the some other factors like nutrition and physiology and hormonal effect *etc.*

CONCLUSION

In biochemical profile, significantly higher levels of serum cholesterol, glucose, triglyceride and uric acid were observed and non-significant changes were observed in serum amylase, bilirubin direct, total bilirubin, creatinine and urea in heifer and adult of sahiwal cows. Also in protein profile, significantly higher levels of serum total protein, globulin and albumin globulin ratio were observed, whereas non-significant change was observed in serum concentration of albumin. It is also observed that levels of serum calcium, phosphorus, copper, zinc were significantly higher in the heifer and adult Sahiwal cows. However, non-significant changes in concentration of magnesium and iron were observed. Therefore, it is concluded that blood profile of Sahiwal heifers and cow varies as per stage of the animal.

Conflict of interest: None.

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