

Some hematological and blood biochemical attributes of Iraqi riverine buffaloes (*Bubalus bubalis*) around calving and postpartum periods

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Abstract

This study was conducted to demonstrate some hematological and blood biochemical profile of Iraqi riverine buffaloes (*Bubalus bubalis*) around calving and two months postpartum (PP) period. Ten out of 22 adult buffaloes of 4.6 ± 0.97 years old were used in this study. Blood samples was collected from each buffalo around calving and 15 days interval during two months PP. The PCV and Hb as hematological parameters and AST, ALT, ALP activities, glucose, cholesterol, total protein, albumin and globulin concentrations, as well as albumin to globulin ratio as biochemical characteristics were determined. The Hb and PCV did not differ significantly at calving and PP period. Although of non-significance, AST and ALT were numerically higher at days 45 and 60 PP as compared with other periods. ALP activity was numerically lower (85.88 ± 9.23 unit/ L) at day 60 PP. Simultaneously, plasma glucose concentration was numerically lower (73.91 ± 1.51 mg/dl) at day 15 PP in comparison with other periods. Plasma total protein and protein fractions did not differ significantly among periods studied. In conclusion, nutritional deficiencies and metabolic disorders at calving and PP periods in riverine buffaloes can be detected by monitoring blood alterations.

بعض المعايير الدمية والكيموحيوية لإناث الجاموس العراقي النهري (*Bubalus bubalis*) خلال

فترة الولادة وما بعدها

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الخلاصة

أجريت هذه الدراسة بهدف بيان بعض القيم الدمية والكيموحيوية لإناث الجاموس النهري العراقي (*Bubalus bubalis*) خلال فترة الولادة وشهرين ما بعد الولادة. تم اختيار عشرة إناث حوامل بمعدل عمر 4.6 ± 0.97 سنة من مجموع 22 أنثى. تم جمع عينات الدم من كل حيوان خلال الأيام القريبة من موعد الولادة وكذلك كل 15 يوم ولمدة شهرين ما بعد الولادة. تم تقدير بعض المعايير الدمية (حجم كريات الدم الحمراء المرصوصة، PCV وتركيز الهيموغلوبين، Hb) والكيموحيوية (فعالية أنزيمات AST و ALT و ALP وتركيز الكلوكون والكوليسترول والبروتين الكلي والألبومين والكلوبوليبيدات إضافة إلى نسبة الألبومين إلى الكلوبيوليبيدات) لإناث الجاموس قيد الدراسة. لم تختلف قيم كل من PCV و Hb معنوياً لجميع الإناث خلال فترة الولادة وما بعدها. على الرغم من عدم وجود فروق معنوية في فعالية أنزيمي AST و ALT خلال فترة الولادة وما بعدها إلا أن هنالك زيادة حسابية في فعاليتها خلال فترة 45 و 60 بعد الولادة. من ناحية أخرى، انخفضت فعالية إنزيم ALP حسابياً عند اليوم 60 بعد الولادة (85.88 ± 9.23 وحدة/ لتر) مقارنةً ببقية الأيام قيد الدراسة. وفي الوقت نفسه، انخفض تركيز الكلوكون حسابياً لدى إناث الجاموس عند اليوم 15 بعد الولادة (73.91 ± 1.51 ملغم/ ديسيلتر) مقارنةً ببقية

الأيام. لم يختلف تركيز كل من البروتين الكلي وأنواع البروتين الأخرى معنوياً بين الأيام التي شملتها الدراسة. يمكن الاستنتاج بان النقص الغذائي والأمراض الايضية التي تتعرض لها إناث الجاموس النهري العراقي خلال فترتي الولادة وما بعدها يمكن التنبؤ بها من خلال مراقبة التغيرات التي تحدث لمعايير الدم المختلفة.

Introduction

Buffalo (*Bubalus bubalis*) is an important source of animal protein in Iraq. It contributes significantly to the food supply in the form of milk (5-8%), meat (1.3%) (1) and leather (2). During the last five years, there has been a pronounced decline in the population of Iraqi buffaloes to a mere 98 thousand (3). Feed shortage and reduced fertility are the main reasons of this decline (4). Inadequate nutrition has a negative effect on the buffalo productive and reproductive efficiency as well as the overall health of buffaloes herd in Iraq. To meet the increasing fetal demands and maternal energy requirements of pregnancy, alterations in the partitioning and utilization of maternal nutrients must occur. These adaptations are regulated by changing blood concentrations of regulatory metabolites and hormones, together with changes in target tissue responsiveness (5). It is well known fact that factors such as breed, sex, age, behavior, handling, physiological changes and the period of the day, can influence the cellular constituents and serum biochemistry of the blood (6). Hagawane et al. (7) found that greater ($P<0.05$) blood glucose and total protein and lower ($P<0.05$) cholesterol concentrations were observed in dry Murrah, Jafarabadi and Nagpuri buffaloes around Parbhani city of India as compared with early and late lactating buffaloes. On the other hand, higher ($P<0.05$) serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities were noted in perpartum than in postpartum (PP) periods for African Dwarf female goats, however, no significant differences were observed in serum total protein, albumin and triglyceride concentration between these periods (8). Similarly, red and white blood cells counts, packed cell volume, hemoglobin concentration and segmented neutrophils were increased after the second period of pregnancy (days 91-180 PP) and decreased in the last period (days 180 onward), while glucose concentration remained unchanged during whole gestation in Sahiwal cows (9). To our knowledge, no other previous study dealt with the hematological and blood biochemical profile around calving and postpartum (PP) periods in riverine buffaloes carried out. Therefore, this study was designed to demonstrate the profile of some hematological and blood biochemical attributes around calving and PP periods in Iraqi riverine buffaloes (*Bubalus bubalis*).

Materials and Methods

Animals: Ten pregnant females out of 22 mated riverine buffaloes of 6.0 ± 0.93 years of age were employed in this study. Animals were naturally mated following estrus detected via monitors both day and night. Pregnancy was checked for these buffaloes by BioPRYN-PSPB technique (4) and assured by rectal palpation on day 61 post-mating (PM). The pregnant buffaloes were selected randomly from the whole pregnant females ($n=14$). Buffaloes were housed at Al-Thahab Al-Abiath village, Abu- Ghraib, Baghdad during the period from August 2005 to November 2007. Buffalo were fed green roughages (alfalfa and a barley: clover mixture) ad libitum in addition to 4 kg/head/day of concentrate consisting of barley grains; 35%, wheat bran; 30%, maize; 17%, cotton; 10%, soybean meal; 5% as well as salt, vitamins and minerals; 3%. Animals were vaccinated against brucellosis, foot and mouth disease, hemorrhagic septicemia and rinderpest.

Blood collection and analyses: Blood sample (10 ml) was collected via heparinized vacutainer tubes were collected from each buffalo from each buffalo at calving and 15 days interval during two months PP. Plasma was immediately harvested from blood following centrifugation of the sample (1409 g for 15 min.) and was stored at -20 °C until assay. The packed cell volume (PCV) was determined according to Archer's method (10), whereas, the hemoglobin concentrations estimated using Cyanmethemoglobin method (11). Glucose (12) and cholesterol (13) concentrations were quantitatively determined. Total protein was assessed using Biuret's method (14). Bush (15) was employed for quantitative determination of albumin concentration. A Globulins concentration was assessed according to Otto *et al.* (16). Albumin to globulins ratio was estimated according to Zvorc *et al.* (17). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were assessed using Reitman and Frankel (18) method. Kind and King (19) was employed to determine alkaline phosphatase activity (ALP).

Statistical analyses: Statistical computations were performed using general linear model (GLM) procedure in the SAS program (20) to investigate the effect of period (gestation and PP) on hematological and blood biochemical parameters. The statistical model for analysis of variance was:

$$Y_{ij} = \mu + P_i + e_{ij}$$

where

Y_{ij} = dependent variable (PCV, Hb, glucose, cholesterol, total protein, albumin, globulins, albumin: globulins ratio).

μ = Overall mean.

P_i = effect of period (P = at calving and 15 days interval for 60 days PP).

e_{ij} = Error term.

Differences among means were computed using the Duncan multiple range test (21).

Results

Hematological parameters: The PCV value did not differ significantly among overall calving or PP periods (Table 1). However, it tended to be higher around calving (39.33 ± 1.26 %) as compared with PP periods (Table 1). Similarly, Hb during and after term did not changed as compared with post-partum periods ranged from 12.22 ± 0.33 to 12.69 ± 0.47 g / 100 ml) (Table 1).

Blood biochemical parameters: Plasma AST activity did not altered around calving and PP periods (Table 1). However, it tended to be higher throughout the post-partum periods (96.52 ± 8.75 - 102.61 ± 11.62 Unit/ L) in comparison with its value at calving (90.06 ± 8.04 Unit/ L) (Table 1). The overall mean of ALT activity was numerically be greater at 60 PP (26.94 ± 2.96 Unit / L) as compared with values around calving and other PP periods (Table 1). In contrast, plasma ALP was numerically and dramatically declined at calving to reach its lowest value (85.88 ± 9.23 Unit/ L) at day 60 PP (Table 1). The differences among means of plasma glucose concentrations around calving and PP periods lacked significance and remained steady throughout the study period (Table 1). Values ranged from 73.91 ± 1.51 mg/ dl (day 15 PP) to 89.17 ± 4.87 mg/ dl around calving (day 300- 310) (Table 1). Plasma cholesterol concentrations did not altered significantly throughout the study periods viewing fluctuated pattern around calving and PP periods (Table 1). The values ranged from 141.81 ± 5.94 mg/ dl (day 60 PP) to 149.20 ± 7.90 mg/ dl (around calving) (Table 1). Mean plasma total protein concentrations were similar around calving and PP periods, being numerically greater ($P = 0.98$) at day 60 PP (9.61 ± 0.47 g/dl) in comparison with

other studied values (Table 2). Similar pattern of non-significance was noticed in plasma albumin concentrations around calving and PP periods (Table 2). Consistent with the total protein pattern, the differences in plasma globulins along the overall studied periods lacked significance (Table 2). Interestingly, plasma globulins tended to be lower around calving namely 4.87 ± 0.41 g/ dl and higher at day 60 PP (6.05 ± 0.47 g/ dl) (Table 2). Concomitant with protein characteristics, the albumin to globulins ratio was similar during the overall studied periods (Table 2). This trait exhibited non-significant increase around calving (0.83 ± 0.09) as comparable to PP periods (Table 2).

Table (1) Some hematological and biochemical parameters of Iraqi riverine buffaloes around calving and post-partum (PP) periods (Mean $\pm$ S.E)

period(day) Trait	Days 300 - 310	Day 15 PP	Day 30 PP	Day 45 PP	Day 60 PP
PCV (%)	a 39.33 ± 1.26	a 37.67 ± 0.90	a 37.11 ± 1.21	a 37.22 ± 1.13	a 37.22 ± 1.00
Hb (g/ 100 ml)	ab 12.69 ± 0.47	ab 12.42 ± 0.38	ab 12.22 ± 0.33	ab 12.29 ± 0.33	ab 12.56 ± 0.42
AST (unit / L)	bcdef 90.06 ± 8.04	abcdef 96.52 ± 8.75	abcdef 101.50 ± 9.64	abcdef 102.50 ± 10.45	abcdef 102.61 ± 11.62
ALT (unit / L)	a 23.11 ± 4.39	a 23.11 ± 3.80	a 23.44 ± 3.43	a 25.78 ± 3.05	a 26.94 ± 2.96
ALP (unit / L)	defgh 103.72 ± 11.19	efgh 99.00 ± 9.90	efgh 93.49 ± 11.11	fgh 89.00 ± 9.23	fgh 85.88 ± 9.23
Glucose (mg / dl)	a 89.17 ± 4.87	a 73.91 ± 1.51	a 82.50 ± 4.39	a 82.24 ± 4.63	a 80.49 ± 4.44
Cholesterol (mg / dl)	a 149.20 ± 7.90	a 145.88 ± 11.82	a 151.53 ± 10.45	a 147.77 ± 6.19	a 141.81 ± 5.94

Means with different superscripts within each row differ significantly ($P < 0.05$).

PCV: Packed cell volume; Hb: Hemoglobin concentration; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase.

Table (2) Total protein and protein fractions of Iraqi riverine buffaloes around calving and post-partum (PP) periods (Mean $\pm$ S.E)

Period (day) Trait	Days 300 - 310	Day 15 PP	Day 30 PP	Day 45 PP	Day 60 PP
Total protein (g/ dl)	a 8.59 ± 0.37	a 8.99 ± 0.47	a 9.56 ± 0.52	a 9.49 ± 0.38	a 9.61 ± 0.47
Albumin (g/ dl)	ab 3.73 ± 0.12	ab 3.48 ± 0.10	ab 3.61 ± 0.06	ab 3.59 ± 0.08	ab 3.55 ± 0.07
Globulins (g/ dl)	a 4.87 ± 0.41	a 5.51 ± 0.43	a 5.95 ± 0.47	a 5.90 ± 0.37	a 6.05 ± 0.47
Albumin: Globulins ratio	a 0.83 ± 0.09	a 0.67 ± 0.05	a 0.63 ± 0.04	a 0.63 ± 0.04	a 0.62 ± 0.06

Discussion

Nutritional deficiencies, metabolic disorder and changes during gestation can be detected by analysis and monitoring of blood and other body fluids (22, 16). Hematological and plasma biochemical values indicate the health status of the dam and any deviation from the normal values in the late pregnancy may reflect the health status of the neonate (23). This study describes for the first time the hematological and plasma biochemical profile in buffaloes at calving and PP periods. In general, the hematological

parameters studied (PCV and Hb) did not differ significantly during the studied periods as depicted in Tables (1). These data are concurs with the previous reports in buffaloes (24, 7) who did not found any hematological profile differences during early and late lactation periods. This fact is in disagreement with the findings in African Dwarf goats (8) and Sahiwal cows (9). In buffaloes, it seems that the demand for nutrients and blood supply of the fetus does not increased during gestation (except for the first trimester) to meet these requirements. AST activity was numerically higher ($96.52 \pm 8.75 - 102.61 \pm 11.62$ Unit/ L) during PP periods in comparison with its value at calving (90.06 ± 8.04 Unit/ L) indicating that hepatic metabolism might be more stressed and tissue catabolism was more pronounced during this period (25). However, because enzyme activities were within the normal range, integrity and functionality of liver tissue was obviously maintained during these periods. A similar trend was observed by Reist *et al.* (26) for Holstein dairy cows, where AST activity was lowest pre - partum and reached its peak during the first week PP. Plasma ALP activity exhibited distinct and gradual decreasing from calving to day 60 PP. This may be attributed to decreasing placental ALP activity in PP period, resulting in decrease plasma ALP activity. ALP normally produced by syncytiotrophoblast cells of placenta and may be involved in migration of primordial germ cell in developing fetus (27). In pregnant cows, increases in ALP may be up 4 times normal during mid and late pregnancy, playing an important role in fetus musculature via transfer of phosphate (28). Similar profiles were also demonstrated by Pizzuti and Salvatori (29) in Italian buffaloes. However, ALP values obtained in that study ($159- 228$ Unit/ L) exceeded those observed currently ($85.88- 103.72$ Unit/ L). Plasma glucose concentration remained steady at calving and PP periods. This steady pattern may indicate lack of changes in the absolute rate of maternal gluconeogenesis and glycogenolysis (30). Glucose is a major limiting nutrient of fetal growth (31). The maternal glucose regulates the expression of placental lactogen (PL) receptors in fetal liver. This PL binding may contribute to the increase in fetal insuline and insulin-like growth factor -1 (IGF-1). PL, insuline and IGF-1 increase glucose and amino acids transport in preadipocytes and fetal myoblasts and stimulates glycogen synthesis in fetal hepatocytes, and thereby enhance fetal growth and development (32). The similar trend of glucose concentration that noticed in embryonic fluids and fetal serum collected during three different gestation periods (33) may confirm this notion. On the other hand, non-significant differences in glucose concentrations during calving and PP periods may explain the decline in maternal circulating insulin concentrations (34). These decreased insulin concentrations during the gestation period in ruminants have been postulated to be the result of the decreased response of the pancreas to insulinotropic agents (35). Circulating peptides such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) secreted from the intestine, as well as glucagon from pancreatic α -cells, are well known to be important physiological insulinotropic agents (36). The insulinotropic effect of these peptides is due to the increase of the cyclic AMP concentration in the β -cells and subsequent activation of protein kinase A, which increases Ca^{2+} influx through the activated of the voltage-dependent Ca^{2+} channels (VDCCs) and enhances Ca^{2+} efficacy in the exocytotic system (36). Reducing insulin secretion during pregnancy is proposed to be beneficial to fetal well-being, through the creation of an environment which supports minimizing peripheral glucose utilization and maximizing glucose extraction of the gravid uterus (37). Furthermore, the hypoglycemia after parturition was attributed to heavy drain of glucose for lactose synthesis (38). The fluctuated pattern of plasma cholesterol during the overall pregnancy periods was inversely associated with the

patterns of plasma progesterone levels (Abdulkareem *et al.*, unpublished data). The cholesterol is a precursor of the most steroid hormones including progesterone. High levels of progesterone during pregnancy are always accompanied with decreasing cholesterol concentrations as a result of cholesterol catabolism to progesterone via cholesterol esterase (39). The hyper-cholesterolemia during pregnancy indicated by approximately 50% increase over the non- pregnant level has been known to influence fetus growth and particularly endocrine functions (40). No alterations in plasma total protein and its fractions were noticed around calving and PP periods. This may denote the stability status of plasma proteins in buffaloes during pregnancy (41). Total protein contents usually used as an appraisal of nutritive status of an animal reflecting feed intake and metabolism (7). Protein synthesis and accretion are the cornerstones of growth that provide the structural framework and enzymatic mechanism necessary for fetal development. The maternal uterine circulation supplies the components that the fetus uses for protein accretion. It is important to explore the regulatory processes within the maternal, placental, and fetal compartments that determine fetal amino acid retention (42). Our data demonstrate for the first time some hematological and blood biochemical profile of Iraqi riverine buffaloes around calving and PP periods. These attributes did not changed obviously during these periods. In contrast, clear changes of these attributes were observed during first, second and third trimesters of pregnancy for Iraqi riverine buffaloes (41). These may reflect the changes of these metabolites during these crucial periods.

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