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Comparative Study on Reference Values for Blood Constituents during Pregnancy in Buffaloes (*Bubalus bubalis*)

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Abstract

Reference values for buffaloes especially those at pregnancy are not yet established. The aim of this study was to establish serum biochemical and hematological reference values for water buffaloes (*Bubalus bubalis*) during pregnancy. In total 409 pregnant buffaloes were examined at buffaloes' farms that belong to Assiut Governorate at the mid of Egypt. Out of them, 107 buffaloes did not meet the selection criteria and were excluded from the study. The remained 302 clinically healthy buffaloes were classified according to the stage of pregnancy into two groups: Group I; included buffaloes till 6 months of pregnancy (No.=146). Group II; included buffaloes after 6 months of pregnancy (No.=156). Three types of samples were collected; serum samples for biochemical analysis, whole blood samples for hematological analysis and fecal samples for parasitological examination. A total of 55 blood variables were measured during this study. The 95% reference intervals for each serum biochemical and hematological constituents were calculated by removing the upper and lower 2.5% of the interval to give the 2.5 and 97.5 percentiles. The present study established the reference intervals for the investigated biochemical and hematological parameters in blood of pregnant buffaloes. Results revealed that most of the measured blood constituents were differed significantly during the period before and after 6 months of pregnancy in buffaloes. In conclusion, the established reference values will be a useful guide for interpreting serum biochemical and hematologic data in pregnant buffaloes.

Keywords: Serum; hematology; buffalo; pregnant; reference values

Introduction

The buffalo (*Bubalus bubalis*) originally Asian animals and distributed mainly in tropical and subtropical Asia. The buffaloes are used for drought power and are found in countries like the Indian sub-continent and the Mediterranean countries (Cockril, 1980). The water buffalo can surpass the cattle genus Bos in its ability to adapt to the hot climates and swampy lands (Webster and Wilson, 1980); therefore, water buffaloes have special importance in milk and meat production in the valley of the River Nile in Egypt (GOVS, 2005).

Both clinical examination and various laboratory diagnostic tests are required for diagnosis of diseases . The major part of the laboratory diagnostic tests is the measurement of serum biochemical and hematological variables that are used to establish normality, to diagnose diseases and physiological alterations (Theodossi *et al*., 1981; Klinkhoff *et al*., 1988; Bailey *et al*., 1989; Pattinson and Theron, 1989). Textbook reference intervals produced by European or United States Veterinary Laboratories are often based on animals living under good husbandry conditions in temperate climates. However, those reference sample groups may differ from those of the developing countries. Differences may be attributed to the environmental temperature, the type and quantity of the ration and the management system (Pritchard *et al*., 2009). Published data propose erratic normal values that are often obtained from a relatively small number of animals, with different nutritional and climatic conditions, which makes it difficult to depend on these published data to interpret results for buffaloes live in Egypt. Reference values are not yet established for the water buffaloes (*Bubalus bubalis*). Therefore, the current study was carried

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out to establish reference values for hematological and serum biochemical constituents during pregnancy in buffaloes.

Materials and methods

Animals

Buffaloes (3-8 years old), were examined at buffaloes' farms (Land of Kheir buffaloes farm at Abnoub city, buffaloes farm at Valley of Sheeh, El-badary city and Bani Sanad buffaloes farm at El-hawatka), that belong to Assiut Governorate, at the mid of Egypt. The study was carried out during the period from August 2011 till June 2012.

Animals were examined carefully and inspected for presence of any abnormal clinical signs. Pregnancy was confirmed by rectal palpation. Only animals that met the selection criteria (Table 1) were included in the study. Pregnant buffaloes were kept together under open half shelter system. Ration received by buffaloes during the study were mixture of silage, hay, roughages, concentrates, and Egyptian clover (*Trifolium alexandrinum*). Water was supplied ad libitum.

Table 1. Selection criteria for the investigated animals

Selection criteria
Clinically healthy buffaloes
None lactating
Pregnant
Good body condition score
General attitude: alert
No loss of skin elasticity
Normal mucous membrane: pink
No diarrhea in previous 7 days
No urogenital abnormalities in previous 7 days
No muscular abnormalities in previous 7 days
No medication in previous 7 days
Absence of skin lesions or alopecia.
Absence of intestinal and blood parasites.

In total 409 pregnant buffaloes were examined. Out of them, 107 buffaloes did not meet the selection criteria described in Table 1, and excluded from the study. The remained 302 animals were clinically healthy, fit with the selection criteria and included in the study. Pregnant buffaloes were classified according to the stage of pregnancy into two groups: Group I; included buffaloes till 6 months of pregnancy (No.=146). Group II; included buffaloes after 6 months of pregnancy (No.=156).

The ear tag number of the individual animal in the farm was recorded in examination sheet. Another serial number was assigned for each individual animal. Tubes used for collection of blood, and cups used for fecal samples were assigned the same serial numbers that was recorded on the examination sheets.

Samples

Samples were collected at 8.00 am prior to feeding. Two blood samples were collected from the jugular vein into vacutainer tubes from all buffaloes under the study; the first blood sample was collected in plain vacutainer tube (10 ml plain vacuum tubes, Biomedica Alex Co., Egypt) and used for obtaining serum. The second blood sample was collected in vacutainer tube (Becton Dickinson vacutainer Tubes, Rutherford, NJ) containing EDTA as anticoagulant and used for hematological analysis. Fecal samples were collected from the rectum of all animals in clean and dry cups. Samples were transported in ice tank within 1- 2 hrs from collection to the research laboratory at Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, Egypt.

Samples were prepared (blood serum) or analyzed (whole blood and fecal samples) directly after receiving them by the research laboratory. Blood samples in plain tubes were centrifuged at 3000 rpm for 15 minutes, and then serum was collected according to standard methods of hematology (Coles, 1986). Serum samples were divided into 4 equal parts in eppendorf tubes, and then stored at -20°C. Samples showing hemolysis were excluded from the study. Serum samples kept in deep freeze were analyzed within a maximum period of two weeks.

Biochemical analysis

Serum biochemical variables were measured using UV spectrophotometer (Optizen 3220 UV, Mecasys Co. Ltd, Korea), reagents and chemicals were supplied with the purchased commercial kits, different methods used for analysis of different serum biochemical variables were summarized in Table 2. Biochemical analysis included measurements of serum total proteins, albumin, globulins, total cholesterol, triglycerides, high density lipoprotein (HDL-C), low density lipoprotein (LDL-C), very low density lipoprotein (VLDL-C), calcium, magnesium, chloride, phosphorus, iron,

total iron binding capacity (TIBC), Unsaturated iron binding capacity (UIBC), sodium, potassium, zinc, copper, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), creatine phosphokinase (CK), blood urea nitrogen (BUN), creatinine, total bilirubin, direct bilirubin and indirect bilirubin levels.

Serum protein electrophoresis

Serum protein electrophoresis was carried out by using cellulose acetate electrophoresis kit (Biotec-Fischer GmbH, Germany) and by Electrophoresis Set (Filipo, Biotec-Fischer GmbH, Germany). Electrophoretic bands were analyzed using Un-Scan-It version 6.1 (Silk Scientific Corporation, USA).

Hematological analysis

Blood film

Air dried smear of fresh blood was prepared directly after collection, fixed and stained with Giemsa stain (Coles, 1986), and then examined for blood parasites and for differential leucocytes counts. Manual differential leucocytes counts were performed to calculate the relative and absolute counts for individual granulocytes (Neutrophils, band cells, eosinophils and basophils), this because, Medonic electronic blood cells counter produced one relative and absolute counts for all granulocytes.

Hematological examination

Hematological examination was performed directly after the samples being received by the research laboratory and within 1-2hrs from collection of blood and by using Medonic Veterinary Hematology analyzer (Medonic CA 620, Sweden). The measured hematological analytes were total red blood cells count (T.RBCs), hemoglobin concentration (HGB), red blood cells distribution width (RDW), red blood cells distribution width absolute (RDWa), hematocrit (HCT), main corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets count (PLT), mean platelets volume (MPV), platelets distribution width (PDW), large platelets concentration ratio (LPCR), plateletcrit (PCT), total white blood cells count (T.WBCs) and total count and percentage of lymphocytes, neutrophils, band cell, eosinophils, monocytes, basophils.

Parasitological analysis

Parasitological analyses of fecal samples were done on the same day of collection using sedimentation and floatation techniques according to Soulsby (1982). Animals that harbored parasites were excluded from the study. The parasitological findings were reported to the farm to treat animals and to take recommended control measures.

Data Analysis

Data analysis was carried out according to the approved recommendations of International Federation of Clinical Chemistry on the theory of reference values (Solberg, 1987). Statistical analysis was performed using Reference Value advisor version 2.1 (Geffré *et al*., 2011). Reference intervals were determined using the non-parametric method. Outliers were determined using Dixon–Reed's and Tukey's tests and removed (Reed *et a*l., 1971). Data were tested for normal distribution according to Anderson and Darling (1954). The 95% reference intervals were calculated by removing the upper and lower 2.5% of the range for each serum biochemical and hematological constituents to give the 2.5 and 97.5 percentiles (Solberg, 1987). Data obtained for hematological and serum biochemical variables from group I (less than 6 months of pregnancy) and group II (more than 6 months of pregnancy) were compared by ANOVA using statistical software SPSS 13.0 for windows (SPSS, Chicago, USA).

Results

Reference intervals for body temperature were 38.24±0.47 ◦C and 37.26-39.09 ◦C respectively for group I, and 38.50±0.33 ◦C and 37.80-39.32 ◦C respectively, for group II.

As shown in Table 3, there were no significant changes between group I and group II in serum total proteins and albumin. Serum globulins (44.4±9.8 g/l) in group II was significantly

Table 2. Method used to measure the serum biochemical variables

Analytes	Methods	Source of Commercial kits
Total proteins	Biuret colorimetric method	Spinreact, GIRONA, Spain
albumin	Bromcresol green colorimetric method	
Total cholesterol	CHOD-POD. Enzymatic colorimetric	
Triglyceride	GPO-POD. Enzymatic colorimetric	
High density lipoprotein	HDL, precipitating method	
Low density lipoprotein	LDL, Enzymatic colorimetric. Liquid method	
Glucose	Glucose Oxidase-peroxidase enzymatic	
Calcium	o-Cresolphtalein. Colorimetric	
Magnesium	Xylidyl Blue. Colorimetric	
Chloride	Thiocyanate-Hg colorimetric	
Phosphorus	Method with molibdenium	Emapol, Gdansk, Poland
Iron	AMSFe1 Colorimetric	AMS International (AMS, UK. Ltd 197
Total iron binding capacity	TIBC, AMSTIBC colorimetric	
Sodium	Uranylthioglycolate Method	Spectrum Diagnostic, Cairo-Egypt
Potassium	Tetraphenylborate Method	
Zinc	5-Br-PAPS method	Centronic GmbH (Wartenberg, Germany)
Copper	3,5-Dibrom PAESA method	
Aspartate aminotransferase	IFCC Enzymatic – UV method	Spinreact, GIRONA, Spain
Alanine aminotransferase	IFCC Enzymatic – UV method	
Gamma glutamyl transferase	Carboxy substrate Kinetic method	
Lactate dehydrogenase	DGKC Kinetic – UV method	
Alkaline phosphatase	DGKC Kinetic optimized method	
Creatine phosphokinase	NAC Kinetic-UV method	
Blood urea nitrogen	Urease-GLDH Kinetic method	
Creatinine	Jaffé Colorimetric-Kinetic method	
Total bilirubin	DMSO - Colorimetric method	
Direct bilirubin	DMSO - Colorimetric method	

(P<0.05) higher than group I, based on colorimetric method. Electrophoretic measurement of serum protein fractions revealed significant decreases (P<0.05) in serum α-globulins and β- globulins (10.8±2.4 and 3.7±1.9 g/l, respectively) for group II compared with their levels in group I (11.6±3.6 and 4.3±2.1 g/l, respectively).

The mean AST value for group I (61.27±19.84 U/l) was significantly higher (P<0.01) than its mean value for group II (55.9±15.85 U/l). Serum ALT from the investigated buffaloes showed a significant increase (P<0.05) in group I (27.63±10.1 U/l), when compared with its level in group II (25.37±9.48 U/l). There was a significant decrease in serum ALP level in group II (155.89±61.77 U/l) when compared with its level in group I (170.74±58.33 U/l). Serum CK and GGT levels in group II was significantly higher (p<0.01) than group I. There was no significant changes in LDH level during pregnancy (Table 4).

Comparing data from group I with group II revealed that serum calcium (P<0.01), chloride (P<0.01), iron (P<0.05) and zinc (P<0.01) levels were significantly higher in group I than their serum levels in group II. On the other hand, there were significant increases in serum phosphorus (P<0.01), magnesium (P<0.05), potassium (P<0.01) and copper (P<0.05) levels in group II compared with group I (Table 5).

Results revealed significant decreases (P<0.01) in serum total cholesterol, HDL-C, LDL-C, glucose and BUN in group II when compared with group I. However, serum creatinine level was significantly higher (P<0.05) in group II (Table 6).

There were significant decreases (P<0.01) in total RBCs count, HGB, HCT and RDW in group II compared to group I. However, MCH and RDWa were significantly higher (P<0.01) in group II than their values in group I. Platelets count and PCT were significantly decreased (P<0.01) in group II (158.4±46.1 $x10^9$/l and 0.10±0.03%, respectively) compared with group I (192.0±41.9 $x10^9$/l and 0.12±0.03%, respectively). On the other hand, PDW and LPCR in group II were significantly higher (P<0.01) than their levels in group I.

Discussion

The International Federation of Clinical Chemistry sets out clear guidelines for the production of ref-

Table 3. Reference values for serum proteins measured both by spectrophotometer and electrophoresis in pregnant buffaloes

		Group I Less than 6 months of Pregnancy		Group II More than 6 months of Pregnancy	
		Mean ± SD	Reference interval	Mean ± SD	Reference interval
Spectrophotometer					
	Total proteins (g/l)	78.2±13.2	60.8-108.8	79.8±10.2	61.8-103.0
	Albumin (g/l)	36.4±6.9	22.8-51.2	35.4±6.1	23.9-46.9
	Globulins (g/l)	41.7±12.2	19.9-69.0	44.4±9.8*	26.4-66.5
	A/G ratio	9.8±4.3	3.9-22.2	8.5±2.7**	3.9-15.2
Protein Electrophoresis					
	Albumin (g/l)	38.3±8.6	24.6-56.4	40.8±7.7**	27.6-57.8
	Total Globulins (g/l)	38.6±11.2	21.4-65.7	39.0±7.2	24.9-53.1
	α-Globulins (g/l)	11.6±3.6	6.2-19.5	10.8±2.4*	6.8-16.4
	β- Globulins (g/l)	4.3±2.1	1.2-10.4	3.7±1.9*	0.8-8.0
	γ- Globulins (g/l)	22.8±8.0	8.1-40.4	24.5±6.0*	12.4-36.5

Reference interval = 2.5 and 97.5 percentiles; reference interval = interval between, and including, two reference limits. Reference intervals were calculated for each reference limit as recommended by PetitClerc and Solberg (1987). *: Significant (P<0.05), **: Highly significant (P<0.01)

erence values and limits. They recommended at least 120 animals being used for establishing the reference values (Grasbeck *et al*., 1979). This study used and carefully selected a relatively large reference population of 302 healthy animals (146 for group I and 156 for group II), which is higher than the number of animals recommended for establishing the reference values (Lumsden and Mullen, 1978; Grasbeck *et al*., 1979; Lumsden and Jacobs 1989; Farver, 1997; Solberg, 1999; Geffré *et al*., 2009). Buffaloes (*Bubalus bubalis*) subjected to study were reared in farms to ensure that they received periodical clinical examination, and their productive and reproductive status were regularly checked and recorded. Also, the physiological condition of the reference sample population was defined and reference intervals were calculated as 0.025 and 0.975 fractiles with 90% confidence intervals for the limits. It is well known that there are profound physiological changes in hematological and serum biochemical constituents in pregnant buffaloes. These changes are not necessarily indicative of disease but reflect physiological variations. Pregnant buffaloes included in the present study were selected precisely based on the established selection criteria stated in Table 1.

In the present study, mean values and reference intervals for body temperature were 38.24±0.47 ◦C and 37.26-39.09 ◦C respectively for group I, and 38.50±0.33 ◦C and 37.80-39.32 ◦C respectively, for group II. Results revealed that body temperature were significantly higher (P<0.05) in buffaloes after 6 months of pregnancy than buffaloes before 6 months of pregnancy, which may be attributed to increased metabolism at late pregnancy with increasing size of fetus. Generally, the observed body temperature agreed with that reported by FAO (1994). The results also were in-accordance with values reported by Radostits *et al*. (2006).

Reference intervals for serum total proteins and fractions were shown in Table 3. The significant increase in colorimetric value of serum globulins levels in group II may be attributed to the significant elevation of γ- globulins (24.5±6.0 g/l) levels. Quayam *et al*. (1990) reported that serum total proteins at 60 days prepartum was ranged from 91.20-93.70 g/l, which is lower than the upper limit of the reference interval for total serum proteins established in both groups at the present study.

Results of the present study revealed that serum albumin measured by electrophoresis was higher than that determined by colorimetric methods. Furthermore, calculated globulins by colorimetric method were higher than globulins measured by electrophoresis. The largest proportion of globulins was in the form of γ-globulins for group I and group II (22.8±8.0 and 24.5±6.0 g/l respectively), followed by α-globulins (11.6±3.6 and 10.8±2.4 g/l respectively) and then β-globulins (4.3±2.1 and 3.7±1.9 g/l respectively), the same was reported by Saleh *et al.* (2008) in none pregnant buffaloes. Mean values for serum globulins from the present study (41.7±12.2 g/l and 44.4±9.8 g/l in group I and group II respectively) was slightly lower than value

reported by Ali *et al*. (2011), who stated that globulins level in late pregnant buffaloes was 52.20±6.50 g/l. Normal ranges for serum total proteins, albumin and globulins reported by Saleh *et al.* (2008) were 58.2-79.7, 27.4-38.1 and 28.5-46.3 g/l, respectively, which is lower than data obtained from the present study. Also, the results of this study for serum proteins and fractions were higher than levels reported by other studies on non-lactating buffaloes (Abd Ellah, 2011). Differences between the current and previous studies may be attributed to variations in the physiological and/or climatic conditions. High serum proteins levels reported in this study compared to previous studies may be attributed to elevation of serum globulins and represent immunological response of the late pregnant buffaloes to provide the newly born calf with sufficient globulins in colostrum. Results from the current study were supported by findings of Larson and Kendall (1957) in cows, who reported that serum total proteins and globulins increased at two months before term and then decreased before parturition.

The present study (Table 4) revealed that, reference intervals for serum AST were 23.54-107.88U/l and 23.24-92.24 U/l for groups I and II respectively. Mean serum AST values for groups I and II from the present study were higher than mean value for serum AST (44.25±3.77 U/l) reported by Serdaru *et al.* (2011), and lower than mean value (72.8±7.2 U/l) reported by Ali *et al*. (2011) in pregnant buffaloes. Ghanem and El-Deeb (2010) reported that mean serum AST level in adult buffaloes was 70.6±4.16 U/l, which is higher than mean AST value from the present study. Mean serum ALT values for groups I and II were higher than its value (21.86±5.34U/l) reported by Abd Ellah (2011) in none pregnant buffaloes. Mean value for serum GGT level were 9.38±3.9U/I and 11.67±4.92 U/l for groups I and II respectively, which was higher than mean GGT value of 7.21 U/I reported in none pregnant buffaloes by Ghanem and El-Deeb (2010). In healthy adult buffaloes, it was reported that serum LDH ranged from 1500.41 to 1603.17 U/l (Grasso *et al*., 2004), which was higher than the upper limit of the reference interval for serum LDH in group I (207.29-1314.93 U/l) and in accordance with the upper limit for group II (212.09-1604.16 U/l) as shown in Table 4.

Results obtained from the present study revealed that reference intervals for serum ALP levels were ranged from 84.04-313.46 U/l and 70.67-329.86 U/l in group I and group II respectively. Normal range for serum ALP was reported to be ranged from 370.11 to 433.12 U/l in adult buffaloes under different housing conditions (Grasso *et al*., 2004), which is higher than serum ALP from this study. There was a significant increase in serum ALP level in group I (170.74±58.33 U/l) when compared with group II, which indicated that serum ALP decrease after 6 months of pregnancy in buffaloes. The results supported by findings of Pizzuti and Salvatori (1993). Serdaru *et al*. (2011) reported that mean serum ALP level was 147.0±24.71 U/l, which is lower than the mean values obtained from this study. Mean serum values for serum Ck levels were 53.46±36.38 U/l and 81.10±69.34 U/l for groups I and II respectively, which are higher than values reported in pregnant buffaloes by Ali *et al*. (2011). The variation in serum enzymes levels between the present study and previous studies may be attributed to variation

Table 4. Reference values for serum enzyme activities in pregnant buffaloes

	Group1 Less than 6 months of Pregnancy		Group II More than 6 months of Pregnancy	
	Mean ± SD	Reference interval	Mean ± SD	Reference interval
AST (U/l)	61.27±19.84	23.54-107.88	55.90±15.85**	23.24-92.24
ALT (U/l)	27.63±10.1	10.15-52.97	25.37±9.48*	8.07-50.79
GGT (U/l)	9.38±3.90	1.16-16.50	11.67±4.92**	2.70-20.93
LDH (U/l)	757.98±278.32	207.29-1314.93	752.12±449.68	212.09-1604.16
ALP (U/l)	170.74±58.33	84.04-313.46	155.89±61.77*	70.67-329.86
CK (U/l)	53.46±36.38	8.47-132.1	81.10±69.34**	13.33-249.0

Reference interval = 2.5 and 97.5 percentiles; reference interval = interval between, and including, two reference limits. Reference intervals were calculated for each reference limit as recommended by PetitClerc and Solberg (1987). Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Gamma glutamyl transferase (GGT), Lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and creatine phosphokinase (CK). *: Significant (P<0.05), **: Highly significant (P<0.01)

Table 5. Reference values for serum minerals and electrolytes in pregnant buffaloes.

		Group1 Less than 6 months of Pregnancy		Group II More than 6 months of Pregnancy	
	Unit	Mean ± SD	Reference interval	Mean ± SD	Reference interval
Calcium	mmol/l	2.89±0.42	2.13-3.68	2.71±0.42	1.89-3.42
	mg/dl	11.59±1.68	8.51-14.72	10.85±1.67**	7.55-13.69
Phosphorus	mmol/l	0.76±0.10	0.51-0.91	0.81±0.12	0.56-1.07
	mg/dl	7.30±1.00	4.88-8.77	7.79±1.13**	5.35-10.27
Magnesium	mmol/l	1.21±0.16	0.91-1.51	1.26±0.18	0.91-1.59
	mg/dl	2.94±0.40	2.22-3.67	3.06±0.44*	2.22-3.86
Sodium	mmol/l	143.83±10.75	121.76-168.09	145.34±9.30	129.84-164.55
Chloride	mmol/l	97.83±7.81	82.62-112.49	94.30±10.11**	74.82-115.83
Potassium	mmol/l	4.55±1.07	2.70-6.72	5.22±0.77**	3.65-6.88
TIBC	µmol/l	34.47±7.10	22.67-51.63	36.87±7.43	23.99-54.79
	µg/dl	192.56±39.44	126.65-288.42	205.97±41.53**	134.04-306.10
Iron	µmol/l	22.26±5.59	11.23-35.25	20.85±5.18	10.76-33.50
	µg/dl	124.37±31.22	62.72-196.95	116.46±28.96*	60.10-187.13
UIBC	µmol/l	12.21±4.83	4.13-23.16	16.02±6.13	4.87-29.41
	µg/dl	68.19±26.99	23.10-129.41	89.51±34.25**	27.19-164.28
Copper	µmol/l	11.26±2.45	7.14-16.97	11.96±2.80	7.85-18.78
	µg/dl	71.72±15.58	45.49-108.12	76.19±17.84*	50.0-119.60
Zinc	µmol/l	14.05±3.50	7.02-21.37	12.33±3.04	7.0-19.89
	µg/dl	91.84±22.87	45.88-139.67	80.59±19.87**	45.80-130.0

Reference interval = 2.5 and 97.5 percentiles; reference interval = interval between, and including, two reference limits. Reference intervals were calculated for each reference limit as recommended by PetitClerc and Solberg (1987). Total iron binding capacity (TIBC), Unsaturated iron binding capacity (UIBC). *: Significant (P<0.05), **: Highly significant (P<0.01)

in age of the animals, and/or stage of pregnancy.

Minerals are essential nutrients bearing a significant role in the animal reproduction, because their excess or deficiency produces detrimental effect on the performance of livestock. Trace elements including copper, zinc and iron, and certain macroelements like calcium, magnesium and phosphorus, and electrolytes like sodium and chloride have been found to be very essential for normal livestock growth (Underwood, 1981). Reference intervals for minerals established in the present study reflected their serum levels during pregnancy in buffaloes (Table 5). The physiological changes in serum mineral levels during pregnancy occurred as a response to increase the nutrients required during different stages of pregnancy in response to increased metabolism. Pathak *et al.* (1987) reported that mean value for serum calcium, phosphorus and magnesium in late pregnant buffaloes were 10.90 mg/dl, 7.23 mg/dl and 3.37 mg/dl respectively, which were agreed with mean serum values for calcium (10.85±1.67 mg/dl) phosphorus (7.79±1.13 mg/dl) and magnesium (3.06±0.44 mg/dl) for buffaloes after 6 months of pregnancy (group II) as shown in Table 5. Furthermore, mean values for serum calcium and phosphorus from the present study were higher than values for calcium (9.85±0.63 mg/dl) and phosphorus (4.33±0.55 mg/dl) recorded in late pregnant buffaloes by Hanif *et al.* (1984). Also, Hanif *et al*. (1984) found that plasma copper and zinc levels were 83.00±4.00 µg/dl and 72.00±6.00µg/dl respectively, which were higher than serum copper level of 71.72±15.58 µg/dl and 76.19±17.84 µg/dl for groups I and II respectively, and lower than serum zinc level of 91.84±22.87µg/dl and 80.59±19.87 µg/dl for groups I and II respectively, reported in the present study. Another study done by Kumar *et al.* (2001) on pregnant Murrah buffaloes, which revealed that the mean values for serum calcium, phosphorus, magnesium and iron concentrations were 11.83±1.17 mg/dl, 4.84±1.44 mg/dl, 1.88±0.26 mg/dl and 93.80±10.36 µg/dl, respectively. Comparing results reported by Kumar *et al*. (2001) with results presented in Table 5, revealed that serum levels of phosphorus, magnesium and iron were lower and serum calcium was higher than values reported in the present study. Mean serum potassium was 4.55±1.07 mmol/l and 5.22±0.77 mmol/l for groups I and II, respectively (Table 5) and was agreed (group I) or higher (group II) than mean value (4.53 mmol/l) reported by Hussain *et al*. (2001) in pregnant buffaloes. Mean serum sodium levels in pregnant buffaloes was 145.71 mmol/l

(Hussain *et al.*, 2001), which agreed with the mean serum sodium of 143.83±10.75 mmol/l and 145.34±9.30 mmol/l for groups I and II respectively, obtained from this study. The differences between serum minerals levels in the present study and previous studies may be attributed to variation in breed, nutritional and climatic conditions.

Large species differences in lipoproteins profiles and the percentage of total cholesterol and triglycerides carried by each lipoprotein class were recorded in different animals. Whereas in human and pigs, the majority of cholesterol is transported as LDL-C. In cattle, cholesterol is equally divided between LDL-C and HDL-C, while in sheep and horses, the majority of cholesterol circulates as HDL (Latimer *et al*., 2003). As shown in Table 6, the decreased serum cholesterol, lipoproteins and glucose levels after 6 months of pregnancy reflected increased demands for cholesterol during late stage of pregnancy to face the requirements of the developing fetus. Mean values of serum total cholesterol, HDL-C, LDL-C and VLDL-C established in the present study were lower than findings of previous studies on none pregnant buffaloes (Abd Ellah, 2011; Tajik and Nazifi, 2011). The present study revealed that serum LDL-C and HDL-C levels were equally distributed during the first 6 months of pregnancy (group1). Equal distribution of LDL-C in group I, agreed with that reported by Tajik and Nazifi (2011) in serum of none pregnant Iranian water buffaloes. According to the results of this study, mean value for serum triglycerides during pregnancy was 24.19±12.56 mmol/l and 23.07±11.45 mmol/l in groups I and II respectively, which were higher than estimated values during lactation (0.1 mmol/l) (Grasso *et al*., 2004). However, mean value for triglycerides obtained from the present study was lower than that reported by Ghanem and El-Deeb (2010), who reported that serum triglycerides was 0.34 mmol/l in none pregnant water buffaloes. In a previous study, mean serum glucose were 40.46 mg/dl as reported by Majeed *et al*. (1990), which is lower than mean glucose level from the present study. Variation in serum triglycerides and glucose levels may be attributed to physiological conditions of buffaloes

Table 6. Reference values for biochemical serum variables in pregnant buffaloes

		Group1 Less than 6 months of Pregnancy		Group II More than 6 months of Pregnancy	
	Unit	Mean ± SD	Reference interval	Mean ± SD	Reference interval
Total Cholesterol	mmol/l	1.78±0.59	0.78-3.08	1.34±0.35	0.70-2.17
	mg/dl	68.82±22.76	29.93-119.20	51.76±13.36**	27.03-83.80
Triglycerides	mmol/l	0.27±0.14	0.07-0.65	0.26±0.13	0.11-0.60
	mg/dl	24.19±12.56	6.51-57.83	23.07±11.45	9.32-53.10
HDL-C	mmol/l	0.85±0.32	0.37-1.56	0.54±0.22	0.22-1.19
	mg/dl	32.65±12.28	14.24-60.06	20.88±8.63**	8.68-45.91
LDL-C	mmol/l	0.81±0.46	0.19-2.21	0.68±0.29	0.14-1.33
	mg/dl	31.34±17.57	7.58-85.36	26.28±11.41**	5.39-51.26
VLDL-C	mmol/l	0.13±0.07	0.03-0.06	0.12±0.06	0.04-0.28
	mg/dl	4.84±2.51	1.30-11.57	4.60±2.35	1.61-10.75
Glucose	mmol/l	3.24±0.85	1.59-4.65	2.88±1.02	1.28-5.41
	mg/dl	58.42±15.31	28.68-83.77	51.96±18.43**	23.03-97.54
Total bilirubin	µmol/l	6.33±2.74	1.88-12.31	7.01±3.42	2.39-15.90
	mg/dl	0.37±0.16	0.11-0.72	0.41±0.20	0.14-0.93
Direct bilirubin	µmol/l	1.71±1.37	0.0-4.79	2.05±1.71	0.0-6.50
	mg/dl	0.1±0.08	0.0-0.28	0.12±0.10	0.0-0.38
Indirect Bilirubin	µmol/l	4.62±2.57	0.17-9.92	5.13±2.91	0.34-11.12
	mg/dl	0.27±0.15	0.01-0.58	0.3±0.17	0.02-0.65
Creatinine	µmol/l	143.21±33.59	60.11-205.97	151.16±31.82	91.94-220.12
	mg/dl	1.62±0.38	0.68-2.33	1.71±0.36*	1.04-2.49
BUN	mmol/l	14.10±4.02	5.48-21.30	12.70±4.74	4.78-22.44
	mg/dl	39.50±11.27	15.36-59.69	35.58±13.27**	13.38-62.86

Reference interval = 2.5 and 97.5 percentiles; reference interval = interval between, and including, two reference limits. Reference intervals were calculated for each reference limit as recommended by PetitClerc and Solberg (1987). High density lipoprotein (HDL-C), low density lipoprotein (LDL-C), very low density lipoprotein (VLDL-C). *: Significant (P<0.05), **: Highly significant (P<0.01)

under this study. Decreased serum BUN level may be attributed to decreased synthesis by the hepatic tissues. Increased serum creatinine level after 6 months of pregnancy may be attributed to decreased excretion by the kidneys in late pregnant buffaloes.

At present, the complete blood cell count can be performed using an automated hematology analyzer, which can increase the throughput of the test. Recently, new indices related to erythrocytes (RDW, RDWa, and platelet (PCT, MPV, PDW, LPCR) have been provided by hematology analyzers (Lombarts *et al.*, 1986). The current study is the first one that provided reference values for these new indices in pregnant buffaloes.

For erythrocyte picture, the physiological decrease in parameters of the erythrocyte and platelets pictures in group II may be attributed to the increased metabolism at late stage of pregnancy that requires the synthesis of more RBCs and platelets. As shown in Table 7, there were significant increases in MCV and MPV that was accompanied with decreased synthesis of RBCS and platelets in late pregnant buffaloes, which reflected increased stress on the bone marrow. Support for this assumption was the significant decreases in WBCs ($P<0.01$), lymphocytes ($P<0.01$) and neutrophils ($P<0.05$) counts in late pregnant buffaloes (group II), when compared with group I.

Reference limits of different hematological analytes developed in the present study (Table 7), were slightly differed from those developed by Cia-

Table 7. Reference values for haematological variables in pregnant buffaloes

	Group1 Less than 6 months of Pregnancy		Group II More than 6 months of Pregnancy	
	Mean ± SD	Reference interval	Mean ± SD	Reference interval
T. RBCs count ($x10^{12}$/l)	7.74±1.45	5.35-10.79	6.71±1.03**	5.25-9.31
HGB (g/l)	123.2±16.3	95.0-155.0	117.8±15.1**	94.0-154.3
HCT (%)	38.07±5.35	28.55-48.90	36.61±4.35**	29.67-47.78
MCV (fl)	49.83±6.54	34.17-61.93	54.96±4.76**	44.87-64.93
MCH (pg)	16.17±1.94	12.60-20.30	17.68±1.51**	14.88-20.82
MCHC g/dl	32.48±1.52	30.60-36.70	32.22±1.23	30.39-35.50
RDW (%)	22.03±2.85	17.67-29.0	20.39±2.10**	16.79-24.43
RDWa (fl)	35.93±4.53	24.84-43.63	39.04±3.98**	30.89-46.38
PLT ($x10^9$/l)	192.0±41.9	117.1-279.3	158.4±46.1**	63.9-245.1
MPV (fl)	6.31±0.41	5.70-7.23	6.70±0.60**	5.70-8.11
PDW (%)	9.63±0.60	8.70-10.93	10.20±0.93**	8.60-12.10
PCT (%)	0.12±0.03	0.07-0.17	0.10±0.03**	0.04-0.15
LPCR (%)	8.06±2.66	4.01-13.89	10.13±3.90**	4.01-20.50
T. WBCs ($x10^9$/l)	10.40±2.72	5.10-15.64	8.77±2.10**	5.30-14.43
Lymphocytes count ($x10^9$/l)	6.33±2.09	2.51-10.13	4.98±1.67**	2.45-9.22
Neutrophiles count ($x10^9$/l)	3.37±1.08	1.56-5.79	3.08±0.98*	1.42-5.37
Band cell count ($x10^9$/l)	0.1±0.10	0.0-0.45	0.08±0.08	0.0-0.29
Eosinophils count ($x10^9$/l)	0.31±0.28	0.0-1.0	0.28±0.21	0.0-0.73
Monocytes count ($x10^9$/l)	0.30±0.23	0.0-0.88	0.34±0.22	0.06-0.91
Basophiles count ($x10^9$/l)	0.0±0.0	0.0-0.0	0.0±0.0	0.0-0.0
Lymphocytes (%)	59.8±9.0	40.7-71.4	56.5±10.1**	34.0-75.1
Neutrophiles (%)	33.2±8.1	21.0-52.0	35.6±9.4*	18.9-57.0
Band cell (%)	0.9±0.9	0.0-4.0	0.9±1.0	0.0-3.0
Eosinophils (%)	3.0±2.7	0.0-10.0	3.3±2.6	0.0-10.0
Monocytes (%)	3.0±2.1	0.0-8.0	3.8±2.0	1.0-8.0
Basophiles (%)	0.0±0.0	0.0-0.0	0.0±0.0	0.0-0.0

Reference interval = 2.5 and 97.5 percentiles; reference interval = interval between, and including, two reference limits. Reference intervals were calculated for each reference limit as recommended by PetitClerc and Solberg (1987). Total red blood cells count (T.RBCs), hemoglobin concentration (HGB), red blood cells distribution width (RDW), red blood cells distribution width absolute (RDWa), hematocrit (HCT), main corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets count (PLT), mean platelets volume (MPV), platelets distribution width (PDW), large platelets concentration ratio (LPCR), plateletcrit (PCT), total white blood cells count (T.WBCs). *: Significant ($P<0.05$), **: Highly significant ($P<0.01$)

ramella *et al.* (2005) in primipara buffaloes. Mean hematological values for group II, from this study were lower than RBCs count (6.9±0.7 x10^{12}/l), Hgb (140±9.8g/l), MCH (19.8±2.1pg) and MCHC (40±1.6g/dl) and higher than HCT (33±0.1%) and MCV (49.6±5.4fl) reported by Ciaramella *et al.* (2005). Reference intervals for platelets count (117.1-279.3x10^9/l and 63.9-245.1x10^9/l, for group I and II respectively) and for MPV (5.70-7.23fl and 5.70-8.11fl, for group I and II respectively) from the present study were different from those previously reported (201-251.8 x10^9/l and 8.8-9.7fl for PLT count and MPV respectively) in lactating buffaloes (Fagiolo *et al*., 2004). Total WBCs count in group I (10.40±2.72x10^9/l) and group II (8.77±2.10x10^9/l) were higher than WBCs count (8.02±0.9x10^9/l) reported by Ciaramella *et al.* (2005). Also, differential leucocytes counts recorded by Ciaramella *et al.* (2005) were slightly different from that obtained from the current study. Differences may be attributed to variations in stage of pregnancy, climatic conditions or breed of buffaloes.

Conclusion

Reference intervals for serum biochemical and hematological variables for buffaloes during pregnancy were established in the present study. Results revealed that most of the measured blood constituents were differed significantly during the period before and after 6 months of pregnancy in buffaloes. The established reference values will be a useful guide for interpreting serum biochemical and hematologic data in pregnant buffaloes.

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