

## Age Related Changes in Blood Biochemical and Hematological Profile of Buffalo in Calves

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### Abstract

The study was conducted to monitor hematological and biochemical changes in Murrah buffalo calves at monthly interval from one to six months of age. Hemoglobin, packed cell volume, total and differential leukocyte count, platelets, aspartate aminotransferase, gamma glutamyl transferase, serum total protein, serum albumin, serum urea nitrogen, plasma fibrinogen, potassium and chloride. Two groups of calves were identified with bimodal distribution in Hb, PCV, TLC and DLC values between 1-3 months of age. Significant age related changes were recorded for serum albumin, serum urea nitrogen, plasma fibrinogen and chloride. This study describes physiological limits of hematological and biochemical values in buffalo calves.

**Keywords:** Buffalo calves; Hematology; Biochemical profile; Fibrinogen; Leucocyte count

### Introduction

Hematological and biochemical variables are very important diagnostic aids in diseases diagnosis. Interpretation of the haemato biochemical variables require comparison with the reference values in healthy population. At present reference ranges for blood biochemical and hematological variables are available in adult animals and more specifically for cattle [1-3]. Although few studies are available on haemato biochemical profile in buffaloes [3,4] and still very few studies are available in pre ruminant buffalo calves [5,6]. So, at present no reliable reference range for biochemical variables is available for young buffalo calves. Above that, differences in method of analysis, geographic location, variation in climatic conditions, nutrition and husbandry practices also modify haemato biochemical profile of healthy animals. Therefore reference values must be established by a local analytical service to provide a more realistic reference values. The aim of this work to follow changes in blood variables and to establish reference ranges in buffalo calves which are commonly used by health specialists in diseases diagnosis

### Materials and Methods

#### Selection of animals

The study was undertaken on Murrah buffalo calves raised at an organised dairy farm. Buffalo calves with which did not develop any clinical illness were selected and complete physical examination was performed to finally select a calf for the study. The study was performed on ten buffalo calves

#### Animal management

The calves were removed from the dams immediately after birth and placed in individual calf pens till the age of 3 months. The calves received their dam's milk for the first 5 days of life and subsequently

pooled milk till 4 months of age at the rate of 10 percent of body weight. Feeding was done two times a day. The dam's first colostrum was hand-fed through pail. From 15 days of age, calves were offered calf starter and by 1 month of age they were offered chaffed green fodder ad-lib. Calves were dewormed by oral administration of fenbendazole (5 mg/kg BW) at 15 day and 2 months of age. They received primary vaccination against foot and mouth disease at 1 month and booster at 2 months of age. Body weight was recorded at birth, first and seventh month of age.

#### Hematological analysis

Blood sample was aseptically collected by jugular venipuncture at 1st, 2nd, 3rd, 4th, 5th and 6th month of age. Four millilitres of blood was collected in K2 EDTA coated vials for complete blood count and five millilitres of blood for harvesting serum. Haemoglobin, packed cell volume, platelets count and total leukocytes count (TLC) were estimated by fully Automatic Laser Based Hematology Analyser (ADVIA® 2120 Hematology system, Siemens Healthcare diagnostics Inc., USA). Differential leukocyte count (DLC) were determined on the Leishman stained blood by counting 100 cells as per Jain [7]. Absolute counts of all the leucocytes were also calculated. Fibrinogen estimation was estimated by heat precipitation method as per Schalm [8].

#### Biochemical analysis

Aspartate amino transferase (U/L), gamma glutamyl transferase (U/L) total protein (g/dL), albumin (g/dL), BUN (mg/dL), potassium (mmol/dL) and Chloride (mmol/dL) were estimated by fully automatic Vitros DT 350 Chemistry system (Ortho Clinical Diagnostics, Johnson & Johnson Company). Statistical analysis was conducted using SPSS 16.0. Age effect was determined using one way ANOVA.

### Results

**Haematological profile:** First three months of age.

Analysis of the haemograms of the individual calves revealed that TLC was high in four calves and comparatively low but within the accepted reference range of cattle in six calves (Table 1). The difference was apparent for first three months of age and it levelled of thereafter. The difference were significant at first and second month of life. This observation was unexpected as it has not been reported previously in buffalo calves. However, these results were in agreement with the findings of Knowles et al. [9] in cow calves that described bimodal distribution in TLC, Haemoglobin, PCV and TEC during first 27 days of life. Undoubtedly, high TLC were often attributed to some disease challenge. The difference in TLC counts appeared to be real because all the animals were without apparent clinical signs of diseases, vital signs were within the normal limits and also, no illness appeared during the period of experimentation. Moreover, daily body weight gain was 0.64 and 0.61 kg respectively in high TLC and low TLC further supporting our view point that buffalo calves with high TLC count were healthy. The difference between the two groups were also unlikely due to sampling or analytical error because the differences continued over three consecutive months and counts were also confirmed by manual method. The values of haemoglobin, PCV were significantly low in high TLC group of calves at first and second month of age.

Parameters	1 Month	2 Month	3 Month
Hb(g/dl)	9.69 ± 0.92	10.2 ± 0.38	10.7 ± 0.53
	(8.00-11.6)	(9.30-11.0)	(9.59-12.2)
PCV (%)	30.1 ± 1.58	30.3 ± 1.11	30.6 ± 1.55
	(25.8-33.4)	(28.0-32.7)	(27.9-35.0)
TLC 10 <sup>3</sup> /μl	23.0 ± 2.18	20.9 ± 1.46	16.6 ± 2.23
	(18.2-28.6)	(16.8-23.7)	(12.9-22.7)
Neutrophil 10 <sup>3</sup> /μl	12.2 ± 0.9	10.1 ± 1.61	5.14 ± 2.17
	(10.2-14.8)	(5.4-13.3)	(1.7-11.3)
Lymphocyte 10 <sup>3</sup> /μl	10.0 ± 1.4	10.0 ± 0.6	11.2 ± 1.4
	(7.28-13.2)	(10.0-12.7)	(8.4-15.5)
Platelet 10 <sup>3</sup> /μl	550 ± 67.0	626 ± 132.1	638 ± 33.1
	(456-748)	(375-858)	(540-679)

**Table 1:** Haematological values in high leucocyte count buffalo calves from 1-3 months (n=4).

**Haematological profile:** Three to six months of age.

Mean haemoglobin and PCV values are given in Table 2. There was a declining trend in mean Hb levels but without any significant difference. Wills also reported haemoglobin values in the similar range in buffalo calves [3]. However, Mohan recorded relatively higher values of haemoglobin in Indian Murrah buffalo calves during 4 to 6 months of age [5]. This difference was ascribed to the method of estimation (Sahli's method) in that the possibility of error is 15 percent. From these results, it was inferred that Hb values in buffalo calves were close to healthy adult buffaloes from 4 months of age. Values of haemoglobin were however, higher than reference range recorded in cow calves [10].

Mean PCV (%) varied from 31.2 to 31.8 without any significant difference with age was similar to observations of Canfield et al. in immature Australian Swamp buffaloes [11]. However, Wills and

Mohan observed higher PCV values but, without any significant change with advancing age in Indian Murrah buffalo calves [3,5]. This difference in PCV value from other studies attributed to the method of estimation.

Mean TLC (10<sup>3</sup>/μl) value varied 11.4 to 12.2 from 4-6 months of age. Mean TLC values in this study were comparable to Kumar et al. in buffalo calves [4]. However, lower TLC value has been reported by Mohan and Wills in buffalo calves [3,5]. The differences in values, of the present study from previous studies, could be ascribed to the methodological variation and or physiological leucocytosis.

Mean absolute neutrophil count showed a gradual decline though the difference was nonsignificant. No values for absolute neutrophil count was available in the studies on normal haematology in buffalo calves. The observed absolute neutrophil count in buffalo calves was in close proximity to reference values accepted in adult buffalo and cattle from months of age [3,12]. Mean absolute lymphocyte count showed an increasing trend from 4-6 months of age which was obviously due to falling neutrophil count, a physiological phenomenon associated with maturity in bovines. Mean absolute lymphocyte was similar to the reference range described in adult cattle [3]. No remarkable difference was appreciable in platelets count from 4 to 6 month of age. The PLT (10<sup>3</sup>/μl) count varied from 529 to 568. No study on platelets count in buffalo calves could be traced out in the scientific literature for comparison. However, Klinkon and Jezek recorded wider range (681-573 × 10<sup>3</sup>/μl) in platelets count in cow calves at 6-20 weeks of age [10]. Brun-Hansen observed platelet count in cow calves were above the reference range of adult cattle until 19-20 weeks of age [13].

Parameters	4 Month	5 Month	6 Month
Hb(g/dl)	11.6 ± 0.3	11.1 ± 0.2	11.0 ± 0.1
	(10.90-13.3)	(9.8-12.2)	(10.0-12.1)
PCV (%)	31.2 ± 0.6	30.4 ± 0.5	30.41 ± 0.4
	(29.1-35.5)	(27.0-33.2)	(30-33.2)
TLC (10 <sup>3</sup> /μl)	12.19 ± 0.12 <sup>c</sup>	12.16 ± 0.8 <sup>c</sup>	11.36 ± 0.6 <sup>c</sup>
	(9590-22720)	(8640-18200)	(8400-15100)
Neutrophil (10 <sup>3</sup> /μl)	4.52 ± 0.1 <sup>bc</sup>	4.05 ± 0.4 <sup>bc</sup>	3.26 ± 0.5 <sup>c</sup>
	(2260-12723)	(2175-6732)	1782 -6240
Lymphocyte (10 <sup>3</sup> /μl)	7.44 ± 0.5 <sup>c</sup>	8.10 ± 0.9 <sup>bc</sup>	8.10 ± 0.5 <sup>bc</sup>
	(4208-9996)	(3787-14924)	(4160-10224)
Platelet(10 <sup>3</sup> /μl)	529 ± 66.0 <sup>ab</sup>	538 ± 87.3 <sup>ab</sup>	568 ± 45.8 <sup>ab</sup>
	(309-880)	(176-880)	(442-854)

**Table 2:** Haematological values in high leucocyte count buffalo calves from 1-3 months (n=4).

**Biochemical profile:** Mean serum AST activity is given in Table 3. There was no significant change in mean AST activity with advancing age. However, a rising trend was appreciable after the 3rd month of age. Individual variability in AST activity was high during early 2 months of age. Very few studies on the plasma/serum activity of AST could be traced in buffalo calves. In the present study serum AST activity was higher than Kumar and Rattan [6]. However, Canfield et al. recorded higher mean values in immature male and female

Australian Swamp buffaloes [11]. Mean serum AST activity was also higher in buffalo calves in present study as compared to the values reported in cow calves [10,14]. In the present study, serum AST activity was higher than reference range in adult cattle [1,15]. However, previous studies showed conflicting findings between calves and adults. Mohri et al. observed AST activity in neonatal calves was within adult reference range up to 56 days of age followed by a rise [14]. On the other hand activity was similar in calves and adult cattle [15]. An increasing trend, in serum AST activity, recorded in this study was similar to observation of Klinkon and Jezek and Mohri et al. [10,14]. The differences in values from other studies was attributed to method of estimation, species, breed or geographic regions and/or rearing system.

Very few studies are available on the serum GGT activity in healthy buffalo calves. Our results were similar to Puri who recorded GGT activity in buffalo calves of an organised dairy farm [12]. However, much lower activity of serum GGT was recorded in immature Australian Swamp buffaloes and adult cattle [1,11]. The activity of GGT was in wider range as had earlier also been reported in cow calves by Klinkon and Jezek [10]. The difference in values from other studies was attributed to method of estimation. Mean serum TP over first six months of age was  $6.15 \pm 0.50$  (g/dL). Current values were similar to the study of Canfield et al. [11] in immature Australian Swamp buffaloes and of Klinkon and Jezek [10] in cow calves in the similar age group. No Significant changes were observed during first 6 months of life. However, total serum protein concentrations in present study were lower than the reference values of adult buffaloes and cattle as had earlier been recorded by Canfield et al [11], Kaneko [1], Kraft and Durr [16] and Puri [12]. Mean of serum albumin level over first six months of age was  $2.25 \pm 0.04$  (g/dL). Higher ( $P < 0.05$ ). Mean serum albumin level was observed at 3rd and 4th month of age. Our results were similar to Zanker et al. who reported albumin level above the reference range after 60 days of age [17]. Mohri et al. observed a significant increase from birth to 84 days of age [14]. However, higher

albumin levels have been reported by Puri [12] in buffalo calves and Klinkon and Jezek [10] in cow calves. Mean of plasma fibrinogen level was  $771.6 \pm 10.6$  (mg/dL) during first six months of age. Plasma fibrinogen levels increased gradually and increase was significant after 3 months of age as has been commented by Wills [3]. Values recorded in the present study were lower than that of Nangia et al. [18] and higher than that of Khan et al. [19]. Present values were comparable to the study of Puri [12] in Indian Murrah buffalo calves and to the report of Mc sherry in cow calves. Mean SUN levels (mg/dL) were  $16.8 \pm 1.01$  during first 6 months of age. Mean values of SUN were comparable to Puri [12]. There was gradual rise in BUN levels and increase was significant after 3 months of age as had earlier been documented by different workers in cow calves [9,10,14]. Serum urea levels were below the adult reference range at first month of life given by Kaneko [1]. Age related increase in the SUN levels may be attributed to change in dietary protein intake.

Mean serum potassium concentration was  $5.34 \pm 0.07$  (mmol/L) during first six months of life. There was no significant changes in potassium levels over first six months of life. Our results were similar to Canfield et al. [11] in immature Australian Swamp buffalo calves Maach et al. [20] in cow calves. However, higher levels of potassium were reported by Puri [12] in buffalo calves and Klinkon and Jezek [10] in cow calves. Serum potassium levels in the present study was similar to adult reference range [1]. Mean serum chloride concentration was  $97.8 \pm 1.24$  (mmol/L) over first six months of life.

A gradual increase in serum chloride was appreciable from first to six months of life. This increase was significant ( $P < 0.05$ ) from 4th month of age. Our results were similar to Reece [21] and Klinkon and Jezek [10] who also, reported that the concentration of Cl increased with age in cow calves. In present study the levels were also similar to that of adult cattle Kaneko [1]. The results of present study suggested that serum chloride level attained adult reference range by 5th month of age.

Parameters	1 month	2 month	3 month	4 month	5 month	6 month
AST(U/L)	$118.7 \pm 13.4^a$	$112.3 \pm 7.3^a$	$118.8 \pm 4.6^a$	$120.3 \pm 5.5^a$	$123.5 \pm 3.2^a$	$120.9 \pm 1.7^a$
	(75-224)	(82-159)	(105-157)	(100-153)	(107-137)	(114-130)
GGT (U/L)	$31.0 \pm 3.4^a$	$35.1 \pm 6.7^a$	$28.6 \pm 3.2^a$	$32.9 \pm 4.8^a$	$36.7 \pm 3.7^a$	$34.7 \pm 2.8^a$
	(17-50)	(18-90)	(18-53)	(16-63)	(16-60)	(18-50)
TP (g/dL)	$6.0 \pm 0.14^a$	$6.2 \pm 0.14^a$	$6.1 \pm 0.13^a$	$6.0 \pm 0.06^a$	$6.3 \pm 0.10^a$	$6.1 \pm 0.12^a$
	(5.2-7.0)	(5.8-7.0)	(5.2-6.8)	(5.8-6.4)	(5.8-6.8)	(5.4-6.8)
ALB (g/dL)	$2.0 \pm 0.05^c$	$2.0 \pm 0.03^c$	$2.5 \pm 0.11^a$	$2.4 \pm 0.12^{ab}$	$2.2 \pm 0.08^{bc}$	$2.2 \pm 0.08^{bc}$
	(1.7-2.3)	(1.9-2.2)	(2.0-3.4)	(2.1-3.3)	(2.0-2.9)	(2-2.9)
Fibrinogen (mg/dL)	$710 \pm 24.9^b$	$760 \pm 22.1^{ab}$	$770 \pm 30.0^{ab}$	$790 \pm 27.6^a$	$800 \pm 25.8^a$	$800 \pm 21.8^a$
	(600-800)	(600-800)	(600-900)	(600-900)	(700-900)	(700-900)
BUN (mg/dL)	$10.1 \pm 0.98^c$	$12.2 \pm 1.5^c$	$15.1 \pm 1.6^{bc}$	$24.5 \pm 3.3^a$	$18.5 \pm 1.7^b$	$20.7 \pm 1.8^{ab}$
	(5-15)	(4-23)	(10-28)	(13-50)	(15-32)	(13-34)
K (mmol/dL)	$5.3 \pm 0.23^a$	$5.6 \pm 0.28^a$	$5.5 \pm 0.13^a$	$5.2 \pm 0.13^a$	$5.2 \pm 0.16^a$	$5.2 \pm 0.18^a$
	(4.6-7.2)	(4.6-7.3)	(4.8-6.3)	(4.3-5.8)	(4.1-5.8)	(4.2-6.0)

Cl (mmol/dL)	87 ± 0.99 <sup>b</sup>	94.1 ± 5.0 <sup>b</sup>	92.6 ± 1.8 <sup>b</sup>	102.2 ± 1.5 <sup>a</sup>	103.9 ± 0.67 <sup>a</sup>	104.3 ± 0.61 <sup>a</sup>
	(81-91)	(84-139)	(88-104)	(89-107)	(100-108)	(100-106)

**Table 3:** Biochemical profile of buffalo calves from 1-6 months of age (n=10).

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