Some Hematological and Blood Biochemical Profile of Iraqi Riverine Buffaloes (*Bubalus bubalis*) During Different Gestation Periods

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Abstract: This study was conducted to demonstrate some hematological and blood biochemical profile of Iraqi riverine buffaloes (*Bubalus bubalis*) during pregnancy. 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 from mating (day 0), day 22-24 PM, 10-12 days interval during gestation period. Hemoglobin (Hb) was greater ($P < 0.05$) at days 103 – 106, 133 – 136 and 238 – 241 of pregnancy than those at 32 -34 days PM. The AST was highest at days 133 – 136 of pregnancy. Higher and lower ALP activity was noted at days 178-181 of gestation. A considerable increase in plasma albumin occurred at days 268–271 of gestation in comparison to days 42 – 44 PM. In conclusion, nutritional deficiencies and metabolic disorders during gestation in riverine buffaloes can be detected by monitoring blood alterations.

Keywords: Hematology, blood biochemistry, gestation, buffaloes.

NTRODUCTION

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%) and leather [1, 2]. Inadequate nutrition has a negative effect on the buffalo productive and reproductive efficiency as well as the overall health of buffaloes herd in Iraq [3]. 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 [4]. Hagawane *et al.* [5] 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. Similarly, red and white blood cells counts, packed cell volume, hemoglobin concentration and segmented neutrophiles were increased after the second period of pregnancy (days 91-180) and decreased in the last period (days 180 onward), while glucose concentration remained unchanged during whole gestation in Sahiwal cows [6].

To our knowledge, no other previous study dealt with the hematological and blood biochemical profile during whole gestation period in riverine buffaloes. Therefore, this study was designed to demonstrate the profile of some hematological and blood biochemical attributes during gestation period in Iraqi riverine buffaloes (*Bubalus bubalis*).

MATERIALS AND MEHODS

Animals

Ten pregnant females of 4.6 ± 0.97 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 [3] on day 22-24 post-mating (PM) and assured by rectal palpation on day 61 PM. Buffaloes 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

The blood sample was collected *via* heparinized vacutainer tubes from each buffalo every 10-15 days from mating (day 0) to calving. 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 [7], whereas, the hemoglobin concentrations estimated using Cyanmethemoglobin method [8]. Glucose [9] and cholesterol [10] concentrations were quantitatively determined. Total protein was assessed

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using Biuret's method [11]. Bush [12] was employed for quantitative determination of albumin concentration. A Globulins concentration and albumin to globulins ratio were assessed according to Otto *et al.* [13]. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were assessed using Reitman and Frankel [14] method. Kind and King [15] was employed to determine alkaline phosphatase activity (ALP).

Statistical Analyses

Statistical computations were performed using general linear model (GLM) procedure in the SAS program [16] to investigate the effect of gestation period on hematological and blood biochemical parameters. The statistical model for analysis of variance was:

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

where

Yij = dependent variable (PCV, Hb, glucose, cholesterol, total protein, albumin, globulins, albumin: globulins ratio).

μ = Overall mean.

Pi = effect of period (P = day of mating "day 0" – calving).

eij = Error term.

Differences among means were computed using the Duncan multiple range test [17].

RESULTS

Hematological Parameters

The PCV value did not differ significantly among overall gestation period (Tables **1-3**). However, they tended to be higher during second (days148 – 166) and third (days 223 – 299) trimesters as compared with its values during first trimester (days 32 – 44 PM). The Hb values were greater ($P < 0.05$) at days 103 – 106 (+12.6 %), 133 – 136 (+13.92 %) and 238 – 241 of pregnancy (+12.3 %) than those at day 32 -34 PM (11.37 ± 0.33 g / 100 ml) which had the lowest value throughout gestation period (Tables **1-3**).

Blood Biochemical Parameters

Plasma AST activity showed highest significant ($P < 0.01$) value (120.78 ± 9.07 Unit / L) at days 133 – 136 of pregnancy (Table **2**) and lowest (77.17 ± 7.94 Unit / L) during the last weeks prior to calving (days 283 – 286 of pregnancy) (Table **3**).

The overall mean of ALT activity did not changed noticeably within the whole study periods (Tables **1-3**). However, it tended to be higher (+18 %) during second

Table 1: Some Hematological and Blood Biochemical Parameters of Iraqi Riverine Buffaloes During First Trimester of Pregnancy (Mean ± S.E)

Gestation length (day) Trait	0	22 - 24	32 -34	42 - 44	58 - 61	73 - 76	88 - 91
PCV (%)	a 38.78 ± 1.53	a 37.89 ± 0.86	a 36.78 ± 1.02	a 36.22 ± 1.52	a 37.67 ± 1.45	a 38.33 ± 1.00	a 37.89 ± 1.62
Hb (g / 100 ml)	ab 12.61 ± 0.59	ab 12.11 ± 0.42	b 11.37 ± 0.33	ab 11.88 ± 0.46	ab 12.60 ± 0.46	ab 12.66 ± 0.33	ab 12.44 ± 0.36
AST (Unit / L)	abcdef 99.56 ± 11.57	abcdef 96.61 ± 7.25	abcdef 103.28 ± 6.10	abcde 107.78 ± 6.29	abc 111.06 ± 7.25	abcd 110.13 ± 5.47	abc 110.33 ± 8.03
ALT (Unit / L)	a 26.33 ± 2.56	a 25.78 ± 2.84	a 25.94 ± 3.46	a 29.17 ± 3.10	a 26.44 ± 4.08	a 25.83 ± 4.08	a 25.06 ± 3.80
ALP (Unit / L)	gh 82.02 ± 9.19	h 80.82 ± 8.74	fgh 86.76 ± 8.04	efgh 94.01 ± 7.75	efgh 101.41 ± 7.94	cdefgh 108.34 ± 8.37	bcdefgh 114.03 ± 9.11
Glucose (mg / dl)	a 84.18 ± 5.88	a 76.30 ± 4.02	a 83.58 ± 3.07	a 81.34 ± 5.21	a 80.79 ± 2.10	a 85.70 ± 3.74	a 80.53 ± 5.40
Cholesterol (mg / dl)	a 142.88 ± 11.97	a 160.69 ± 12.04	a 155.26 ± 9.44	a 147.90 ± 15.38	a 140.70 ± 7.23	162.25 ± 12.25	a 149.70 ± 10.97

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: Some Hematological and Biochemical Parameters of Iraqi Riverine Buffaloes During Second Trimester of Pregnancy (Mean ± S.E)

Gestation length (day) Trait	103 - 106	118 - 121	133 - 136	148 - 151	163 - 166	178 - 181	193 - 196
PCV (%)	a 38.00 ± 1.40	a 37.56 ± 1.67	a 38.89 ± 1.73	a 39.56 ± 1.54	a 39.44 ± 1.42	a 37.67 ± 1.20	a 37.56 ± 1.26
Hb (g / 100 ml)	a 13.00 ± 0.45	ab 12.78 ± 0.60	a 13.21 ± 0.60	ab 12.77 ± 0.67	ab 12.85 ± 0.47	ab 12.36 ± 0.35	ab 11.82 ± 0.42
AST (Unit / L)	ab 116.39 ± 10.29	ab 117.94 ± 9.50	a 120.78 ± 9.07	abcdef 105.22 ± 7.97	abcdef 99.83 ± 10.01	abcdef 92.78 ± 8.59	abcdef 92.44 ± 7.79
ALT (Unit / L)	a 26.06 ± 2.51	a 27.67 ± 2.79	a 29.61 ± 3.42	a 27.56 ± 4.07	a 28.33 ± 4.45	a 28.83 ± 4.65	a 28.00 ± 4.51
ALP (Unit / L)	bcdefgh 114.24 ± 9.33	abcde 127.19 ± 10.57	abc 138.26 ± 11.19	ab 148.42 ± 13.79	a 152.51 ± 13.79	a 154.49 ± 14.23	ab 145.72 ± 10.87
Glucose (mg / dl)	a 84.02 ± 3.00	a 81.67 ± 2.89	a 79.30 ± 4.80	a 80.30 ± 3.10	a 79.61 ± 5.57	a 85.74 ± 8.01	a 79.79 ± 4.93
Cholesterol (mg / dl)	a 156.48 ± 11.57	a 153.05 ± 4.26	a 141.86 ± 9.70	a 147.41 ± 6.32	a 151.47 ± 7.27	a 153.89 ± 12.61	a 145.22 ± 8.99

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 3: Some Hematological and Biochemical Parameters of Iraqi Riverine Buffaloes During Third Trimester of Pregnancy (Mean ± S.E)

Gestation length (day) Trait	208 - 211	223 - 226	238 - 241	253 - 256	268 - 271	283 - 286	292 - 299
PCV (%)	a 38.67 ± 1.43	a 39.67 ± 1.63	a 39.56 ± 1.09	a 38.56 ± 1.25	a 38.44 ± 1.32	a 39.00 ± 1.08	a 39.56 ± 1.50
Hb (g / 100 ml)	ab 12.42 ± 0.43	ab 12.66 ± 0.48	a 12.97 ± 0.38	ab 12.83 ± 0.39	ab 12.70 ± 0.39	ab 12.71 ± 0.41	ab 12.88 ± 0.41
AST (Unit / L)	cdef 86.67 ± 9.91	cdef 85.00 ± 8.40	cdef 84.28 ± 6.23	ef 80.50 ± 5.93	edf 80.72 ± 6.81	f 77.17 ± 7.94	cdef 86.94 ± 8.47
ALT (Unit / L)	a 26.17 ± 4.55	a 27.06 ± 4.79	a 26.06 ± 4.75	a 23.00 ± 5.01	a 23.17 ± 4.74	a 23.22 ± 4.53	a 24.11 ± 4.22
ALP (Unit / L)	ab 143.73 ± 10.30	ab 142.77 ± 9.32	abcd 135.86 ± 10.14	abcdef 120.67 ± 9.57	bcdefg 116.43 ± 9.75	cdefgh 107.98 ± 11.17	cdefgh 106.40 ± 11.22
Glucose (mg / dl)	a 82.91 ± 3.00	a 79.68 ± 4.61	a 81.94 ± 1.97	a 84.70 ± 4.07	a 78.15 ± 4.47	a 84.45 ± 3.00	a 80.68 ± 4.57
Cholesterol (mg / dl)	a 159.61 ± 6.20	a 146.43 ± 10.05	a 157.75 ± 6.32	a 154.13 ± 10.35	a 146.35 ± 6.24	a 144.57 ± 12.71	a 145.60 ± 9.50

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.

trimester at days 133 – 196 (Table **2**) as compared with third trimester (Table **3**).

Plasma ALP activity was gradually increased (P < 0.01, + 47 %) from 82.02 ± 9.19 Unit / L (day 0, Table

Table 4: Plasma Protein and their Fractions in Iraqi Riverine Buffaloes During First Trimester of Pregnancy (Mean ± S.E.)

Gestation length (day) Trait	0	22 - 24	32 -34	42 - 44	58 - 61	73 - 76	88 - 91
Total protein (g / 100 ml)	a 9.65 ± 0.41	a 9.32 ± 0.43	a 9.14 ± 0.42	a 9.20 ± 0.44	a 8.58 ± 0.39	a 9.05 ± 0.54	a 8.79 ± 0.43
Albumin (g / 100 ml)	ab 3.56 ± 0.14	ab 3.48 ± 0.21	ab 3.45 ± 0.15	b 3.29 ± 0.12	ab 3.45 ± 0.10	ab 3.31 ± 0.05	ab 3.32 ± 0.12
Globulins (g / 100 ml)	a 6.09 ± 0.38	a 5.84 ± 0.56	a 5.70 ± 0.44	a 5.92 ± 0.51	a 5.12 ± 0.44	a 5.73 ± 0.55	a 5.47 ± 0.47
Albumin: Globulins ratio	a 0.60 ± 0.04	a 0.68 ± 0.11	a 0.64 ± 0.07	a 0.60 ± 0.07	a 0.72 ± 0.07	a 0.63 ± 0.07	a 0.65 ± 0.06

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

1) up to 154.49 ± 14.23 Unit / L (178 – 181 of pregnancy) (Table **2**).

The differences among means of plasma glucose concentrations over the entire gestation period lacked significance and remained steady throughout the study period (Tables **1-3**). The highest non – significant value (162.25 ± 12.25 mg / dl) was detected during days 73 – 76 of gestation, registered 12.57 % increasing percentage in comparison with the lowest value (141.86 ± 9.70 mg / dl) noticed at days 133 – 136 of gestation (Tables **1** and **2**).

Mean plasma total protein concentrations were similar during the whole gestation period (Tables **4-6**). It was numerically (P = 0.98) greater at day 0 (+10.9 %, 9.65 ± 0.41 g / 100 ml) as compared with those at days 58-61 PM (8.58 ± 0.39 g / 100 ml), which represent the highest and lowest values respectively (Table **4**).

Excluding data of days 42 – 44 PM and 268 – 271 of gestation, plasma albumin did not vary significantly during the whole study period (Tables **4-6**). A considerable increase in plasma albumin (+13 %) was occurred at days 268 – 271 of gestation (3.79 ± 0.30 g / 100 ml) (Table **6**) in comparison to first trimester at days 42 – 44 PM (3.29 ± 0.12 g / 100 ml) (Table **4**).

The differences in plasma globulins along the entire periods lacked significance (Tables **4-6**). Interestingly, plasma globulins tended to be higher (+20 %, P = 0.98) at day 0 (6.09 ± 0.38 g / 100 ml) (Table **4**) as compared to second trimester at days 133 – 136, namely 5.05 ± 0.29 g / 100 ml (Table **5**).

Concomitant with protein characteristics, the albumin to globulins ratio was similar during the whole periods (Table **4-6**), exhibiting non – significant lowest values at day 0 (0.60 ± 0.04) and 42-44 PM (0.60 ± 0.04) (Table **4**).

Table 5: Plasma Protein and their Fractions in Iraqi Riverine Buffaloes During Second Trimester of Pregnancy (Mean ± S.E.)

Gestation length (day) Trait	103 - 106	118 - 121	133 - 136	148 - 151	163 - 166	178 - 181	193 - 196
Total protein (g / 100 ml)	a 8.96 ± 0.43	a 8.85 ± 0.43	a 8.71 ± 0.26	a 8.86 ± 0.42	a 9.06 ± 0.46	a 9.09 ± 0.45	a 8.75 ± 0.50
Albumin (g / 100 ml)	ab 3.62 ± 0.13	ab 3.40 ± 0.15	ab 3.66 ± 0.22	ab 3.65 ± 0.15	ab 3.49 ± 0.09	ab 3.59 ± 0.14	ab 3.60 ± 0.13
Globulins (g / 100 ml)	a 5.34 ± 0.49	a 5.45 ± 0.46	a 5.05 ± 0.29	a 5.21 ± 0.42	a 5.58 ± 0.48	a 5.50 ± 0.41	a 5.15 ± 0.50
Albumin: Globulins ratio	a 0.74 ± 0.10	a 0.67 ± 0.08	a 0.75 ± 0.08	a 0.74 ± 0.06	a 0.67 ± 0.07	a 0.68 ± 0.05	a 0.79 ± 0.13

Table 6: Plasma Protein and their Fractions in Iraqi Riverine Buffaloes During Third Trimester of Pregnancy (Mean ± S.E.)

Gestation length (day) Trait	208 - 211	223 - 226	238 - 241	253 – 261	268 - 271	283 - 286	292 - 299
Total protein (g / 100 ml)	a 8.74 ± 0.54	a 9.10 ± 0.44	a 9.29 ± 3.24	a 9.22 ± 0.36	a 9.26 ± 0.37	a 9.05 ± 0.39	a 9.01 ± 0.40
Albumin (g / 100 ml)	ab 3.66 ± 0.17	ab 3.48 ± 0.08	ab 3.76 ± 0.20	ab 3.67 ± 0.15	a 3.79 ± 0.30	ab 3.47 ± 0.10	ab 3.47 ± 0.15
Globulins (g / 100 ml)	a 5.08 ± 0.55	a 5.62 ± 0.39	a 5.53 ± 0.50	a 5.59 ± 0.43	a 5.47 ± 0.41	a 5.58 ± 0.36	a 5.55 ± 0.39
Albumin: Globulins ratio	a 0.82 ± 0.13	a 0.64 ± 0.04	a 0.76 ± 0.12	a 0.70 ± 0.07	a 0.74 ± 0.10	a 0.64 ± 0.04	a 0.65 ± 0.06

DISCUSSION

Nutritional deficiencies, metabolic disorder and changes during gestation can be detected by analysis and monitoring of blood and other body fluids [13]. 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 [5].

This study describes for the first time the hematological and plasma biochemical profile in buffaloes during the pregnancy. In general, the hematological parameters did not differ significantly during gestation period as depicted in Tables (**1-3**). However, Hb was increased in the second trimester of pregnancy as compared with the first trimester. These data are concurs with the previous reports in buffaloes [5, 18] who did not found any differences during early and late lactation periods. This fact is in disagreement with the findings in Sahiwal cows [6]. 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 relatively higher during the first trimester indicating that hepatic metabolism might be more stressed and tissue catabolism was more pronounced during this period [19]. The AST activity was the lowest during the last trimester period. Decreasing metabolism may be linked with these lower concentrations [19].

Plasma ALP activity exhibited gradual increases as pregnancy progressed. This may be attributed to increasing placental ALP activity, resulting in increased plasma ALP activity. The ALP normally produced by syncytiotrophoblast cells of placenta and may be involved in migration of primordial germ cell in developing fetus [20]. 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 [21]. Similar profiles were also demonstrated by Pizzuti and Salvatori [22] in Italian buffaloes. However, ALP values obtained in that study (159 – 228 Unit/ L) exceeded those observed currently (80.82 – 154.49 Unit/ L).

The steady pattern of plasma glucose concentrations may indicate lack of changes in the absolute rate of maternal gluconeogenesis and glycogenolysis. 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 [23]. Similar trend of glucose concentration that noticed in embryonic fluids and fetal serum collected during three different gestation periods [24] may confirm this notion. 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 [25].

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. 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 function [26].

Pregnancy did not influence the proteinogram of blood plasma in water buffalo. This may denote the stability status of plasma proteins in buffaloes during pregnancy. Total protein contents usually used as an appraisal of nutritive status of an animal reflecting feed intake and metabolism [5]. It is important to explore the regulatory processes within the maternal, placental, and fetal compartments that determine fetal amino acid retention [27].

Our data demonstrate for the first time that plasma AST and ALT activities were higher during the second trimester of pregnancy and lowered prior to calving of Iraqi riverine buffaloes. In contrast, other hematological and biochemical characteristics did not changed obviously during the whole gestation period, and these may reflect the changes of these metabolites during these crucial periods.

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