Journal of Advanced Veterinary Research

http://advetresearch.com/index.php/avr/index

Reference Values for Serum Biochemical and Hematological Constituents in Lactating Pregnant Buffaloes

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ARTICLE INFO

Original Research

Accepted:
30 September 2015

Keywords:

Serum
Hematology
Buffalo
Lactating
Reference values

ABSTRACT

Serum biochemical and hematological reference values are used to establish normality and to diagnose disease and physiological alterations. Up till now there are no reference values for different blood biochemical and hematological variables in lactating pregnant buffaloes, consequently the current study considered the first one that established those values in lactating pregnant water buffaloes (Bubalus bubalis). Animals were examined at buffalo farms that belong to Assiut Governorate, Egypt. A total of 148 pregnant lactating buffaloes (5-10 years old) were examined, of these, 20 buffaloes did not meet the selection criteria and excluded from study. The remained animals (N.=128) were clinically healthy and included in the study. A total of 55 hematological and serum biochemical variables were measured in blood of lactating buffaloes. Mean reference values, reference intervals (2.5 and 97.5 percentiles) and their 90% confidence intervals, for the measured hematological and serum biochemical constituents were statistically calculated. Data for the serum biochemical and hematological variables were presented in tables and compared with previously published data. The established reference values will be a useful guide for interpreting serum biochemical and hematologic data in lactating pregnant water buffaloes.

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, 1977, 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 (Theodossi et al., 1981; Klinkhoff et al., 1988; Bailey et al., 1989; Pattinson and Theron, 1989). The major part of the laboratory diagnostic tests is the measurement of serum biochemical and hematological variables that are used to establish normality and to diagnose disease and physiological alterations. 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

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ISSN: 2090-6277/2090-6269/

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 out to establish reference values for hematological and serum biochemical constituents in pregnant lactating buffaloes.

Materials and methods

Animals

Pregnant and none pregnant lactating buffaloes (Age 5-10 years) were examined at buffaloes' farms (Land of Kheir buffaloes farm at Abnoub city, buffaloes farm at Valley of Sheeh, El-badary City and buffaloes farm at Bani Sanad, El-hawatka), that belong to Assiut Governorate, at the mid of Egypt. The study was carried out during the period from August 2011 till April 2012. Lactating buffaloes were kept together under open half shelter system, milked two times per day using milking machine, the amount of milk produced per day were from 6-8 liter. Ration received by buffaloes during the study were mixture of silage, hay, roughages and concentrates. Water was supplied *ad libitum*.

Animals were examined carefully and inspected for presence of any abnormal clinical signs. Their inclusion in the study were based on numbers of selection criteria, which were as follow: Animals were lactating pregnant buffaloes till 6 months of pregnancy, clinically healthy, had good general body condition score, alert, no loss of skin elasticity, 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, pink and normal mucous membranes and absence of intestinal and blood parasites.

In total 148 lactating buffaloes were examined, of these, 20 buffaloes did not meet the selection criteria and excluded from study. The remained animals (N.=128) were clinically healthy, fit with the inclusion criteria and included in the study.

The ear tag number of individual animal in the farm was recorded in examination sheet. Another serial numbers were assigned for 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 study; the first blood sample was collected in plain vacutainer tube and used for obtaining serum. The second blood sample was collected in vacutainer tubes containing EDTA as anticoagulant and used for hematological analysis. Fecal samples were collected from the rectum of all animals in clean, dry and sterile cups. Samples were transported in ice tank directly after collection to the research laboratory (Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, Egypt) within 1- 2 hours from collection of samples.

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, after which serum was harvested according to standard methods (Coles, 1986), and then divided into 4 equal parts in eppendorf tubes, stored at -20°C, and were used for measuring serum biochemical constituents. Samples showed hemolysis were excluded from the study. Serum samples were analyzed within a maximum period of two weeks.

Biochemical analysis

Serum biochemical analytes

Serum biochemical analytes 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 biochemical analytes were summarized in Table 1. 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), 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) and 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 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, Sweeden). Hematological variables measured 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

Table 1. Method used to measure serum biochemical variables in lactating buffaloes

Analyte	Method	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 colorimetric method	
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	Egyptian Co. for Biotechnology, Obour City Industrial Area, 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 glutamultransferase	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	

(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 count and percentage of lymphocytes, neutrophils, band cell, eosinophils, monocytes and basophils.

Parasitological analysis

Parasitological analyses of fecal samples were done on the same day of collection using sedimentation and floatation techniques (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 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 al., 1971). Data were tested for normal distribution according to Anderson and Darling (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).

Results

During the study, body temperature was measured for all pregnant lactating buffaloes, mean value and reference intervals were 38.3±0.3◦C and 37.50-39.00◦C respectively.

Results of statistical analysis for data of different biochemical and hematological analytes were arranged in Tables 2, 3, 4, 5 and 6, and included mean values and standard deviation (SD), and the upper and lower limits for the reference intervals.

Table 2. Reference values for serum proteins measured both by spectrophotometer and electrophoresis in lactating buffaloes

		Mean ± SD	Reference interval
Spectrophotometer	Total proteins (g/l)	78.9±11.6	57.7-101.8
	Albumin (g/l)	35.32±5.31	25.13-47.56
	Globulins (g/l)	43.6±12.1	23.4-68.2
	A/G ratio	0.89±0.33	0.43-1.66
Protein Electrophoresis	Albumin (g/l)	38.9±7.40	27.1-54.5
	Total Globulins (g/l)	40.0±8.70	25.2-56.3
	α-Globulins (g/l)	10.5±2.7	5.8-16.7
	β- Globulins (g/l)	3.7±1.8	0.8-7.1
	γ- Globulins (g/l)	25.8±6.4	14.4-41.1

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 (PetitClerc and Solberg, 1987).

Table 3. Reference values for serum enzyme activities in lactating buffaloes

	Mean ± SD	Reference interval
AST (U/l)	61.36±33.3	18.14-138.03
ALT (U/l)	33.52±13.49	11.98-65.51
GGT (U/l)	10.04±4.46	1.77-19.17
LDH (U/l)	621.19±369.73	170.53-1289.26
ALP (U/l)	180.12±79.52	51.72-343.27
CK (U/l)	78.51±47.92	15.94-191.90

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 (PetitClerc and Solberg, 1987). Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Gamma glutamyl transferase (GGT), Lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and creatine phosphokinase (CK)

Table 4. Reference values for serum minerals and electrolytes in lactating buffaloes

	Unit	Mean ± SD	Reference interval
Calcium	mmol/l	2.67±0.41	2.0-3.60
	mg/dl	10.66±1.64	8.02-14.41
Phosphorus	mmol/l	2.75±0.47	1.85-3.63
	mg/dl	8.52±1.46	5.73-11.23
Magnesium	mmol/l	1.30±0.25	0.89-1.79
	mg/dl	3.16±0.62	2.17-4.36
Sodium	mmol/l	151.27±12.18	121.88-172.75
Chloride	mmol/l	96.85±10.10	82.03-119.30
Potassium	mmol/l	5.59±0.6	4.22-6.77
Tibc	µmol/l	30.01±7.33	18.78-45.10
	µg/dl	167.68±40.97	104.91-251.88
Iron	µmol/l	16.99±5.21	8.10-29.02
	µg/dl	94.96±29.11	45.20-162.1
Uibc	µmol/l	13.10±5.14	3.91-24.37
	µg/dl	72.97±28.73	21.84-136.13
Copper	µmol/l	12.13±4.26	7.17-23.39
	µg/dl	77.23±27.11	45.65-148.97
Zinc	µmol/l	13.44±4.19	6.52-21.59
	µg/dl	87.82±27.38	42.62-141.11

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 (PetitClerc and Solberg, 1987). Total iron binding capacity (TIBC), Unsaturated iron binding capacity (UIBC).

Table 5. Reference values for biochemical serum variables in lactating buffaloes

	Unit	Mean ± SD	Reference interval
Total Cholesterol	mmol/l	15.58±0.39	0.89-2.40
	mg/dl	60.93±15.08	34.27-92.56
Triglycerides	mmol/l	0.28±0.15	0.37-0.66
	mg/dl	24.84±13.39	3.26-58.75
HDL-C	mmol/l	0.73±0.25	0.28-1.28
	mg/dl	28.27±9.75	10.9-49.5
LDL-C	mmol/l	0.72±0.31	0.29-1.47
	mg/dl	27.69±11.92	11.18-56.63
VLDL-C	mmol/l	0.19±0.1	0.02-0.3
	mg/dl	4.97±2.68	0.65-11.75
Glucose	mmol/l	3.23±0.88	1.66-4.89
	mg/dl	58.21±15.77	29.96-87.72
Total bilirubin	µmol/l	6.50±2.9	1.9-12.5
	mg/dl	0.38±0.17	0.11-0.73
Direct bilirubin	µmol/l	1.9±1.54	0.0-5.64
	mg/dl	0.11±0.09	0.0-0.33
Indirect Bilirubin	µmol/l	4.8±2.2	1.2-10.9
	mg/dl	0.28±0.13	0.07-0.64
Creatinine	µmol/l	127.3±24.8	78.77-182.10
	mg/dl	1.44±0.28	0.89-2.06
BUN	mmol/l	17.59±5.24	9.25-28.51
	mg/dl	49.27±14.68	25.91-79.87

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 (PetitClerc and Solberg, 1987). High density lipoprotein (HDL-C), low density lipoprotein (LDL-C), very low density lipoprotein (VLDL-C)

Table 6. Reference values for haematological variables in lactating buffaloes

	Mean ± SD	Reference interval
T. RBCs count ($x10^{12}$/l)	6.57±0.96	5.13-9.42
HGB (g/l)	118.3±15.6	96.2-161.8
HCT (%)	35.79±4.46	28.70-48.02
MCV (fl)	54.78±4.61	45.56-62.74
MCH (pg)	18.1±1.37	15.02-20.80
MCHC g/dl	33.12±1.43	30.32-35.60
RDW (%)	20.59±2.13	17.34-25.48
RDWa (fl)	39.1±3.82	30.1-45.94
PLT ($x10^9$/l)	146.3±51.3	48.0-235.2
MPV (fl)	6.89±0.52	6.0-8..10
PDW (%)	10.46±0.79	9.22-12.24
PCT (%)	0.09±0.03	0.03-0.17
LPCR (%)	11.81±3.89	5.36-20.53
T. WBCs ($x10^9$/l)	9.41±2.24	5.41-14.87
Lymphocytes count ($x10^9$/l)	5.21±1.76	2.52-9.45
Neutrophiles count ($x10^9$/l)	3.47±1.54	1.68-6.15
Band cell count ($x10^9$/l)	0.05±0.05	0.0-0.24
Eosinophils count ($x10^9$/l)	0.3±0.26	0.0-0.98
Monocytes count ($x10^9$/l)	0.34±0.17	0.0-0.74
Basophiles count ($x10^9$/l)	0.0±0.0	0.0-0.0
Lymphocytes (%)	55.0±9.9	32.0-75.0
Neutrophiles (%)	37.6±9.7	18.0-60.
Band cell (%)	0.6±0.6	0.0-3.0
Eosinophils (%)	3.2±2.7	0.0-10.0
Monocytes (%)	3.6±1.6	0.0-7.0
Basophiles (%)	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 (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).

Discussion

The International Federation of Clinical Chemistry sets out clear guidelines for the production of reference 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 128 animals, 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 selected from farms to ensure that they received periodical clinical examination and their productive and reproductive status were regularly checked and recorded. Also, the selection of buffaloes was based on numbers of selection criteria and the physiological condition of the reference sample population was defined and reference intervals were calculated as 0.025 and 0.975 fractiles for the limits. It is well known that there are profound physiological changes in hematological and serum biochemical constituents in lactating and pregnant buffaloes. These changes are not necessarily indicative of disease but reflect physiological variations.

In the present study, mean values and reference intervals for body temperature were 38.3±0.3◦C and 37.50-39.00◦C respectively. Generally, the observed body temperature agreed with previously reported data (FAO 1994; Radostits et al. 2006).

Reference values for serum total proteins and fractions were shown in Table 2. Mean values for serum total proteins, albumin and globulins obtained from the present study were agreed with normal values (73.2±1.05, 31.0±0.4, 42.2±0.7 g/l respectively) for Iranian water buffaloes (Tajik et al. 2012). Reference intervals for serum total proteins, albumin and globulins were higher than normal range reported in a previous study, which were 58.2-79.7, 27.4-38.1 and 28.5-46.3 g/l, respectively (Saleh et al. 2008). 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). Serum total proteins at 60 days prepartum was ranged from 91.20-93.70 g/l (Quayam et al., 1990), which is lower than the upper limit of the reference interval for total serum proteins established in both groups at the present study. An average of 62.6 g/l (Nawaz and Siddique 1974) and 64-85 g/l (Khurshid et al. 1992) for serum total proteins level were found in blood of lactating buffaloes, which were relatively lower than the total serum protein concentration recorded in the present study. The largest proportion of globulins was in the form of γ-globulins (25.8±6.4g/l), followed by α-globulins (10.5±2.7g/l) and then β-globulins (3.7±1.8g/l), the same was reported in none pregnant buffaloes (Saleh et al. 2008). Mean values for serum globulins from the present study was slightly lower than value reported in a previous study, where globulins level in late pregnant buffaloes was 52.20±6.50g/l (Ali et al. 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 the current study compared to previous studies may be attributed to elevation of serum globulins and represent immunological response for lactating and pregnant buffaloes.

Lactation period is one of the physiological conditions that associated with high variability in the blood activity of serum enzymes (Pizzuti and Salvatori 1993). The present study (Table 3) revealed that, reference interval for serum AST was18.14-138.03U/l. The upper limit for the reference interval of serum AST established in the current study was lower than value reported in adult buffaloes (Bertoni et al. 1994; De Rosa et al. 2001), where serum AST varied from 145 U/l (Bertoni et al. 1994), and 160 U/l (De Rosa et al. 2001). Mean serum AST activityfor the present study was higher than mean value for serum AST (44.25±3.77 U/l) (Serdaru et al. 2011), and lower than mean value of 72.8±7.2 IU/l (Ali et al. 2011) that reported in pregnant buffaloes. Ghanem and El-Deeb (2010) reported that mean serum AST level in adult buffaloes was 70.6±4.16U/l, which is higher than mean AST value from the present study. Mean serum ALT (25.37±9.48U/l) values from the investigated buffaloes was higher than its value of 21.86±5.34U/l reported in none pregnant buffaloes (Abd Ellah 2011). In the early months of lactation, observed serum ALT activity was ranged from 83 to 116 U/l (De Rosa et al. 2001), which is higher than serum ALT (13.61-57.18U/l) obtained from the present study. Mean value for serum GGT activities was 9.5±4.5U/I, which was higher than mean GGT value of 7.21U/I that reported in none

pregnant buffaloes (Ghanem and El-Deeb 2010), and lower than mean value of 21.2 U/I reported in adult buffaloes (Bertoni et al. 1994). 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 (170.53-1289.26U/l) as shown in Table 3. Serum LDH activity was reported to be ranged from 1272 to 1741 U/l (Terzano et al. 2000) and from 713 to 1047 U/l in lactating buffaloes (Fagiolo et al., 2004). Serum ALP ranged from 200 to 650 U/l was reported in adult buffaloes under different housing conditions (Terzano et al. 2000), which is higher than serum ALP from this study. Mean serum value for serum Ck from the studied animals was 78.51±47.92 U/l, which is higher than values reported in pregnant buffaloes (Ali et al. 2011). The variation in serum enzymes levels between the present study and previous studies may be attributed to variation in age of the animals, stage of pregnancy or lactation.

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 in pregnant lactating in buffaloes (Table 4). The changes in serum mineral levels may be attributed to the physiological changes during pregnancy or lactation as a response to increase or decrease the demand for nutrients. Phosphorus levels in buffaloes have been found to be quite stable at 6 mg/dl (Campanile et al. 1997), increased at the postpartum period to reach a level of 7.9 mg/dl at 160 days of lactation (Montemurro et al. 1997). Its physiological range for during lactation was recorded to be 1.2-1.4 mmol/l in buffaloes (Bertoni and Piccioli-Cappelli 1999), which was slightly different than the reference range for serum phosphorus obtained from the current study. Mean serum calcium obtained from the studied animals was 11.3±1.8 mg/dl, which is higher than value reported in a previous study (9.7±0.3mg/dl) on lactating buffaloes (Ramkrishna 1991). The measured serum copper in the investigated buffaloes was 11.4±3.5µmol/l, which is higher than that measured in adult buffaloes (9.1±0.8µmol/l) (Tajik et al. 2010). In a previous study, some serum minerals were measured in fifty eight lactating buffaloes, the authors found that the mean serum calcium, inorganic phosphorus, sodium, potassium and chloride levels were 9.9±0.2mg/dl, 4.9±0.2mg/dl, 144.0±1.3mmol/l, 4.6±0.1mmol/l and 97.8±1.1mmol/l (Kulkarni et al. 1984), comparing these findings with our results, revealed that the present study showed higher serum calcium, phosphorus, sodium, potassium and lower chloride levels.

Mean values for serum calcium, phosphorus and magnesium in late pregnant buffaloes were 10.90mg/dl, 7.23mg/dl and 3.37mg/dl respectively (Pathak and Janakiraman, 1987), which were agreed with mean serum values for calcium (10.66±1.64mg/dl) and magnesium (3.16±0.62mg/dl) for lactating pregnant buffaloes obtained from this study. Furthermore, mean vales for serum calcium and phosphorus from the present study were higher than values for calcium (9.85±0.63mg/dl) and phosphorus (4.33±0.55 mg/dl) recorded in late pregnant buffaloes (Hanif et al. 1984). Also, it was found that plasma copper and zinc levels were 83.00±4.00µg/dl and 72.00±6.00µg/dl respectively (Hanif et al. 1984), which were higher than serum copper level (77.23±27.11 µg/dl) and lower than serum zinc level (87.82±27.38 µg/dl) from the present study. Another study done 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 (Kumar et al. 2001), comparing these results with results presented in Table 4, 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 5.59±0.6 mmol/l (Table 4), which was higher than mean value of 4.53mmol/l reported in pregnant buffaloes (Hussain et al. 2001). It was concluded that mean serum sodium levels in pregnant buffaloes was 145.71mmol/l, which was lower than mean serum sodium (151.27±12.18 mmol/l) obtained from the present study. The differences between levels serum minerals reported in the present study and previous studies may be attributed to variation in breed, nutritional, climatic and physiological conditions.

Blood lipids play an important role in the synthesis of fatty acids of milk from dairy animals (Kaneko 2008). The blood stream is the main sources of fatty acids in milk (Iverson et al. 1995; Tripathi et al. 2010). 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 5, 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 lactation. Equal distribution of LDL-C was agreed with that reported in serum of none pregnant Iranian water buffaloes (Tajik and Nazifi 2011). According to the results of this study, mean value for serum triglycerides was 0.28±0.15 mmol/l, which was higher than recorded values during lactation (0.1mmol/l) (Bertoni et al. 1997). However, mean value for triglycerides obtained from the present study was lower than serum triglycerides of value 0.34 mmol/l in none pregnant water buffaloes (Ghanem and El-Deeb 2010). Plasma triglycerides levels for lactating buffaloes were dependent on the energy level (ranging between 0.10 mmol/l and 0.12 mmol/l) and varied before and after meals (Bertoni et al. 1997). The current study reported that mean serum triglycerides level were agreed with studies on lactating buffaloes (Tripathi et al. 2010; Monteiro et al. 2012). In a previous study, mean serum glucose were 40.46 mg/dl (Majeed et al. 1990), which is lower than mean glucose level from the present study. Mean value for serum glucose level was reported to be 4.08 mmol/l in lactating buffaloes (Bertoni et al. 1994; Campanile et al. 1991), which is slightly higher than mean glucose values of 3.23±0.88 mmol/l reported in the present study. The variations may be attributed to species differences or may be due to different environmental temperature (Satriani et al. 2001).

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 hematologic analyzers (Lombarts et al. 1986). The current study is the first one that provided a reference values for these new indices in lactating buffaloes. Reference limits of hematological analytes developed in the present study to define lactating buffaloes differ from those previously developed (Jain et al. 1982). The latter used only 50 lactating buffaloes for the study (Table 6), which is less than the recommended number for establishing the reference values (Solberg 1987).

Reference intervals obtained from the present study for total RBCs count, Hgb, HCT, MCV and MCH had higher upper limits than those reported previously (Jain et al. 1982) for the same analytes, which were 5.1-8.3 x1012/l, 90.0-135.0g/l, 26.0-34.0%, 40.6-55.2fl and 13.5-20.5pg respectively, and also than those normal ranges reported previously, which were 5.8-7.7x1012/l,11.4-13.6g/l, 32.6-40.7%, 53-56fl, 17.8-19.5pg respectively (Fagiolo et al., 2004). Reference intervals for MCHC had a lower upper limit than the range 20.9-38.5g/dl for MCHC recorded in a previous study (Tajik et al. 2012), and agreed with another study on adult water buffaloes (Fagiolo et al., 2004), the latter reported that normal range of MCHC in lactating buffaloes was 33.5-34.8g/dl. Reference limits of hematological analytes developed in the present study (Table 6), were slightly differed from those developed in primipara buffaloes (Ciaramella et al. 2005). Reference intervals for platelets count and MPV from the present study were different from those previously reported in lactating buffaloes (Fagiolo et al., 2004), which were varied from 201-251.8 x109/l and 8.8-9.7fl for PLT count and MPV respectively. Total WBCs count from this study was 9.41±2.24x109/l, which was higher than reported WBCs count (8.02±0.9x109/l) reported previously (Ciaramella et al. 2005). Also, differential leucocytes counts recorded (Ciaramella et al. 2005) were slightly different from that obtained from the current study. Differences may be attributed to stage of pregnancy or lactation, climatic conditions or breed of buffaloes.

In this study, the majority of the obtained hematological values (Table 6) were different to those reported in Indian none-lactating water buffaloes (Ciaramella et al. 2005) and free range buffalo (Syncerus caffer) species (Beechler et al. 2009) re-

spectively, except for some similarity in total RBCs count and HCT % when compared with data from Indian none-lactating water buffaloes (Ciaramella et al. 2005).

Conclusion

Reference intervals for serum biochemical and hematological variables for lactating buffaloes were established in the present study. The established reference values will be a useful guide for interpreting serum biochemical and hematologic data in lactating none or pregnant water buffaloes.

Acknowledgement

This work was supported by Basic and Applied Research Project (ID: 2947) from Science and Technology Development Fund (STDF), Ministry of Scientific Research, Egypt. The authors greatly appreciate the help of the members at the Department of Animal Health Care at the Veterinary Directorate for facilitating the examination of animals and collection of samples from buffaloe farms.

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