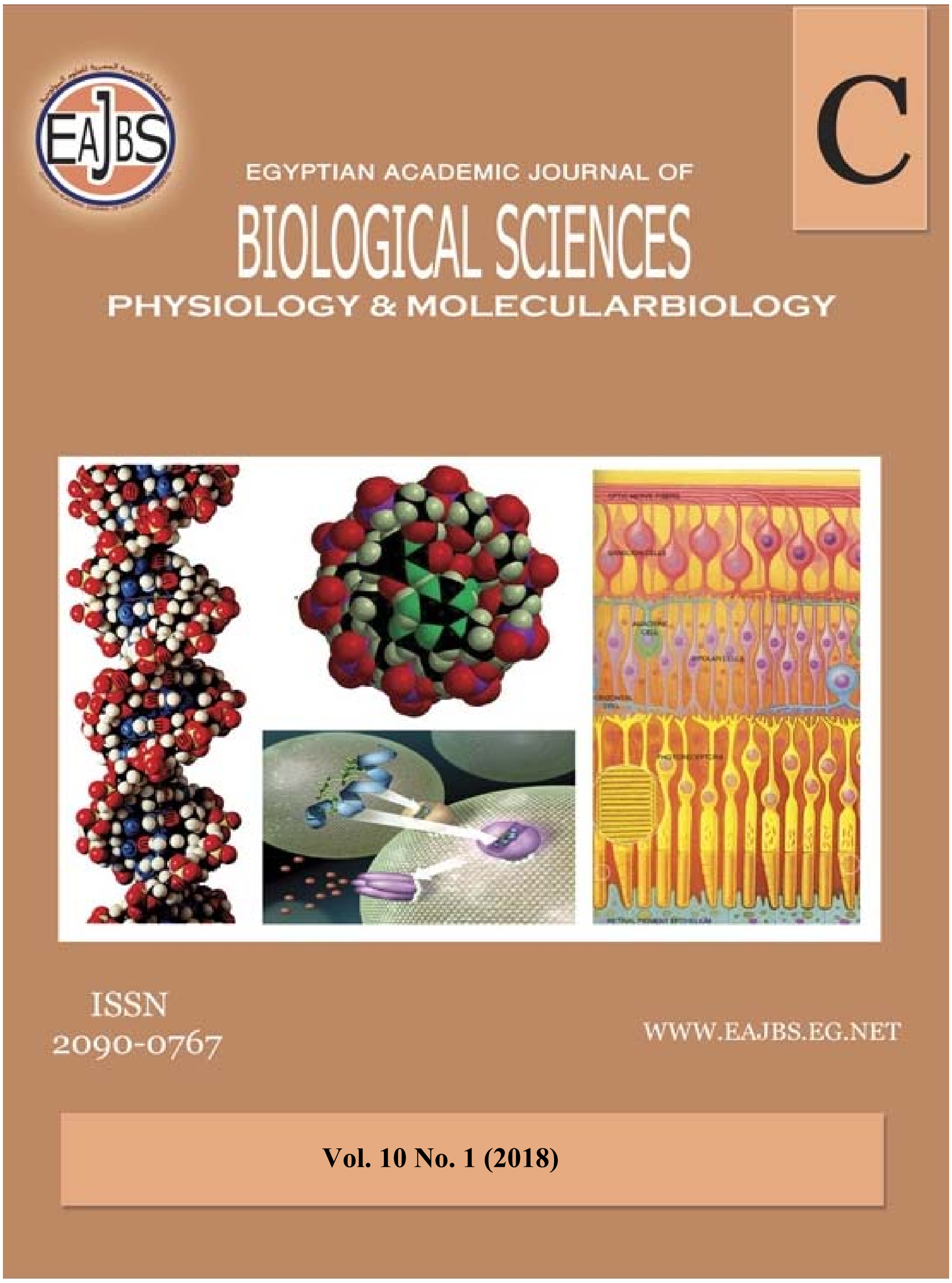

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Hematological and Metabolic Alterations in Egyptian Buffaloes During Transition Period

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ABSTRACT

Transition period is a very critical period for buffaloes that extends three weeks before calving up to 3 weeks after calving. Few published papers about the changes in Egyptian buffaloes' metabolic and hematologic parameters are reported at this period. The current study was designed to investigate the physiological variations in hematological and some metabolic parameters in Egyptian buffaloes during transition period. Fifteen pluriparous pregnant Egyptian buffaloes were subjected to weekly blood sampling at 21 days before calving (-21), at calving day (0), and at 21 days post calving (+21). Complete blood picture (CBC) was determined in whole blood samples. Leptin, non-esterified fatty acids (NEFA), total cholesterol (TC), high density lipoprotein (HDL), triglycerides (TG), blood glucose values, albumin, alanine aminotransferase (ALT), and blood urea nitrogen (BUN) were analyzed. Levels of malondialdehyde (MDA) and nitric oxide (NO) were also estimated. Hematological analysis revealed significant ($P<0.05$) increase in total leukocytes count accompanied with neutrophilia and monocytosis during postpartum period. Serum NEFA, TC, ALT, BUN, MDA and NO were significantly increased during postpartum period while leptin, TG and albumin were significantly ($P<0.05$) declined than prepartum period. From these results, the physiological adaptations of the buffaloes under the study suggest that negative energy balance during postpartum period needs efficient management to prevent metabolic disorders and their associated diseases.

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INTRODUCTION

Water buffaloes (*Bubalus bubalis*) considered the major milk producing animals in some countries (Cockrill, 1981). Water buffaloes have a great contribution in milk and meat production in Egypt (Abd Ellah et al., 2013). They have unique physiological adaptation than cattle where it can claim with hot environmental conditions and swampy lands (Ruthenberg, 1980). Therefore, more attentions had been paid for understanding the physiological adaptations of buffaloes (Abd Ellah et al., 2013; Fiore et al., 2017).

The transition period of bovine species was defined as the period extending from 3 weeks before parturition till 3 weeks after parturition (Lean and DeGaris, 2010; Su et al., 2013; Pande et al., 2016). Transition period is distinguished by marked physiological, nutritional, and metabolic modifications (Quiroz-Rocha et al., 2009). These modifications occurred as a result of changes in the animal's endocrine status to support the late gestation and the onset of milk production as well as events of parturition in between (Sundrum, 2015). There is a gradual slump in dry matter intake which begins 2–3 weeks before parturition together with sharp increase in nutrient demand with the beginning of lactation. All these events could contribute to negative energy balance (NEB) that required interactions of various body metabolic organs including liver and adipose tissue (Herdt, 2000) that share in lipolysis in periparturient period (Ingvartsen and Andersen, 2000).

Leptin is a polypeptide hormone that is primarily produced by fat cells (Henry and Clarke, 2008) and has an effective role in energy homeostasis as well as its effective role in modulating feed intake (Giblin et al., 2010). Furthermore, it could be considered like a barometer inside the body that provides a critical link between appetite, energy homeostasis and reproductive function (Zieba et al., 2008).

The increase in metabolic activities during transition period and the accompanied modification of animal's energetic status could increase oxygen consumption. This is a casual factor for the generation of reactive oxygen species (ROS). The over production of ROS and exhaustion of antioxidant reserve lead to oxidative stress (Trevisan et al., 2001). Therefore, detection of oxidants and antioxidants had become of great importance in clinical medicine as a complementary instrument for the assessment of metabolic status (Castillo et al., 2005). Oxidative stress occurred during periparturient period contributed to some metabolic disorders (Pintea et al., 2008).

Biochemical and haematological values are usually used to detect the physiological homeostasis and to adjust physiological alterations (Abd Ellah et al., 2013). As buffaloes show a quiet different metabolic pattern in comparison with other ruminants (Fiore et al., 2017) and studies on physiology of the transition period in buffaloes are limited (Abdulkareem, 2013; Fiore et al., 2017). Therefore, current study aimed to put insights on the physiology of transition period in Egyptian buffaloes in relation to hematological parameters, lipid, hepatic biomarkers as well as oxidative status during this period. This will enable us to predict with the future health status of neonates as well as their dams thus increasing the profitability of buffaloes.

MATERIALS AND METHODS

1. Animals:

The study was implemented on fifteen pluriparous pregnant Egyptian buffaloes (*Bubalus bubalis*) weighing 410- 450 Kg. The animals were apparently healthy and free from any disease. The buffaloes were managed in the Educational farm of Suez Canal University, Ismailia Egypt. The diet of experimental buffaloes was (15.5 % CP) in concentrated ration and roughage.

Concentrated diet consisted of 34% yellow corn, 11.5% cotton seed meal, 18% corn gluten feed (16% CP), 15% soybean meal, 15% beet, 1.5% sodium bicarbonate, 1.5% lime stone, 2% vitamin and mineral premix, and 1.5% sodium chloride. Roughages were available all time. Buffaloes were fed concentrate diet two times/ day in a rate of 6 kg daily for late pregnant buffaloes and 10 kg for early lactating ones. Buffaloes were milked manually twice daily. Ethics Research Committee of Suez Canal University approved all the experimental procedures of the experiment.

2. Blood sampling:

Totally 105 blood samples were collected from jugular vein of 15 buffaloes. They were collected weekly along the transition period as follows: three weeks before calving (-21 days, n= 45), where one sample was collected from each buffalo for 3 weeks. At calving (n= 15), and three weeks after calving (+21 days, n= 45), where one sample was collected from each buffalo for 3 weeks. Samples were collected in triplicates; the first one in ethylene diamine tetra acetic acid (EDTA) for complete blood count (CBC). Second one in sodium fluoride tubes for glucose estimation. The third one in plain tubes that were centrifuged after clotting at 3000 rpm for 20 min. for separation of sera. The harvested sera were stored at-20°C till be analyzed for metabolic parameters.

3. Complete blood count (CBC):

Whole blood samples collected in EDTA tubes were subjected to red blood corpuscles (RBCs) count, hemoglobin concentration (Hb), total leukocytes count (TLC), differential leukocytic count (DLC), and platelets count according to Benjamin (2001). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Wintrobe (2009).

4. Biochemical analysis:

Plasma collected in sodium fluoride tubes were submitted for calormetric glucose estimation (Diamond Co., Egypt). Sera were subjected for estimation of NEFA (Wako Co., Japan), total cholesterol, High density lipoproteins (HDL), triglycerides (Spectrum Co., Germany), blood urea nitrogen (BUN) (Diamond Co., Egypt), albumin (Spectrum Co., Germany), and alanine aminotransferase (ALT) (Diamond Co., Egypt). All the previous parameters were analyzed using spectrophotometer (V-730 UV-Vis, JASCO, USA) according to manufacturers' protocol. Serum leptin hormone level was assayed using specific enzyme linked immunosorbent assay (ELISA) kit (Cat. No. MBS703027, MyBioSource Co., USA) according to maufacturer's protocol.

5- Oxidative biomarkers:

Nitric oxide (NO), as oxidative stress marker, and malondialdehyde (MDA), as lipid peroxidation biomarker, were assayed using kinetic enzymatic method according to the manufacturers' instructions (BioAssay Systems Co. USA).

6. Statistical analysis:

All data were statistically analysed by Graphpad prism software (version 7.0, San Diego, USA). Data along the transition period were presented as Mean±SE and analyzed statistically using one-way analysis of variance (ANOVA). Further comparisons among different periods were done by Duncan's multiple comparison test. Differences were considered significant at P value ≤ 0.05.

RESULTS

1. Complete blood picture:

Table (1) revealed non significant changes in RBCs count, PCV, Hb, MCV, MCH, MCHC, and platelets count along transition period. However, TLC showed significant ($P<0.05$) increase at calving and postpartum period compared to prepartum period. Neutrophils % showed significant ($P \leq$

0.05) increment in postpartum period than prepartum period. Lymphocytes % showed significant ($P \leq 0.05$) elevation in prepartum period when compared to calving time. However, postpartum lymphocytes % were not significantly altered when compared to prepartum period and time of parturition. Monocytes % showed significant ($P \leq 0.05$) increase at calving when compared to their percentage in prepartum and postpartum period. Eosinophils % demonstrated non significant changes along the transition period of buffaloes (Table 1).

2. Metabolic profiles:

The results of metabolic profiles along the transition period were declared in Table (2). Serum levels of leptin, NEFA, TC, TG, albumin, glucose, and BUN varied significantly ($P \leq 0.05$) along the transition period. Leptin , TG and albumin levels were significantly ($P \leq 0.05$) declined in postpartum period when compared to calving and prepartum periods. However, concentrations of NEFA, TC , ALT and BUN elevated significantly ($P \leq 0.05$) in the postpartum period as compared to prepartum period. Moreover at calving, level of TC, BUN and glucose showed significant ($P \leq 0.05$) elevation and highly significant ($P \leq 0.001$) increase in glucose level when compared to their levels in the pre and postpartum periods. HDL level showed non significantly altered values among different periods of the transition period.

3. Oxidative stress markers:

High levels ($P \leq 0.05$) of serum MDA and NO were recorded in the postpartum period in comparision to prepartum period (Table 2).

Table 1: Complete blood count of Egyptian buffaloes along transition period.

Parameter	Prepartum (-21 days)	At calving	Postpartum (+21 days)
RBC (x10^6/ μl)	3.88 ± 0.11	3.93 ± 0.02	3.857 ± 0.10
Hb (gm%)	10.26 ± 0.25	10.35 ± 0.38	11.28 ± 0.37
PCV (%)	33.97±1.21	45.67 ±1.42	34.39 ± 1.43
MCV (fl)	86.93 ± 1.95	87.14 ± 0.29	88.28 ± 2.35
MCH (pg)	26.49 ± 0.57	26.35 ± 1.11	29.94 ± 1.57
MCHC (%)	30.35 ± 0.51	30.25 ± 1.38	32.29 ± 0.59
Platellets (x10^3/μL)	218.60 ± 18.75	143.00 ± 14.24	163.10 ± 13.88
TLC (x10^3/μL)	5.30 ± 0.55^b	8.19 ± 0.54^a	7.86 ± 0.32^a
Neutrophils (%)	42.43 ± 2.82^b	46.5 ± 1.01^{ab}	52.29 ± 4.01^a
Lymphocytes (%)	58.17 ± 0.99^a	47.33 ± 2.0^b	53.00 ± 3.17^{ab}
Monocytes (%)	4.00 ± 0.37^b	6.38 ± 0.59^a	3.00 ± 0.47^b
Eosinophils (%)	1.89 ± 0.31	2.00 ± 0.58	1.43 ± 0.29
Basophils (%)	00.00	00.00	00.00

Data were represented in means±SE.
Different superscripts within the same raw considered significant at P<0.05

Table (2): Metabolic profiles and oxidative markers of buffaloes along the transition period.

Parameter	Prepartum (-21 days)	At calving	Postpartum (+21 days)
Leptin (ng/ml)	7.707± 0.24^a	7.213 ± 0.33^a	5.927 ± 0.23^b
NEFA (mlEq/L)	0.39 ± 0.07^b	0.53 ± 0.04^{ab}	0.63 ± 0.00^a
HDL (mg/dL)	29.67 ± 1.72	34.43 ± 3.32	30.44 ± 2.97
TG (mg/ dL)	41.66 ± 3.11^a	40.97 ± 3.02^a	35.49 ± 3.87^b
TC (mg/dL)	48.25 ± 1.93^c	96.67 ± 2.86^a	64.58 ± 2.75^b
Glucose (mg/dL)	54.00 ± 2.68^b	66.14 ± 1.48^a	58.17 ± 1.25^b
Albumin (g/dL)	3.62 ± 0.07^a	3.39 ± 0.07^{ab}	3.27 ± 0.06^b
ALT (U/L)	22.93 ± 1.45^b	23.96 ± 1.54^b	33.62 ± 1.19^a
BUN (mg/dL)	43.64 ± 2.33^c	48.07 ± 0.54^b	55.51 ± 3.30^a
MDA (μMol/L)	0.19 ± 0.00^b	0.20 ± 0.00^{ab}	0.21 ± 0.00^a
NO (μMol/L)	58.63 ± 2.43^b	63.31 ± 1.31^{ab}	69.18 ± 0.88^a

Data were represented in means±SE.
Different superscripts within the same raw considered significant at P<0.05

DISCUSSION

Dairy animals have merits for physiological adaptation to the high energy demands during transition period specially after parturition for milk production. However, the individual animals vary tremendously in their own adaptive success (Sundrum, 2015). The failure of physiological adaptation was subsequently expressed as subclinical or clinical diseases (Broom, 1993).

In the current study, hematological and biochemical parameters were analysed to judge the physiological modifications during transition period to face the higher energy demands for expecting the health status of neonates as well as milk production (Abdulkareem, 2013).

The present results demonstrated non significant alteration in RBCs count, PCV, Hb, MCV, MCH, MCHC, and platelets count along transition period. These results were in agreement with the previous record of Abdulkareem (2013) while contradict with those of sharma et al. (2017). The observed elevation in TLC at calving could be attributed to the maximum rise in cortisol durring parturiton that incresed TLC due to stimulation of bone marrow (Kim et al., 2005). The increment in TLC was continued in the postpartum period due to the stressful conditon resulted by continious cortisol production and manifested by the high levels of NO and MDA. The same explaination was assumped to the increament of neutrophils % at the postpartum period. Where as stressfull conditions lead to an increase in capacity of bone marrow to produce neutrophils that is considered the first line of defense in the body. However, the functional capacity of these neutrophils was impaired as they were immature (Pathan et al., 2015). On the opposite side, cortisol surge at calving caused lymphocytopenia (Jacor et al., 2001) and monocytosis (Pande et al., 2016) in the blood of buffaloes used in the study.

The higher energy demands during postpartum period were demonstrated in the present study by the reduction in leptin concentration as well as the increment in NEFA level. These results were in accordance to those obtained by Pande et al. (2016) and Nagre and Kuralkar (2017). The increment in energy demands during lactation can create NEB that leads to fat mobilization and lipolysis to compunsate such deficit (Khan et al., 2011). The lipolysis leads to elevation of NEFA serum level and reduction in leptin hormone level that is primarily produced by adipose tissue (Accorsi et al., 2005). The reduced leptin levels during postpartum period could influence feed intake in these buffaloes (Gabai et al., 2002) beside diverting energy from reproductive organs especially ovaries causing their functional impairments (Nagre and Kuralkar, 2017).

Serum TG demonstrated lower values in postpartum period than the prepartum period and calving. This result was in harmony with Fiore et al. (2017). This could be attributed to the upregulation of mammary gland lipoprotein lipase (Arfuso et al., 2016). This enzyme favours transfer of TG to mammary gland for milk fat component and meeting energy required for milk production (Fiore et al., 2014). Triglycerides precipitation in liver which is characteristic in such energy deficit and could contribute to serum TG reduction (Turk et al., 2005). The levels of HDL were non significantly altered along the transition period however, TC was significantly increased at calving and postpartum period. These results were concurred with El-Maghraby and Mahmoud (2016). The reduced cholesterol values before parturition may be assumed to the usage of cholesterol by ovaries and placenta for steroidogenesis during gestation (Arfuso et al., 2016). The increased concentration of TC was ascribed to lipid catabolism to meet the higher energy requirement for milk production during postpartum period (Ashmawy, 2015) that was in harmony with the elevated NEFA.

The elevated glucose level that was observed at calving was coincided with El-Maghraby and Mahmoud (2016). Hyperglycemia is a result of the elevation of both cortisol and epinephrine due to excitement at calving (Quiroz-Rocha et al., 2009).

In current study, the serum albumin level was significantly reduced in postpartum period; whereas the hepatic ALT was significantly elevated that were in agreement with Fiore et al. (2017). Hereby results were indicative for hepatic injury where, serum albumin was indicative for hepatic synthetic function (Youssef et al., 2010) and ALT elevation have been documented for hepatic injury. The results suggested hepatic accumulation of TG, manifested by TG reduction in postpartum period therefore led to some hepatic functional impairments and metabolic disorders (Fiore et al., 2015). This is predictive for the impairment in cholesterol homeostasis where liver play a potential role through integrating hepatic enzymes and proteins (Della Torre et al., 2016).

Current data demonstrated significant increase in serum BUN at calving and at postpartum period that were coincided with (El-Maghraby and Mahmoud, 2016). The increment in BUN levels may be suggestive to the occurrence of NEB that resulted in excessive deamination (Oliva et al., 1991) or due to increased crude protein intake (Kida, 2003). Usually BUN has been used as an indicator for dietary concentrate intake (Toharmat et al., 1999). Higher dietary concentrates during postpartum period leads to promotion of rumenal propionic acid production that increased microbial protein supply (Heck et al., 2009). Moreover, the higher dietary protein intake led to increment in ammonia production which was effeciently transformed to urea by hepatic microsomes (Campanile et al., 2006). The excessive load exerted by liver in ammonia transformation could predispose hepatic injury observed by elevated serum ALT.

At the same trend, serum MDA and NO were significantly increased in postpartum period. Whereas the NEB during the postpartum period led to promotion of fat mobilization, fatty acids oxidation (Ashmawy, 2015), and increased NEFA levels. Fatty acids oxidation is a fundamental source of free radicals that exceed the normal antioxidant body capacity thus increasing lipid peroxidation and NO levels over the normal antioxidant scavenging capacity (Li et al., 2016). The oxidative status biomarkers have been used as indicators for the occurrence of pathological processes and metabolic disorders where they are implicated in and can cause biohazard to individual cells (Castillo et al., 2005). Moreover, the observed promotion of oxidative load in postpartum period was in accordance with the reduced serum albumin values in the present study; where albumin is considered to possess antioxidant properties (Quinlan et al., 1998; Rostoker et al., 2011; Taverna et al., 2013).

Conclusion:

The metabolic profile during transition period and the oxidative status of Egyptian buffaloes can serve as an efficient indicator for the health condition and managemental procedures to improve profitability. Leptin hormone, NEFA, lipid profile, glucose, and BUN were interplayed together toward compensation of NEB during postpartum period. The NEB during postpartum period creates hepatic load by increasing ALT as well as oxidative load manifested by elevated MDA and NO. Buffaloes should be managed carefully during this period to avoid occurrence of metabolic disorders and their subsequent reproductive failure and diseases.

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ARABIC SUMMARY

التغيرات الدموية و الأيضية في الجاموس المصري خلال الفترة الانتقالية

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الفترة الانتقاليه هي فترة حرجة للغاية فى الجاموس و التى تمتد ثلاثة أسابيع قبل الولادة إلى ٣ أسابيع بعد الولادة .كما يوجد عدد قليل من الأبحاث المنشورة حول التغيرات في الدلالات الأيضية والدموية للجاموس المصري في هذه الفترة .صُممت الدراسة الحالية لدراسة التغيرات الفيسيولوجية في الدلالات الدموية و الأيضية للجاموس المصرى خلال الفترة الانتقالية .تم تجميع عينات الدم من خمسة عشر انثى من اناث الجاموسً المصرى الحوامل التى سبق لها الولادة في اليوم الحادى و العشرون قبل الولادة (-٢١) وفي يوم الولادة (٠) وفي اليوم الحادى و العشرون بعد الولادة (+٢١) بواقع عينة اسبوعيا . تم تحليل صورة الدم كاملة بالاضافة الى كل من هرمون الليبتين و الاحماض الدهنيه الغير مأسترة و الكوليسترول و الدهون عالية الكثافة و الدهون الثلاثية والجلكوز و زلال الدم و انزيم ناقل امين الالانين و اليوريا. كما تم تقدير الدهيد المالون و اكسيد النيتريك. افاد فحص صورة الدم زيادة معنوية فى العدد الكلى لكريات الدم البيضاء مصحوبة بزيادة فى الخلايا عدلة الحبيبات و الخلايا الوحيدة خلال فترة ما بعد الولادة. علاوة على ارتفاع معدل كل من الاحماض الدهنيه الغير مأسترة و الكوليسترول و انزيم ناقل امين الالانين و اليوريا و الدهيد المالون و اكسيد النيتريك بشكل معنوى فى مصل الدم خلال فترة ما بعد الولادة بينما انخفض معنويا معدل كل من الليبتين و الدهون الثلاثية و زلال الدم مقارنة بفترة ما قبل الولادة. يتضح من النتائج السابقة أن الاقلمة الفسيولوجية خلال الفترة الانتقالية للجاموس تحت الدراسة تؤدى الى حدوث خلل سلبى فى اتزان الطاقة فى فترة ما بعد الولادة مما يوجب رعايه مثلى للجاموس خلال هذه الفترة لمنع حدوث الخلل الايضى و ما يترتب عليه من أمراض.