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RELATIONSHIP AMONG ACUTE PHASE PROTEINS IN TRANSITION PERIOD IN BUFFALOES

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ABSTRACT

The objectives of this study were to record the changes in some acute phase proteins (APPs) either positive or negative during transition period and to demonstrate the correlation between different APPs in the transition period. To achieve these objectives, 29 pregnant buffaloes were selected at 2nd and 3rd milk season from Ard El -Kheir farm. The study was carried out in Assuit Governorate, Whole blood samples were collected from these buffaloes for determination of fibrinogen, blood plasma samples were collected for determination of vit. A and blood serum samples were collected for determination of serum albumin, serum total cholesterol, serum total bilirubin and serum haptoglobin). Blood samples were collected as the following: (-21d: -15d), (-14d: -8d), (-7d: -1d), calving day, 3 d P.P. 7 d P.P. 14 d P.P and 28 d P.P. The study revealed significant changes in the concentration of some acute phase proteins, whether positive or negative. The mean values of fibrinogen revealed significant changes with maximum increase at day 3 P.P. Non significant changes in the mean values of haptoglobin with maximum increase one week pre partum. Significant increase in the mean values of total bilirubin at calving day and post partum periods compared with pre partum periods, reached maximum at day 14 P.P. The mean values of albumin revealed significant changes with maximum increase one week pre partum. A significant decrease in the mean values of total cholesterol at pre partum compared with post partum reached maximum at calving day compared with pre and post partum periods. A significant decrease in the mean values of vit. A reached maximum at day 7 P.P.

Key words: acute phase proteins, buffaloes, haptoglobin, total cholesterol, fibrinogen, pre partum, Assuit, post partum

INTRODUCTION

The Egyptian buffalo plays a crucial role in Egypt economy by their livestock productivity from milk and meat. The water buffalo is the major source for milk in Egypt as it contributes more than 50% of the annual milk production in Egypt (Agriculture economic institute, 1997).

The transition period, as defined by Drackley (1999), is a stressful time for the health and productivity of dairy animals, and the most challenging stage in the lactation cycle when most metabolic disorders and infectious diseases occur. It is the period around calving constitutes late pregnancy and early lactation, generally 3 weeks before and 3 weeks after parturition. This period is characterized by tremendous metabolic and endocrine adjustments that the animals must

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experience from late gestation to early lactation (Drackley *et al.*, 2001 and DeFrain *et al.*, 2005) having a significant effect on the animal's health (Lippolis, 2008). This period is marked by changes; some of these are related to alterations increases in energy requirements driven by both fetal needs and lactogenesis, endocrine and metabolic preparing animals for childbirth and lactation (Morgante *et al.*, 2012 and Piccione *et al.*, 2012).

In periparturient dairy animals, in addition to the increase in ROS production, animals are typically in an inflammatory-like condition especially in the liver (Bertoni *et al.*, 2008 and Trevisi *et al.*, 2010& 2012), even without signs of microbial infections or other pathologies (Petersen *et al.*, 2004 and Sordillo *et al.*, 2009).

Parturition, changes in homeostasis, metabolic and physiological challenges occurring in this stressful period as well as other external and internal harmful stimuli may contribute to the activation of the host immune system, including the initiation of

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inflammatory responses (Tóthová *et al.*, 2014). These inflammatory markers are elevated at parturition and the days following in all animals, although these increases appear to be more pronounced in animals that go on to have more serious health complications (Huzzey *et al.*, 2009). All dairy animals suffer from an inflammatory condition during their transition period, exerts several negative effects on metabolism, performances and fertility (Trevisi *et al.*, 2011).

Acute phase response consists of a large number of behaviors. physiological, biochemical. and nutritional changes (Ceciliani et al., 2012). The most important metabolic changes include the highly increased or decreased production of a large family of proteins from the liver (the acute phase proteins) (Ceciliani et al., 2002 and Murata et al., 2004). Acute phase proteins (APPs) are blood proteins primarily synthetized by hepatocytes as part of the acute phase response (Cray et al., 2009) which released into the blood stream in response to a variety of stressors. In general, the main function of APPs is to protect the host against pathological damage, assist in the restoration of homeostasis and in the regulation of different stages of inflammation (Petersen et al., 2004).

The APPs can be produced by both hepatocytes and peripheral tissues, and can be classified according to their concentration in positive APPs, if they increase, or negative APPs if they decrease (Petersen *et al.*, 2004, Ceron *et al.*, 2005 and Eckersall & Bell, 2010).

The plasma changes of +APPs (e. g. haptoglobin, ceruloplasmin) are useful indices to describe the severity of the inflammatory status, also in case of a subclinical form. While the assessment of -APPs (e. g. albumins, retinol binding protein, apolipoproteins) describe the extent and the consequences of the response to inflammation (Ceron *et al.*, 2005, González *et al.*, 2008 and Grossi, 2011).

Acute phase proteins (APPs) are blood proteins primarily synthesized by hepatocytes as part of the acute phase response. The acute phase response is a non-specific complex reaction of an organism, triggered by different stimuli (Cray *et al.*, 2009). The APPs consist of negative and positive proteins that show a decrease and an increase in levels, respectively, in response to challenge or stressors (Eckersall, 2004, Murata *et al.*, 2004 and Gruys *et al.*, 2005).

Changes in acute phase proteins during transition period in dairy cattle have been widely investigated, however; few researches were carried out in buffaloes. To the best of our knowledge, the current study is considered as one of the first in the field to address water buffalo transition period by using APPs. Studies on APPs in buffaloes are scarce in Egypt. So, the main objectives of this study were to record the changes in some acute phase proteins (APPs) during transition period and to demonstrate the correlation between different APPs in the transition period.

MATERIALS AND METHODS

1- Animals:

The study was carried out on 29 clinically healthy, multiparous buffaloes at transition period (3weeks before calving and 4 weeks after calving), with average age of 2-4 years old from a private farm belongs to Assuit Governorate (Ard El- Kheir). Samples were collected from May to October, 2015. Buffaloes were fed daily on meal composed of corn, soybeans, wheat bran, silage, little amount of sunflower, hay, multi vitamins, mineral mixture, antioxidant, buffer, dibasic phosphate" only before calving"). Protein in ration usually 11%, but at dry period reach 14- 16%. Water was supplied ad libitum.

2- Clinical examination:

All animals in the study were subjected to general clinical examination, where the clinical examination including body temperature, respiratory and pulse rates, ruminal movement, visual inspection of the udder, appetite as well as examination of mucous membranes was conducted according to Rosenberger (1990).

3- Samples:

Blood samples were collected in early morning; avoid stress or physical efforts as possible, avoid sick buffaloes, samples were kept in ice box and centrifuged within 2 h of collection. Three blood samples were collected by jugular vein puncture in vacuum tubes (Vacuette, Greiner Austria) containing no anti-coagulant, lithium-heparin, and K3EDTA.

Several blood samples were collected from each animal before calving, according to calving day, only samples at the (- 21), (-14) and (-7) days pre calving were used, then at calving day, 3^{rd} , 7^{th} , 14^{th} and 28^{th} days post calving.

4- Biochemical analysis:

Measurement of fibrinogen by using the routine heat precipitation technique (Millar *et al.*, 1971). Measurement of Retinol binding Protein (RBP, measured as retinol (vit. A) chemically by spectrophotometric method using Cecil Digital

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Ultraviolet Cambridge, England (CE, 292, Series2) (Carr and Price, 1926). Measurements of albumin, lipoproteins (indirectly measured as total cholesterol) and total bilirubin by using commercial test kits supplied by diamond diagnostics (Diamond diagnostics, Hannover, Germany) measured spectrophotometry using UV spectrophotometer (Optizen 3220 UV, Mecasys Co. Ltd, Korea). Spectrophotometeric assay was performed using spectrophotometer in the department of Animal Medicine- Faculty of Veterinary Medicine Assiut University, Assuit Egypt. Measurement of haptoglobin using Haptoglobin test kit (Catalog. No: WBE- 030 WKEA med supplies) by ELISA microplate reader (Elson, 1974 and Makimura & Suzuki, 1982) in molecular biology unit Assiut University, Assuit Egypt.

5- Statistical analysis:

Values are presented as means \pm standard error of the mean (SEM). Statistical analysis of obtained data was carried out using general linear model (GLM procedure) of SAS System (2004). Two-way analysis of variance (ANOVA) with repeated measurements was carried out to study the effect of periods. The statistical significance of difference between period's means was determined using Duncan's Multiple Range Test (DMRT) (Steel and Torrie, 1980). All the variables were considered significant at (p < 0.05). Pearson's coefficients of correlation were evaluated using the CORR procedure of SAS.

RESULTS

I-Clinical examination:

All examined animals were inspected for presence of any abnormal clinical signs. Clinical examination of the animals in this study revealed that body temperature, respiratory and pulse rates and ruminal movement within normal reference range. Also, udder and mucous membranes were apparently healthy. No abnormal clinical signs were recorded during the study period.

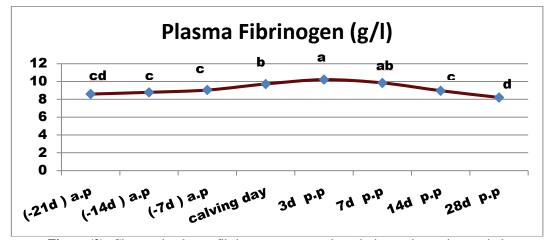
II- Biochemical analysis:

Results of selected measured positive acute phase proteins (Plasma fibrinogen, serum haptoglobin and total bilirubin) are illustrated in Table (1) and Figure (2, 3 and 4). Mean values revealed significant (p < 0.05) increase in plasma fibrinogen at calving day compared with pre calving periods, reached maximum at day 3 Post partum (P.P), followed by a significant (p < 0.05) decrease at days14 and 28 P.P, a non significant difference in serum haptoglobin during transition period but maximum increase occurred at (-7d) pre partum and significant (p < 0.05) increase in serum total bilirubin at calving day and post calving periods compared with pre calving periods, reached maximum at day 14 P.P. Except one week before calving.

Parameters Fibrinogen (g/l) Haptoglobin (µg /ml) Total Bilirubin (µmol/l) **Days relative** (n=29)(n=9)(n=29)to calving (-21 d) 8.59 ± 0.12 ^{cd} 21.88 ± 3.44 a 0.86 ± 0.15 c a.p (-14 d) 8.78 ± 0.12 ^c 25.28 ± 5.36 ^a 1.04 ± 0.15 ° a.p (-7 d) 9.04 ± 0.11 c 36.09 ± 11.92 a 1.40 ± 0.19 bc a.p $4.04\ \pm 0.70\ ^{ab}$ 9.72 ± 0.15^{b} 29.53 ± 3.90^{a} Calving day $4.74\ \pm 0.86\ ^a$ 3 d p.p 10.21 ± 0.22 a 27.44 ± 2.77^{a} $9.84 \pm 0.22^{\ ab}$ 7 d p.p 29.31 ± 3.93 ^a 4.88 ±1.33 ^a $8.96 \ \pm 0.14 \ ^{c}$ 27.02 ± 2.89^{a} $5.79\ \pm 1.56\ ^{a}$ 14 d p.p 28 d p.p $8.21 \pm 0.09^{\text{d}}$ $21.38 \pm \ 1.44^{\ a}$ $5.61\ \pm 1.47\ ^{a}$

 Table 1: Changes in selected positive acute phase proteins during transition period in buffaloes.

Values= mean \pm standard error, means of different superscript letters in the same column differ significantly (p < 0.05). a.p., ante partum; d p.p., day post partum



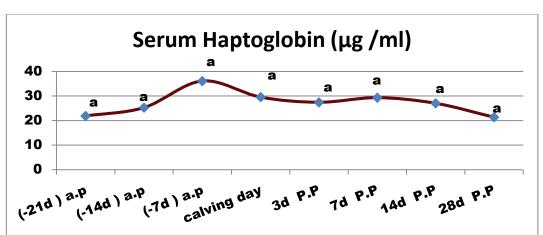


Figure (2): Changes in plasma fibrinogen concentrations during periparturient period.

Figure (3): Changes in serum haptoglobin concentrations during periparturient period.

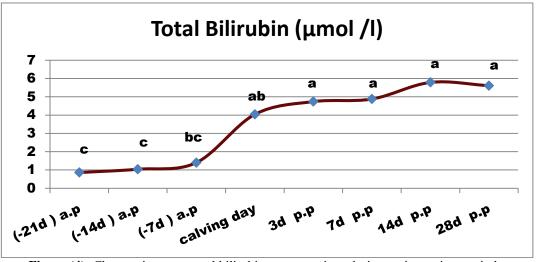


Figure (4): Changes in serum total bilirubin concentrations during periparturient period

Results of selected measured negative acute phase proteins (serum albumin, total cholesterol and vit. A) are illustrated in Table (2) and Figure (5,6 and 7). Mean values revealed significant (p<0.05) increase in serum albumin reached maximum at (-7d) ante partum followed by significant ($p \le 0.05$) decrease at calving day reached maximum at day 28 P.P,

significant (p<0.05) decrease in serum total cholesterol at calving day compared with ante partum and post partum periods followed by significant (p<0.05) increase reached maximum at day 28 P.P., significant (p<0.05) decrease in serum vit. A reached maximum at day 7 P.P. followed by significant (p<0.05) increases at day 28 P.P.

parameters Days relative to calving	Albumin (g/l) (n=29)	Total Cholesterol (mmol/l) (n= 29)	Vit. A (µg / dl) (n= 29)
(-21d) a.p	$42.51\pm~0.73~^{\circ}$	$1.46\pm0.05^{\ b}$	$49.95\pm0.68^{\ b}$
(-14d) a.p	$42.72~\pm~0.55~^{\rm c}$	$1.40\ \pm 0.03\ ^{b}$	47.04 ± 0.70 ^c
(-7d) a.p	$49.35\pm1.27^{\ a}$	1.18 ± 0.03^{c}	$45.08 \pm \ 0.61 \ ^{c}$
Calving day	$46.77~\pm1.34~^{ab}$	$0.82~\pm0.04^{-d}$	$39.79\pm0.67^{\ d}$
3 d p.p	$44.65 \ \pm 0.85 \ ^{bc}$	1.06 ± 0.04 ^c	36.83 ± 0.75 °
7 d p.p	$43.61\ \pm 1.04\ ^{\text{c}}$	1.08 ± 0.04 ^c	$34.44\ \pm 0.71\ ^{\rm f}$
14 d p.p	$43.49\ \pm 0.76\ ^{\circ}$	$1.42\ \pm 0.07\ ^{\text{b}}$	$45.67\ \pm 0.88\ ^{\rm c}$
28 d p.p	$43.03 \pm 0.70 \ ^{c}$	1.85 ± 0.08 ^a	$52.04\ \pm 0.90\ ^{a}$

Table 2: Changes in selected negative acute phase proteins during transition period in buffaloes.

Values= mean \pm standard error, means of different superscript letters in the same column differ significantly (p < 0.05), a.p., ante partum; d p.p., day post partum

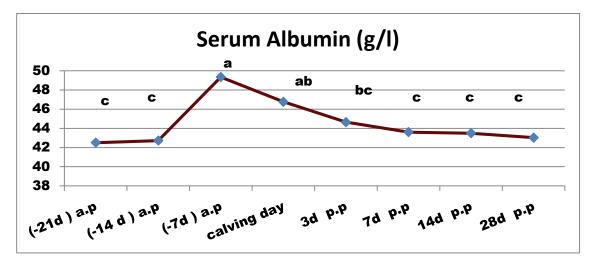


Figure (5): Changes in serum albumin concentrations during periparturient period.

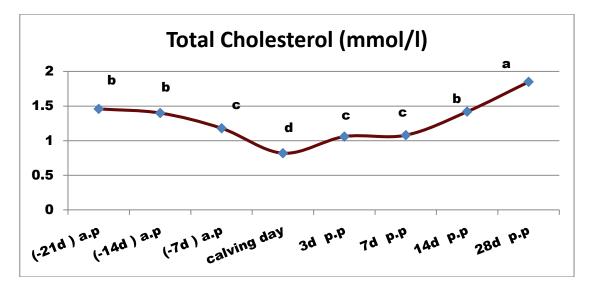
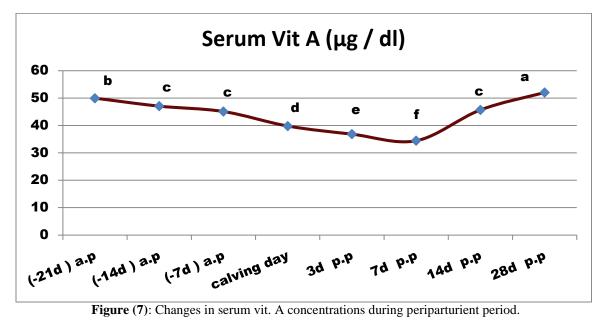


Figure (6): Changes in serum total cholesterol concentrations during periparturient period.



The obtained results showed that haptoglobin was positively correlated with fibrinogen. Total cholesterol and vit. A were negatively correlated with albumin, total bilirubin, fibrinogen and haptoglobin.

Table (3): Correlations among blood biochemical parameters in periparturient buffaloes

	Albumin	T .Chol	T.Bilirubin	Fibrinogen	Vit. A	Haptoglobin
Albumin	1.000	r = - 0.205	r =0.129	r =0.342	r = - 0.267	r = 0.2097
	232	0.0017 **	0.0490*		<0.0001 **	0.0770
		n =232	n=232	<0.0001**	n =232	n =72
				n =232		
T. Chol		1.000	r= - 0.0812	r=- 0.414	r= 0.556	r= - 0.1715
		n=	0.2180	<0.0001**	<0.0001**	0.1498
		232	n=232	n=232	n=232	n=72
T.Bilirubin			1.000	r=0.1485	r=- 0.2443	r=0.1367
			n= 232	0.0236*	0.0002**	0.2512
				n=232	n =232	n=72
Fibrinogen				1.000	r=- 0.6213	r= 0.2482
				n= 232	<0.0001**	0.0355 *
					n=232	n=72
					4 000	o 400
Vit. A					1.000	r=- 0.199
					n= 232	0.0931
						n= 72
Haptoglobin						1.000
n = Number of sa	mples					

n = Number of samples

* =significant at (P < 0.05)

** =highly significant at (P< 0.01)

DISCUSSION

1- Plasma fibrinogen (g/l):

There was a significant increase (p < 0.05) at calving day and reached maximum at day 3 P.P. The obtained result was lined with Zargham Khan *et al.* (1994) who reported that the plasma fibrinogen concentrations were higher in lactating than non lactating buffaloes.

There was a significant decrease (p < 0.05) at days14 and 28 P.P. when compared with calving day and day 3 P.P. The obtained result may be attributed to the involvement of fibrinogen in homeostasis, providing a substrate for fibrin formation and in tissue repair, providing a matrix for the migration of inflammatory related cells (**Thomas, 2000**).

2- Serum haptoglobin (µg/ml):

In our study, we noticed non significant changes in serum haptoglobin during periparturient period and its concentration reached the maximum value at (-7d) a.p and then decreased gradually after calving. This result was lined with Ametaj *et al.* (2005) and Kováč *et al.* (2009) who reported that serum Hp concentrations increased during pregnancy in animals and especially near parturition, probably because of up-regulation of its expression by cortisol and non-steroid fatty acids.

Results of HP in our study were lower than those reported by Tajik *et al.* (2011) who reported that the reference value for serum Hp of healthy water buffaloes was 0.129 ± 0.06 g/l. This could be due to the different study conditions such as diet, climate, age, geographical location (Iranian study) and good management of the farm in the present study.

3- Total bilirubin (µmol/l):

In the present study, there was a significant increase (p < 0.05) in the mean values of serum total bilirubin at calving day compared with (-21d) and (-14d) a.p. It can be mainly attributed to the lower synthesis of enzymes responsible for its clearance (Bertoni and Trevisi, 2013). This result was agreed with Omur *et al.* (2016) who reported that the level of total bilirubin increased at parturition and decreased after 3 weeks. Such increase may have resulted from the minimal level of cellular damage emerged in the liver due to the lipomobilization based on the energy deficit arising as the birth approaches.

Also, we noticed a significant increase (p < 0.05) in the mean values of serum total bilirubin at post calving periods compared with pre calving periods. This increase may be resulted from the deficiency in liver functions developed due to the Negative Energy Balance (NEB) in that period (Bobe *et al.*, 2004). This result was lined with Djokovic *et al.* (2011) who reported that the mean bilirubin concentration was significantly and markedly increased (P < 0.05) in the early lactating compared to the late pregnant animals. Liver damage induces an increase in the serum total bilirubin, and the haemic compound is considered as a sensitive indicator for liver injury (Lubojacka *et al.*, 2005). Also, it was lined with Celeska *et al.* (2015) who reported a significant increase (p < 0.05) of serum total bilirubin in dairy animals at day 5 post calving compared with day 5 pre calving and day 30 post calving. The author attributed an increased value of total bilirubin day 5 relative to calving, because the pathobiochemical processes of gluconeogenesis, glucogenolysis, phosphorilation, beta oxidation happened simultaneously in hepatocytes.

4-Serum albumin (g/l):

In the present study, serum albumin levels showed a significant increase (p < 0.05) at (-7 d) a.p compared with (-14 d) and (-21d) a.p. This result could be attributed to endogenous production of antioxidants, such as albumin, in an attempt to cope with the increase in lipoperoxide production, especially one week before calving and early post-partum (Ghada *et al.*, 2015). This result was lined with Montemurro *et al.* (1997) who reported high serum albumin levels in buffaloes at the end of pregnancy. Also, it was agreed with Cavestany *et al.* (2005), Djokovic *et al.* (2011) and Djokovic *et al.* (2013) who reported that albumin concentrations are known to be higher during the prepartum period than after parturition.

We noticed also that serum albumin level decreased at calving day compared to (-7d) a.p. It may be attributed to increase albumin consumption to avoid imbalance in oxidant/antioxidant status. The current study reported a significant decrease (p < 0.05) in serum albumin level at post calving periods compared with (-7d) a.p. This mentioned decrease may be caused by the transfer of albumin and γ globulins from blood to the mammary gland (Kupczynski and Chudoba-Drozdowska, 2002), or it is probably due to decreased synthesis in the liver and increased utilization in the udder (Celeska et al., 2015). This result was lined with Tóthová et al. (2008) who stated that in postpartum period, the serum total protein and albumin levels were low as compared to the pre-partum and parturient periods. Also, it was lined with Celeska et al. (2015) who reported a significant decrease (p < 0.05) in serum albumin at day 10 post calving compared with day 5 pre calving and day 30 post calving. Albumin and total protein concentrations showed changes at day 10 after calving, probably due to decreased synthesis in the liver and increased utilization in the udder. Seifi et al. (2007) also noticed that albumin was at lowest levels at day 8 post partum. There was a tendency for animals to have lower concentrations of serum albumin shortly after calving than at any other stage of the lactational cycle. This fall was explained

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in four possible ways: decreased synthesis of albumin by the liver, an increase in the rate of albumin catabolism, a dilution of albumin in the serum by an increase in blood volume, or an increase in the loss of albumin into the gut or extra vascular fluids including milk.

5- Total cholesterol (mmol/l):

There was a significant decrease (p < 0.05) at (-7d)a.p., calving day, days 3 and 7 P.P compared with (-21d) and (-14d) a.p. The lowest value was noticed at calving day followed by significant increase (p <0.05) and reached maximum level at day 28 P.P. The decrease in serum total cholesterol concentration during the last stage of pregnancy is likely due to the increased requirement of fetal tissues as well as maternal glands for steroid hormone synthesis (Pysera and Opalka, 2000). This result was lined with Castillo et al. (2005), Nath et al. (2005). Seifi et al. (2007). Hagawane et al. (2009) and Quiroz-Rocha et al. (2009) who observed that the cholesterol level was low in dry pregnant buffaloes compared with lactation buffaloes. Also, it was lined with Ashmawy (2015b) who reported that the cholesterol level was low in the buffaloes near parturition. The lower cholesterol level in these species near parturition could be attributed to the increased utilization for steroid synthesis around parturition (Saeed et al., 2009 and Saeed & Khan, 2012). Ling et al. (2003) and Yaylak et al. (2009) also found that the cholesterol level was low in the dry period and begin to increase gradually after calving to the peak at the mid lactation in dairy animals.

Also, Celeska *et al.* (2015) reported a significant increase (p < 0.05) in serum total cholesterol at days 5 ,10 and 30 post calving compared with day 5 pre calving. Hypocholesteremia was maintained during the ante partal period in dairy animals, as a result of low "de novo" synthesis of cholesterol. During the post partum period, serum cholesterol concentration significantly increased, thus 30 and 60 days after calving they reached the highest value. Also, the liver ability for synthesis of apo lipoproteins for cholesterol transport was maintained and the highest significant cholesterol concentration was reached at day 30 and day 60 after calving (Celeska *et al.*, 2015).

Piccione *et al.* (2012) reported that serum total cholesterol and triglycerides were significantly affected by the physiological status. There was a significant decrease (p < 0.05) at days 3 and 7 P.P compared with day 28 P.P. It could be attributed to the switch in synthesis of lipoproteins from the hepatocytes in postpartum period as there was reduction in VLDL-c contain the large proportion of cholesterol among the lipoprotein (Mohammad, 2011). The higher level of cholesterol with advancement of lactation was a physiological

adjustment to meet the lactation requirements (Ashmawy, 2015a).

The current study revealed lower levels of serum total cholesterol in buffaloes when compared with those reported by Tajik & Nazifi (2011). Also, it was lower than those reported by Ghada *et al.* (2015). It may be attributed to lower blood cholesterol during summer than during winter season, in lactating Murrah buffaloes (Verma *et al.*, 2000). This shows that cholesterol concentration decreases markedly with the increase in environmental temperature (Marai *et al.*, 1995 and Habeeb *et al.*, 1996). The marked decrease in cholesterol concentration may be due to dilution as a result of the increase in total body water or to the decrease in acetate concentration, which is the primary precursor for the synthesis of cholesterol (Verma *et al.*, 2000).

6- Serum Vit. A (µg/dl):

In the present study, we noticed that there was a significant decrease (p < 0.05) in serum vit. A level and reached maximum at day 7 P.P. It may be due to the transfer of large amounts of retinol and its derivatives into colostrum (Goff *et al.*, 2002). This result was lined with Van Merris *et al.* (2004), Debier *et al.* (2005), Rezamand *et al.* (2007) and Abd Eldaim *et al.* (2010) who found that plasma retinol and Beta carotene levels in animals decrease at the end of gestation reach their lowest values around parturition, increase again during the first week of lactation and return to basal levels by 15 days after parturition.

The obtained results showed that haptoglobin was positively correlated with albumin, total bilirubin and fibrinogen. Total cholesterol and vit. A were negatively correlated with albumin, total bilirubin, fibrinogen and haptoglobin. These results showed the liver's response to avoid tissue damage, health problems and diseases that may occurred during transition period - indicated by increased production of natural antioxidant (albumin, haptoglobin and total bilirubin) and decreased production of lipoproteins due to enhanced lipomobilization and negative energy balance during the mentioned period. The different degree of correlation between various proteins is not surprising, particularly when the long period is considered (Bertoni *et al.*, 2008).

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العلاقة بين بروتينات المرحله الحاده خلال المرحله الانتقاليه بالجاموس

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استهدفت هذه الدراسة توضيح التغيرات في بروتينات المرحلة الحادة سواء كانت موجبة أو سالبة وحساب معامل الارتباط بين القياسات البيوكيميائية ولتحقيق هذا الهدف اجريت الدراسة في محافظة أسيوط في مزرعة تابعة لجمعية أرض الخير. اشتملت الدراسة على ٢٩ جاموسة في الفترة الاخيرة من العشر (مرحلة التجفيف) ، وخضعت الحيوانات محل الدراسة الى معاملات غذائية وتربية موحدة واعتمدت المعلومات المتعلقة بالتغذية وانتاجية اللبن على سجلات المزرعة. ولاجراء التحاليل البيوكيميائية تم جمع عينات الدم الكامل لتعيين مستوي الفيبرينوجين وعينات بلازما الدم لتعيين فيتامين أ، وعنات مصل الدم لتعيين الألبيومين، الكولسترول الكلى والبلير وبين الكلي والهابتوجلوبين في الفترات التالية: (٢٠) يوم ، (-١٤) يوم ، (-٢) يوم قبل الولادة ، يوم الولادة ،اليوم الثالث بعد الكامل لتعيين مستوي الفيبرينوجين وعينات بلازما الدم لتعيين فيتامين أ، وعينات مصل الدم لتعيين الألبيومين، الكولسترول الكلى والبلير وبين الكلي والهابتوجلوبين في الفترات التالية: (٢٠) يوم ، (-١٤) يوم ، (-٢) يوم قبل الولادة ، يوم الولادة ،اليوم الثالث بعد معنوية في تركيز بعض بروتينات المرحلة الحادة ، سواء كانت موجبة او سالبة اوضحت النتائج وجود تغيرات معنوية للفيبرينوجين الولادة ، اليوم الثالث البلادة ، من وجود تغيرات معنوية في الولادة ، اليوم الثالث بعد معنوية في تركيز بعض بروتينات المرحلة الحادة ، سواء كانت موجبة او سالبة اوضحت النتائج وجود تغييرات معنوية للفيبرينوجين مع زيادة قصوى في اليوم الثالث بعد الولادة، تغيرات غير معنوية في الهابوجلوبين مع زيادة قصوى في الأسبوع الاخيرقبل الولادة. كما وجدت زيادة معنوية في البيليروبين في يوم الولادة وفترات ما بعد الولادة مقارنة بالفترات ما قبل الولادة ، وما وجدت زيادة معنوية في البيليروبين في يوم الولادة وفترات ما بعد الولادة مقارن الفاقي ولولادة ، ووصلت إلى الولادة ورحمات ما قبل الولادة ، ووم الرابع عشر بع م وما وجدت زيادة معنوية في البيليروبين في يوم الولادة وفترات ما بعد الولادة مقارنة بالفترات ما قبل الولادة ، ووصلت إلى الحد وما وجدت زيادة معنوية في البيليروبين في في قبرات ما ولادة وبلغ أقصى انخفاض في يوم ولادة مقارنة معنوات ما قبل وم بعا وجد وي الدفاض معنوى للكوليسترول الكلي في قدرات ما قبل الولادة وبلغ أقصى انخفاض في ما ولادة مقارات ما قبل وم