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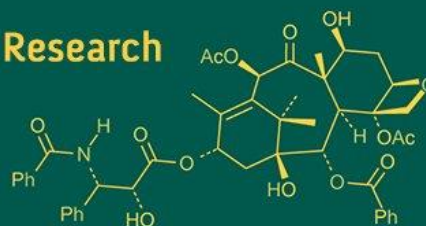
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Investigation on blood biochemicals and enzymes profile of Jaffarabadi buffalo calves

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Abstract
An attempt was made to investigate the profile of blood biochemicals and enzymes in Jaffarabadi buffalo calves. The study involved 24 calves and was conducted at the Cattle Breeding Farm, Kamdhenu University, Junagadh, following approval from the Institutional Animal Ethics Committee. Iso-managerial condition was ensured. Pelleted concentrate was offered to meet protein requirements feeding standards. A total 3 blood collections from each calf, at 8th, 91st and 182nd day of age were made. The blood biochemicals *viz.*, glucose, cholesterol, triglycerides, total protein, albumin and globulin, blood urea nitrogen (BUN), bilirubin, creatinine, insulin like growth factor (IGF-1): and the enzymes like lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and asparate amino-transferase (AST) and antioxidants like super-oxide dismutase (SOD) and catalase activity were measured using standard kit method. The alterations in the biochemicals and enzymes up to 6 months of age, early (day-8 to 91) and late (day-91 to 182) post-natal phases, were ascertained. Overall mean values of glucose, cholesterol, triglycerides, total protein and albumin were 74.56±1.83 mg/dL, 77.00±1.24 mg/dL, 29.37±0.52 mg/dL and 7.15±0.03 g/dL and 3.34±0.07, respectively. The levels of glucose, cholesterol, triglycerides, and albumin marginally, non-significantly declined by -8.75, -7.81, -14.90, -10.38 respectively; however, these were within the normal physiological range. Total protein and globulin levels increased by 1.90% and 11.84%, respectively. Serum urea nitrogen, bilirubin and creatinine averaged 13.84±0.20,0.17±0.01 and 1.17±0.03 mg/dL, the levels remained almost static and in normal range. The overall mean of the IGF-1 was 45.05±2.65 ng/ml. A highly significant ($p<0.001$) elevation of 45.11 and 33.39% was observed in serum IGF-1 level during 8 to 91 and 91 to 182 days, respectively, total rise being 93.56%. The enzyme levels lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and asparate amino-Transferase (AST) and antioxidant like super-oxide dismutase (SOD) non-significantly marginally varied but within the normal physiological range. Corresponding overall values were 411.97±2.04,137.20±1.38, 68.60±0.65 and 20.63±0.08 IU/L, respectively. The catalase activity concentration 51.04±0.27, 54.92±1.24 and 56.79±1.77ng/ml at the 8th, 91st and 182nd day of age, overall mean being 55.02±1.38 ng/ml. A highly significant differences (p≤0.001) were observed among all the age groups. An elevation of 7.59 and 3.41% was observed in serum catalyse level during 8 to 91 and 91 to 182 days, total rise being 11.26%, but was within the normal physiological range.

Keywords: Blood, biochemical, enzymes, Jaffarabadi breed, buffalo, calves

Introduction

In dairy bovine production, the period from birth to weaning in artificial rearing or entire suckling phase under natural method is the most critical, because it directly adds to cost with no financial return to the producer. In addition, feeding management during the early stage influences the body growth and development, function of the immune system and in turn to lifetime performance. Economical and efficient calf rearing refers to the maintaining overall health and well-being, which includes factors such as achieving desired body weight, body biometry with optimum growth rate and fulfilment of balanced feed & DM intake along with feed conversion ratio. Certain blood biochemical profile referred to the levels of different metabolites *viz.*, Glucose, Cholesterol, Triglycerides, Total Protein, Albumin and Globulin, Blood Urea Nitrogen (BUN), Bilirubin, Creatinine, Insulin like Growth Factor (IGF-1) are important in maintain health. The blood enzymes like Lactate Dehydrogenase (LDH), Alkaline Phosphatase (ALP) and Asparate Amino-Transferase (AST) and antioxidants like Super Oxide Dismutase (SOD) and Catalase activity also contribute to overall health and

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well-being. Normal blood haematological profile of Hb, PCV and RBC, WBC and Platelet counts reflect health status of growing animals. Some researchers (Hossain *et al*., 2012; Kumar *et al*., 2015; Singh *et al*., 2016; Dar *et al*., 2018: Singh *et al*., 2018; Omran *et al*., 2020; Varada *et al*., 2022; Sharma *et al*. 2023) [5, 7, 13, 1, 14, 9, 17, 11] have attempted to study alterations in blood biochemicals, enzymes and haematocrit of growing cattle and buffalo. Jaffarabadi buffalo native to south saurashtra agroclimatic region of Gujarat are famous for larger body size, heavy body weight and high milk fat content. There is a dearth of information on blood biochemicals, enzymes and haematocrit of this buffalo breed. Therefore, blood biochemicals, enzymes and haematocrit profile were investigated in Jaffarabadi buffalo calves during early and late post-natal phases.

Materials and Methods

An investigation was undertaken on blood biochemicals, enzymes and haematocrit profile of Jaffarabadi buffalo calves during early and late post-natal phases. The study involved 24 calves and was conducted at the Cattle Breeding Farm, Kamdhenu University, Junagadh, following approval from the Institutional Animal Ethics Committee (Protocol No. KU-JVCIAEC-LA-99-22).

The experimental period spanned from day 8 to 182 of age. The calves were selected based on birth weight, dam parity, previous and current average milk yield of the dam and calf sex, ensuring equal distribution (50:50 male and female calves) and maintained in iso-managerial condition. Pelleted concentrate was offered to meet protein requirements as per

feeding standards and mineral mixture @10-15 g/h/. Feed and fodder samples were periodically tested for proximate principles for meeting the dietary needs across two post-natal phases: early (2nd -13th week) and late (14th -26th week) stages.

The blood samples were collected within the specified period (Aug 2022 to July 2024) for the approved protocol and all the experimental animals were maintained as per protocol outline.

Blood Collection: Total 3 blood collections from each calf, at initiation (0 day) of experiment, at mid-experiment on 84th day and at the end of 175th day of the experiment. (At 8th, 91st and 182nd day of age), Approximately 5 ml whole blood was collected in vacutainer Na-EDTA vial from each calf in all the groups for haematological parameters (Plate 8). For separation of serum, 7 ml blood was collected in another tube without anticoagulant and kept in slanting position. The serum was separated from blood by centrifugation of blood samples at 2000 rpm for 10 minutes and stored in properly sterilized 2 ml plastic storage vials at -20°C in deep freezer until analysis.

Blood biochemical parameters: All the listed biochemical parameters (Table A) were estimated from serum samples using standard kits on automatic biochemistry analyzer (Dia-chem 240 PLUS, Diatek, China) at the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh.

Table A: Details of serum biochemical parameters and methods of estimation(N=24)

Sr. No.	Parameters	Unit	Method
1	Glucose	mg/dL	Standard Analysis Kits
2	Total Cholesterol	mg/dL	CHOD-POD
3	Triglyceride	mg/dL	GPO-POD
4	Total Protein	g/dL	Biuret
5	Albumin	g/dL	BCG
6	Globulin (Total Protein- Albumin)	g/dL	-
7	Total bilirubin	mg/dL	DC
8	Blood urea nitrogen	mg/dL	GLDH
9	Creatinine	mg/dL	Jaffe's kinetic
10	IGF-1	ng/ml	ELISA Kit

Blood enzymes parameters: Following serum levels for antioxidant activity and enzymes were determined using commercially standard analytical kits (Table B).

Table B: Particulars on Serum enzyme parameters and methods of estimation(N=24)

Sr. No.	Parameters	Unit	Method
1	Superoxide dismutase (SOD)activity	ng/ml	Marklund & Marklund (1974).
2	Catalase activity	ng/ml	Goth (1991)
3	Lactate dehydrogenase	IU/L	IFCC UV-Kinetic
4	Alkaline phosphatase	g/dL	IFCC
5	Aspartate aminotransferase	IU/L	UV-Kinetic

Statistical analysis: The data were subjected to analysis for mean ± standard error of means. (Snedecor & Cochran, 1994) [15]. Means were considered significantly different at p≤0.05, *i.e*., at 5% level of significance. The significance of the differences among the ages was assessed by Duncan's Multiple Range Test (Duncan, 1955) [2].

Results and Discussion
Profile of blood biochemicals of experimental Jaffarabadi buffalo calves

The profile of various blood biochemicals *viz*., Glucose, Cholesterol, Triglycerides, Total Protein, Albumin and Globulin, Blood Urea Nitrogen (BUN), Bilirubin, Creatinine, Insulin like Growth Factor (IGF-1) of Jaffarabadi buffalo calves was investigatedat the starting (8th day of age) and then again at the age of 91st and 182nd day. The data are presented in Table 1 and the trend is also illustrated in Figure 1 and 2.

Alteration (%) in levels of blood biochemical parameters of the growing Jaffarabadi calves is detailed in the Table 2.

Serum glucose (mg/dL) concentration: Mean glucose level of Jaffarabadi buffalo calves at the initiation of the experiment (8th day of age) was 77.64±1.23 mg/dL, while at the 91st day (mid of the experiment), also it was almost similar and higher, 77.57±2.96 mg/dL; while at the end 182nd day, the calves had a glucose level of 70.84±3.14

mg/dL indicating no significant (*p*>0.05) difference among the means of glucose level at ages. The overall mean serum glucose level across all periods was 74.56±1.83 mg/dL. Although a total of 8.75% reduction in the level occurred, greater being in 91 to 182 days (Table 2), all the values were in optimum limits, indicating proper management and health.

Present results with respect to values and trend of change of blood glucose are confirmed by the findings reported by Hossain *et al*. (2012) [5] in Kankrej calves, Noori *et al*. (2016) [8] in Holstein calves, Dar *et al*. (2017) [1] in crossbred calves, Sri Lekha *et al*. (2021) [16] in Murrah buffalo calves, Wang *et al*. (2021) [18] in Holstein calves, Raza *et al*. (2022) [10] in pre-weaning male Holstein-Friesian calves. These researchers have found varying results on values of blood glucose level, with respect to effect of age, sex, breeds and treatments or feeding /supplementation protocols.

Serum cholesterol (mg/dL) level: Average serum cholesterol (mg/dL) concentration in the Jaffarabadi buffalo calves was 79.97±1.69, 77.77±1.53, and 73.72±1.94 mg/dL, at the start (8th day of age), 91st and 182nd day of age, respectively, indicating a declining trend with an overall average being 77.00±1.24 mg/dL. There was no significant (*p*>0.05) difference in the serum cholesterol at different ages.

Triglycerides (mg/dL) level: At 91st and 182nd day of age, the triglyceride levels of the calves in the were 31.91±0.34 at the start, while the levels at 91st and 182nd day of age were slightly lower, 29.16±0.64 and 27.16±0.56 mg/dL, respectively; with overall mean value of 29.37±0.52 mg/dL. These differences among values at different age were not statistically significant (*p*>0.05). As evident from Table 2, although a total of 14.9% reduction occurred, all the values of triglyceride were in optimum limits, indicating proper management and health.

The findings of this study, with respect to values and trend of change of this blood biochemical, align with the similar results of El-Mehanna *et al*. (2017) [4] in lambs for serum cholesterol and Singer *et al*. (2023) [12] in Baladi goats for serum triglycerides.

Serum Protein (g/dL) concentrations: At the 8th, 91st and 182nd day of age, the calves had averages total protein level of 7.12±0.06, 7.17±0.05 and 7.25±0.09g/dL, respectively; overall mean value being 7.15±0.03 mg/dL. The age difference did not have a significant effect (*p*>0.05) on the serum total protein levels in the Jaffarabadi buffalo calves. Total protein level was maintained within the optimum range throughout the study period (Table 2).

Serum albumin (g/dL) levels varied between 3.09±0.09 and 3.48±0.10 at different ages, with overall mean of 3.34±0.07 g/dL. Similarly, serum globulin (g/dL) ranged from 3.63±0.10 to 4.06±0.15, overall average of 3.81±0.07g/dL. Serum albumen level reduced overall to the tune of 10.4% while serum globulin concentration increased by 11.8%, resulting in a static level of total protein (Table 2).

Present findings, with respect to values and trend of change of this blood biochemical, are confirmed by the results of Hossain *et al*. (2012) [5] in Kankrej calves, Duvvu *et al*. (2018) [3] and Sri Lekha *et al*. (2021) [16] and Sharma *et al*.

(2023) [11] in Murrah buffalo calves, Raza *et al*. (2022) [10] in Holstein-Friesian calves and Singer *et al*. (2023) [12] in goats.

Serum urea nitrogen (mg/dL) level: The mean serum urea nitrogen values of the calves were 14.26±0.21, 14.10±0.16 and 13.21±0.40 mg/dL at the 8th, 91st and 182nd day of age, respectively, with no significant difference (*p*>0.05) found amongst the groups during the experiment. The overall mean serum urea nitrogen value across all ages was 13.84±0.20 mg/dL.

Present findings are supported by similar results of Duvvu *et al*. (2018) [3] in Murrah buffalo calves and Raza *et al*. (2022) [10] in growing calves.

Serum bilirubin (mg/dL) concentration: The level serum bilirubin marginally varied from 0.15±0.00 to 0.19±0.01 with an overall mean of 0.17±0.01 and no statistically significant difference was observed in serum bilirubin levels amongst the age groups. A net reduction of -17.57% was observed during the study period; however, the values were in the normal range (Table 2).

Serum creatinine (mg/dL) level: At the initiation, 8th day of age, the average value for the control group was 1.29±0.05, while at 91st and 182nd day of age, serum creatinine (mg/dL) levels were lower and almost similar (1.11±0.07 and 1.12±0.03). There were no statistically significant differences found among the age groups. The calves recorded 1.17±0.03 overall 1.19 ± 0.04 mg/dL serum creatinine level. As evident from Table 2, a reduction of -14.51% was observed in creatinine level during the study period, however, the values were in normal range.

Present findings are in accordance with Dar *et al*. (2017) [1] in crossbred calves, who reported a non-significant (*p*>0.05) effect of supplementation on serum creatinine (mg/dl).

Serum insulin-like-growth Factor (IGF-1, ng/ml) levels: At the 8th day of age (initiation of the experiment), the IGF-1 concentration values was 30.81±0.63 ng/ml, at the 91st day of age, IGF-1 concentration values were 44.71±2.24 ng/ml and at the end of the experiment (on 182nd day of age), IGF-1 concentration values were 59.64±5.16 ng/ml. A highly significant differences (p≤0.001) were observed among all the age groups. It is well known that this biochemical is associated with glucose uptake by animal cells and contributes to energy metabolism in livestock. The overall mean of the observations was 45.05±2.65 ng/ml. An elevation of 45.11 and 33.39% were observed in serum IGF-1 level during 8 to 91 and 91 to 182 days, total rise being 93.56%. (Table 2).

The present result, with respect to values and trend of change of this blood biochemical, is supported by the findings of some of the earlier researchers. El-Mehanna *et al*. (2017) [4] experimented to assess the effect of synbiotic (lactic acid bacteria along with aqueous dandelion extract, a prebiotic) on Noemi male lambs and observed that IGF-I concentrations was significantly higher in synbiotic group of lambs than control group. Wang *et al*. (2022) [18], while supplementing Holstein calves with probiotics, did not find any significant change (*p*>0.05) in serum IGF-I concentrations (ng/ml).

Table 1: Means ± SE(s) of blood biochemical parameters of the Jaffarabadi calves at 8th, 91st and 182nd day of age (N=24)

Sr. No.	Blood biochemical parameters	Concentration estimated on			Overall
		Day- 8	Day-91	Day-182	
1	Serum glucose (mg/dL)	77.64±1.23	77.57±2.96	70.84±3.14	74.56±1.83
2	Serum cholesterol (mg/dL)	79.97±1.69	77.77±1.53	73.72±1.94	77.00±1.24
3	Serum triglycerides (mg/dL)	31.91±0.34	29.16±0.64	27.16±0.56	29.37±0.52
4	Serum total protein (g/dL)	7.12±0.06	7.17±0.05	7.25±0.09	7.15±0.03
5	Serum albumin (g/dL)	3.44±0.06	3.48±0.10	3.09±0.09	3.34±0.07
6	Serum globulin (g/dL)	3.63±0.10	3.65±0.07	4.06±0.15	3.81±0.07
7	Serum urea nitrogen (mg/dL)	14.26±0.21	14.10±0.16	13.21±0.40	13.84±0.20
8	Serum bilirubin (mg/dl)	0.19±0.01	0.17±0.01	0.15±0.00	0.17±0.01
9	Serum Creatinine (mg/dl)	1.29±0.05	1.12±0.03	1.11±0.07	1.17±0.03
10	IGF-1(ng/ml) Sign.($P < 0.01$)	30.81[a]±0.63	44.71[b]±2.24	59.64[c]±5.16	45.05±2.65

Table 2: Alteration (%) in levels of blood biochemical parameters of the growing Jaffarabadi calves

Sr. No.	Blood biochemical parameters	Alteration (%) during		
		Day- 8 to 91	Day-91 to 182	Day-8 to 182
1	Serum glucose (mg/dL)	-0.09	-8.67	-8.75
2	Serum cholesterol (mg/dL)	-2.74	-5.22	-7.81
3	Serum triglycerides (mg/dL)	-8.62	-6.88	-14.90
4	Serum total protein (g/dL)	0.70	1.19	1.90
5	Serum albumin (g/dL)	1.02	-11.29	-10.38
6	Serum globulin (g/dL)	0.48	11.30	11.84
7	Serum urea nitrogen (mg/dL)	-1.16	-6.28	-7.36
8	Serum bilirubin (mg/dl)	-9.46	-8.96	-17.57
9	Serum Creatinine (mg/dl)	-13.15	-1.56	-14.51
10	IGF-1 (ng/ml)	45.11	33.39	93.56
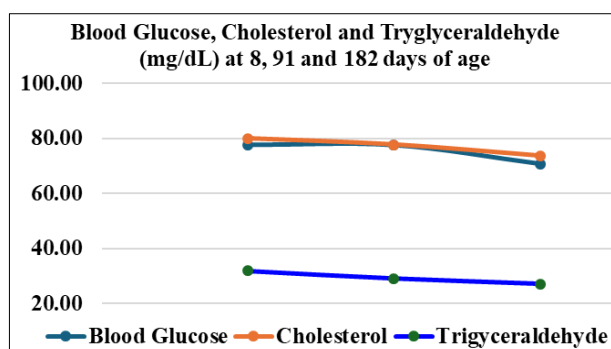
Fig 1: Blood glucose(mg/dL), cholesterol (mg/dL) and tryglyceraldehyde (mg/dL) profile in Jaffarabadi calves
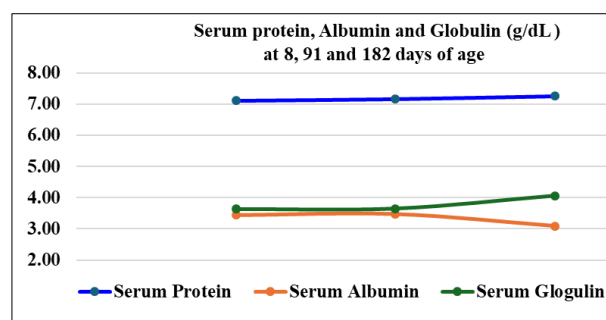
Fig 2: Serum protein(g/dL), Albumin(g/dL) and globulin(g/dL) profile in Jaffarabadi calves

Table 3: Means ± SE(s) of blood enzymes of the Jaffarabadi calves at 8th, 91st and 182nd day of age (N=24)

Sr. No.	Blood enzymes	Concentration estimated on			Overall
		Day- 8	Day-91	Day-182	
1	Lactate Dehydrogenase LDH (IU/L)	420.63±1.84	412.20±1.07	404.15±2.86	411.97±2.04
2	Alkaline Phosphatase ALP (IU/L)	139.26±0.79	137.41±2.14	134.94±2.09	137.20±1.38
3	Aspartate aminotransferase (AST, IU/L)	70.65±0.61	68.33±0.63	66.83±0.78	68.60±0.65
4	Superoxide dismutase (SOD) activity, (IU/L)	21.95±0.24	20.43±0.21	20.02±0.34	20.63±0.08
5	Catalase activity (ng/mL) Sign.($P < 0.01$)	51.04[a]±0.27	54.92[b]±1.24	56.79[c]±1.77	55.02±1.38

Table 4: Alteration (%) in levels of blood enzymes of the growing Jaffarabadi calves

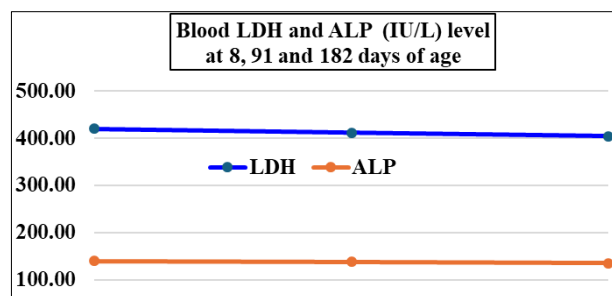| Sr. No.	Blood enzymes	Alteration (%) during		
		Day-8 to 91	Day- 91 to 182	Day-8 to 182
1	Lactate Dehydrogenase, LDH (IU/L)	-2.00	-1.95	-3.92
2	Alkaline Phosphatase, ALP (IU/L)	-1.33	-1.79	-3.10
3	Aspartate Aminotransferase (AST, IU/L)	-3.28	-2.20	-5.40
4	Superoxide dismutase, SOD, (IU/L)	-6.95	-1.98	-8.79
5	Catalase activity (ng/mL)	7.59	3.41	11.26

Fig 3: Lactate Dehydrogenase (LDH, IU/L)) and Alkaline Phosphatase (ALP, IU/L) profile in Jaffarabadi calves
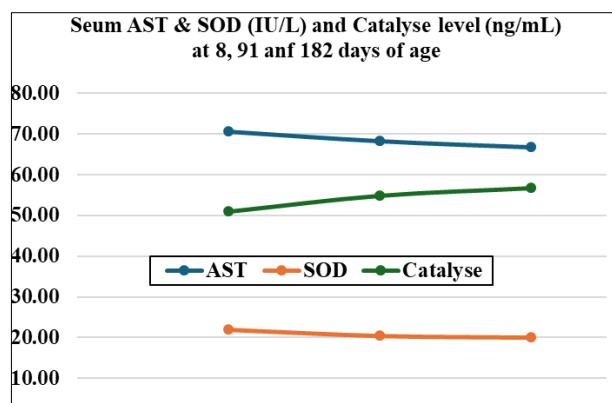

Fig 4: Asparate Amino-Transferase (AST, IU/L), Super Oxide Dismutase (SOD, IU/L) and Catalase activity (ng/mL) profile in Jaffarabadi calves

Profile of blood enzymes and antioxidant activity of experimental Jaffarabadi buffalo calves
Table 3 furnishes details on the information on the blood enzymes like Lactate Dehydrogenase (LDH), Alkaline Phosphatase (ALP) and Asparate Amino-Transferase (AST) and antioxidants like Super Oxide Dismutase (SOD) and Catalase activitymean of Lactate Dehydrogenase (LDH, IU/L) concentration of experimental Jaffarabadi buffalo calves measured at the start (day-8) and then at day-91st day and 182nd day of age. The trend of change of these enzymes and antioxidant activity are illustrated in Figure 3 and 4.
Alteration (%) in levels of blood enzymes and antioxidant activity of the growing Jaffarabadi calves is detailed in the Table 4.

Lactate dehydrogenase (LDH, IU/L) concentration: The mean lactate dehydrogenase values of the calves were 420.63±1.84, 412.20±1.07 and 404.15±2.86 IU/L at the 8th, 91st and 182nd day of age, respectively, with no significant difference ($p>0.05$) found amongst the groups during the study. This enzyme showed a declining trend, with almost similar magnitude, -2.00% in early and late phases. The overall mean serum urea nitrogen value across all ages was 411.97±2.04 IU/L (Table 4). The findings of Wang *et al.* (2022) [18] in Holstein calves supported the present results.

Alkaline phosphatase (ALP, IU/L) concentration: The blood alkaline phosphatase level marginally decreased from 139.26±0.79 IU/L at 8th day of age to 134.94±2.09 IU/Lat 182nd day of age, an overall mean being 137.20±1.38 IU/L and no statistically significant difference was observed in serum alkaline phosphatase levels amongst the age groups.

A net marginal reduction of -3.10% was observed during the study period; however, the values were in the normal range (Table 4).
The results of the present study align with those of Hossain *et al.* (2012) [5] in Kankrej calves, Wang *et al.* (2022) [18] in Holstein calves and Sharma *et al.* (2023) [11] in buffalo calves with respect to values and/or trend of change of this enzyme.

Aspartate Aminotransferase (AST, IU/L) level: Like ALP, the blood aspartate aminotransferase level also revealed a declining tendency from 70.65±0.61 IU/L at 8th day of age to 66.83±0.78 IU/Lat 182nd day of age, an overall mean being 68.60±0.65 IU/L.The means were at par in aspartate aminotransferase level amongst the age groups. A net marginal reduction of -5.40% was observed during the study period; however, the values were in the normal range (Table 4).
Confirming to present results, Hossain *et al.* (2012) [5] and Dar *et al.* (2017) [1] in crossbred calves, reported similar findings.

Superoxide dismutase (SOD, IU/L) Concentration: The level of superoxide dismutase marginally varied from 20.02±0.34 at the age of day 182nd to 21.95±0.24 at the age of day 8th, with an overall mean of 20.63±0.08 and no statistically significant difference was observed in serum bilirubin levels amongst the age groups. A net reduction of -8.791% was observed during the study period; however, the values were in the normal range (Table 4).

Catalase activity (ng/ml) Concentration: At the 8th day of age (start of the experiment), the catalase activity concentration values was 51.04±0.27 ng/ml, at the 91st day of age, Catalase activity concentration values were 54.92±1.24 ng/ml and at the end of the experiment (on 182nd day of age), Catalase activity concentration values were 56.79±1.77ng/ml. A highly significant differences ($p\leq0.001$) were observed among all the age groups. It is well known that this enzyme is associated with removal of harmful elements/ metabolites from the body of livestock resulting in improvement of health and well-being. The overall mean of the observations was 55.02±1.38 ng/ml. An elevation of 7.59 and 3.41% were observed in serum Catalase activity level during 8 to 91 and 91 to 182 days, total rise being 11.26%. (Table 4).
The results of the present study with respect to values and trend of change of superoxide dismutase and catalase activity concentration are conformed with those of Varada *et al.* (2022) [17] and Sharma *et al.* (2023) [11] in buffalo calves.

Conclusion
The overall results of the study tended to indicate that values of all the blood biochemicals and enzymes investigated in 24 Jaffarabadi buffalo calves were in the normal physiological range. Since only three collections, initially at 1week, at 3 month and 6 month of age was taken, as per approval of IAEC, the trend of change during 8 to 91 and 91 to 182 days furnished some idea regarding tendency of change in the blood profile. It may be inferred that, study of blood profile at monthly intervals and from still larger number of calves would furnish more concrete results.

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