



## Hemato-Biochemical Profiling in Relation to Metabolic Disorders in Transition Dairy Cows

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### ABSTRACT

This study was aimed to provide a complete picture of dynamics of selected hematological and serum biochemical parameters in dairy cows from late pregnancy to early postpartum period, giving new and useful information about the guidelines for the management strategies during different physiological phases. This study was carried out on thirty clinically healthy Holstein Friesian dairy cows; in good nutritional condition. Blood samples were collected two weeks before the expected parturition, during the 1<sup>st</sup> post-partum week and during the 3<sup>rd</sup> week after parturition. Cows under investigation were clinically examined including body temperature, pulse, respiration, mucous membranes and ruminal movement. Two blood samples were collected: whole blood samples for hematological picture, while serum samples for biochemical analysis which included total proteins, albumin, globulin, AST, ALT, glucose, Creatinine, Blood urea nitrogen (BUN), Total cholesterol, Beta hydroxy butyrate ( $\beta$ HBA), triglycerides, Nitric Oxide (No), Malondialdehyde (MDA), total antioxidant capacity (TAC), serum calcium, magnesium, sodium, potassium, chloride and phosphorus. Significant changes between pre and post-partum period were observed in values of hemogram and biochemical parameters including RBCs, PCV%, hemoglobin, MCV, MCHC, MCH, WBCs count and differential leucocytic count, total proteins, albumin, globulin, glucose, BUN, Total cholesterol, triglycerides, calcium, phosphorus, magnesium, sodium, chloride, No, MDA and TAC, while non-significant changes were observed in AST, ALT, Potassium, Creatinine and  $\beta$ HBA levels. It can be concluded that late pregnancy and the onset of lactation were accompanied by marked changes in hemato-biochemical and oxidative stress markers which have great impact on dairy cow's health, immunity and disease susceptibility.

### Key words:

Transition period, dairy cattle, hemato-biochemical, antioxidants.

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### 1- INTRODUCTION:

Peri-parturient or transition period including the change from a gestational non-lactating to a non-gestational lactating state (Morgante et al. 2012). It is characterized by alterations in both metabolic and immune functions in animals (Piccione et al. 2012). Some of these changes are related to increases in energy requirements needed by fetus and lactogenesis. Due to metabolic, behavioral and hormonal changes that occur around parturition; cows had a marked decrease in dry matter intake (Grummer et al. 2004) resulting in a negative energy balance (NEB) that

further aggravated by withdrawal of nutrient to the mammary gland (Leroy et al. 2008). This energy reduction results in increased fat mobilization followed by generation of lipid peroxides and reactive oxygen species (ROS). These ROS are normally neutralized by sufficient antioxidant levels of living organisms (Trevisan et al. 2001). Peri-parturient disorders in dairy cows occur due to imbalance between increased production of lipid peroxides, ROS and antioxidant defense of the body. Reduced availability of antioxidant defenses near the time of parturition increases oxidative stress (Waller, 2000; Gitto et al.,

2002). So the health problems affect cows after birth resulted from poor nutritional management immediately after calving (Bell, 1995). These problems include milk fever, fatty liver, ketosis, retained placenta, displacement of the abomasum and severely suppressed immune function (Goff and Horest, 1997). Hemato-biochemical profiling of dairy cattle during transition period has been reported to provide a reliable method to monitor animal health during this critical period with the main purpose to predict a herd's susceptibility to production diseases (Kevin and Ellen, 2012). So, the present study was designed for monitoring of dairy cows during peri-parturient period (3 weeks pre-partum till three weeks post-partum) for evaluation clinical, hematological and biochemical changes, oxidant-antioxidant status during this critical period.

## 2- MATERIAL AND METHODS:

### 2.1. Animals:

Thirty apparently healthy highly lactating multiparous Friesian dairy cows from a private farm belong to Behira governorate, Egypt. The average age 4- 8 years, weighting  $480 \pm 5.86$  kg, the average body condition score (BCS) of cows was 4.0, with daily milk yield  $15.25 \pm 0.22$  kg per lactating animal where cows were milked automatically two times daily. Cows were fed daily on diet consisting of 30 Kg berseem (*T. alexandrum*), 3 Kg rice straw and were fed concentrate diet (16% protein) two times daily and their amount was differ according to her productive status where the amount for late pregnant animal was 7 kg daily while for early lactation was 10 kg daily per individual animal. Minerals and vitamin block and tap water were available all the time. All cows were kept in a free-stall barn under the same environmental condition. Cows were divided into 3 groups :( 1<sup>st</sup> group was at the last 2 weeks before parturition (-14days) while, the 2<sup>nd</sup> group was at the first week of parturition (+7 days) and the 3<sup>rd</sup> group was at the third week (+21days) after calving).

### 2.2. Sampling:

Two blood samples were collected from each animal. First blood samples (coagulated blood) Were used for determination of serum total proteins , albumin , globulin ( by subtracting the albumin from the total protein values ) , AST, ALT, glucose, Creatinine, Blood urea nitrogen (BUN) , Total cholesterol, Beta hydroxy buterate ( $\beta$ HBA) ,triglycerides, Nitric Oxide (No), Malondialdehyde (MDA), total antioxidant

capacity (TAC), serum calcium, magnesium , sodium , potassium, chloride and phosphorus while, second blood samples (non-coagulated blood on EDTA) were used for hematological examination.

### 2.3. Clinical examination:-

Complete thorough clinical examination of all cows under investigation was performed according to the method described by (Kelly, 1984). Including temperature, pulse, respiration and ruminal movement. Also, Periodical rectal examination was applied to detect the expected parturition date on all cows under investigation according to the method described by (David et al., 2001).

### 2.4. Hematological examination:-

Red blood Cell count (RBCs  $\times 10^6/100\text{ml}$ ), hemoglobin concentration (Hb g/l), packed cell volume (PCV%), mean corpuscular volume (MCV fl), mean corpuscular hemoglobin (MCH pg), mean corpuscular hemoglobin concentration (MCHC g/dl), WBCs count( $\times 10^3/100\text{ml}$ ) and differential leucocytic count were determined by using of fully automated veterinary hematology analyzer, Exigo, Boule medical AB., Sweden in the central laboratory, Faculty of veterinary medicine, Alexandria University.

### 2.5. Biochemical analysis:-

Determination of serum concentrations of calcium , phosphorus , magnesium , sodium , potassium, chloride, AST, ALT, Albumin, Total proteins glucose and Creatinine were carried out by using commercial test kits supplied by (Bio-labo, France ) according to the method described by Clark et al., (1975) , Daly and Ertingshausen (1972), Khayam-bashi et al., (1977) , Mathieu and Sfbc (1976) , Murray (1984) , Dumas and Biggs (1972), Curtis et al., (1975) and Young (1995) respectively. While analysis of  $\beta$ HBA , BUN, Total cholesterol and triglycerides were carried out by using commercial test kits supplied by (Ben-Biochemical Enterprise, Italy) and (Vitro Scient., Egypt) according to the method described by Parry et al., (1980), Rock et al., (1987) and Balistreri and Shaw (1987) respectively. Total anti-oxidant capacity (TAC), Malondialdehyde (MDA) and Nitric oxide (NO) were analyzed using test kits supplied commercially by (Bio-Diagnostic, cairo , Egypt) according to the method described by Koracevic *et al.*, (2001), Satoh (1978) and Montgomery and Dymock (1961) respectively.

The free radical defense system in these dairy cows was assessed by measuring total antioxidant capacity (TAC) which was considered the cumulative effect of all anti-oxidants present in the blood and in body fluids (Miller and Rice-Evans, 1996) not just the antioxidant capacity of a single compound.

## 2.6. Statistical analysis:-

Data were analyzed using the packaged SPSS program for windows version 10.01 (SPSS, 2000 Inc., Chicago, IL). Data were presented as mean  $\pm$  standard Error (SE). Differences between groups were determined by the one way analysis of variance (ANOVA). Significance level was set at  $P \leq 0.05$ .

## 3- RESULTS

### 3.1. Clinical findings:

Clinical findings of the examined cows (Table 1) showed a non-significant increase ( $P \leq 0.05$ ) in temperature during the period from 2 weeks pre-partum to 3 weeks postpartum. The respiratory and pulse rates showed a significant increase in late gestational period when compared to early lactation stages. Pallor m.m. was observed during pre-parturient period. The ruminal movements showed a significant decrease during 2 weeks pre-partum and 1<sup>st</sup> week of lactation compared to advanced postpartum stage.

### 3.2. Hematological examination:

RBCS count, PCV%, hemoglobin, MCV, MCHC, MCH, WBCS count, neutrophils and lymphocytes was significantly higher in peri-parturient cows at 2 weeks before calving than in post-parturient cows at the first and third week after calving. On the other hand the total number of MID "eosinophils, basophils and monocytes" showed non-significant changes at the three stages of the transition cycle. Tables (2, 3, 4).

### 3.3. Biochemical findings:-

Serum calcium level was significantly lower post calving than pre-parturient period, while phosphorus level showed a significant decrease in its mean value at the first week post-partum than its level before parturition and the third week after calving, level of magnesium in the third week after calving was

significantly higher than those at the second week before parturition and the first week post-partum. (Table 5)

Analysis of the serum sodium showed that its mean value was significantly decreased at the first week after parturition as compared with its mean value at the last 2 weeks before parturition and the third week after calving, while the mean value of chloride showed significant increase at the last 2 weeks before parturition as compared either with its mean value at the first week after parturition or at the third week after calving. On contrary potassium level was not significantly changed either before or after calving. Table (6).

Serum total protein, albumin and globulins values were significantly lower at 1<sup>st</sup> and 2<sup>nd</sup> group when compared with the 3<sup>rd</sup> one. Table (9)

The mean value of blood urea nitrogen was significantly increased at the third week after calving as compared to the last two weeks before calving and the first week after calving. On other hand the mean value of creatinine showed non-significant changes between all groups. Table (11)

The serum glucose estimation revealed that its mean value was significantly lower in cow's serum at the first week post-partum than its level at the second week before parturition and the third week after calving, while levels of AST and ALT were not significantly changed either before or after calving. Table (10)

The mean value of total cholesterol showed a significant increase at the third week after calving, while triglycerides was significantly higher at 2 weeks before calving, on contrary BHBA showed no significant changes at the three stages of the production cycle. Table (8)

Serum level of TAC at the second week before parturition and in the first week after calving were significantly lower than those at the third week post-partum, in contrast to the mean value of No and MDA were significantly higher at the 2 weeks before parturition and in the first week after calving than those at the third week post-partum. Table (7)

**Table (1):** Mean values ( $\pm$ SE) of some clinical examination parameters of transition cows:

Parameters	Group (1)	Group (2)	Group (3)
Body temperature °C	38.6 $\pm$ 0.6a	39.0 $\pm$ 0.5a	38.3 $\pm$ 0.4a
Respiratory rate / min.	23.5 $\pm$ 0.5a	21.5 $\pm$ 0.5b	19.5 $\pm$ 1.0c
Pulse rate / min.	69.5 $\pm$ 0.5a	60.5 $\pm$ 0.7b	58.0 $\pm$ 0.9c
Mucous membrane	Pale	Pale	Pale
Ruminal movement / 2 min.	2 $\pm$ 0.00	2 $\pm$ 0.00	3 $\pm$ 0.00

**Table (2):** Mean values ( $\pm$ SE) of RBCS ( $\times 10^6$ /ml), Hb (mg/100ml) and HCT (%) in Transition dairy cows:

Group	RBCS ( $\times 10^6$ /ml)	Hb (mg/100ml)	PCV (%)
1	5.69 $\pm$ 0.10a	10.94 $\pm$ 0.27a	32.60 $\pm$ 0.60a
2	4.82 $\pm$ 0.10b	9.90 $\pm$ 0.28b	28.56 $\pm$ 0.50b
3	5.07 $\pm$ 0.09b	9.73 $\pm$ 0.19b	29.50 $\pm$ 0.47b

Means bearing different letters are significantly differed at ( $P < 0.05$ ).

**Table (3):** Mean values ( $\pm$ SE) of MCH (Pg.), MCHC (g/dl) and MCV (FL) in transition Dairy cows:

Group	MCH (Pg.)	MCHC (g/dl)	MCV (FL)
1	16.45 $\pm$ 0.18a	36.28 $\pm$ 0.72a	49.72 $\pm$ 1.26a
2	14.82 $\pm$ 0.63b	34.90 $\pm$ 0.26b	46.87 $\pm$ 0.92b
3	15.08 $\pm$ 0.45b	35.75 $\pm$ 0.60b	48.05 $\pm$ 0.52b

Means bearing different letters are significantly differed at ( $P < 0.05$ ).

**Table (4):** Mean values ( $\pm$ SE) of WBCS ( $\times 10^3$ /ml) , Lymphocytes, MID and Neutrophils ( $\times 10^3/\mu$ l) in transition dairy cows:

Group	WBCS ( $\times 10^3$ /ml)	Lymphocytes ( $\times 10^3/\mu$ l)	MID( $\times 10^3/\mu$ l)	Neutrophils ( $\times 10^3/\mu$ l)
1	12.25 $\pm$ 0.46a	7.07 $\pm$ 0.33a	0.98 $\pm$ 0.09a	5.30 $\pm$ 0.92a
2	10.93 $\pm$ 1.19b	3.95 $\pm$ 0.70b	0.99 $\pm$ 0.05a	4.01 $\pm$ 0.46b
3	11.10 $\pm$ 0.83b	5.52 $\pm$ 0.94b	0.98 $\pm$ 0.09a	4.95 $\pm$ 1.26b

Means bearing different letters are significantly differed at ( $P < 0.05$ ).

**Table (5):** Mean values ( $\pm$ SE) of serum calcium (mg/dl), phosphorus (mg/dl) and Magnesium (mg/dl) in Transition dairy cows:

Group	Ca (mg/dl)	Ph (mg/dl)	Mg (mg/dl)
1	8.92 $\pm$ 0.31a	4.78 $\pm$ 0.34a	2.08 $\pm$ 0.10b
2	7.11 $\pm$ 0.22b	3.53 $\pm$ 0.19b	1.99 $\pm$ 0.05b
3	7.08 $\pm$ 0.29b	5.29 $\pm$ 0.30a	2.73 $\pm$ 0.11a

Means bearing different letters are significantly differed at ( $P < 0.05$ ).

**Table (6):** Mean values ( $\pm$ SE) of serum sodium, potassium and chloride (mmol/l) in Transition dairy cows:

Group	Na (mmol/l)	K (mmol/l)	CL (mmol/l)
1	149.73 $\pm$ 7.90a	3.65 $\pm$ 0.34a	103.99 $\pm$ 1.54a
2	123.02 $\pm$ 2.08c	3.84 $\pm$ 0.20a	94.92 $\pm$ 1.45b
3	133.03 $\pm$ 2.34b	3.77 $\pm$ 0.17a	96.46 $\pm$ 1.49b

Means bearing different letters are significantly differed at ( $P < 0.05$ ).

**Table (7):** Mean values ( $\pm$ SE) of oxidants /antioxidant profile in transition dairy cows:

Group	TAC (Mm/l)	NO ( $\mu$ mol/l)	MDA (nmol/ml)
1	0.54 $\pm$ 0.10b	15.11 $\pm$ 0.36a	14.45 $\pm$ 0.73b
2	0.50 $\pm$ 0.11b	16.39 $\pm$ 1.02a	23.18 $\pm$ 0.45a
3	0.83 $\pm$ 0.04a	13.08 $\pm$ 0.40b	8.02 $\pm$ 0.24c

Means bearing different letters are significantly differed at (P<0.05).

**Table (8):** Mean values ( $\pm$ SE) of serum Cholesterol, Triglycerides and BHBA (mg/dl) in Transition dairy cows:

Group	Cholesterol (mg/dl)	Triglycerides(mg/dl)	BHBA(mg/dl)
1	150.95 $\pm$ 4.78b	34.39 $\pm$ 1.83a	17.79 $\pm$ 0.59a
2	139.65 $\pm$ 6.77b	29.04 $\pm$ 1.05b	19.33 $\pm$ 0.63a
3	162.05 $\pm$ 13.49a	24.18 $\pm$ 1.25c	17.44 $\pm$ 0.70a

Means bearing different letters are significantly differed at (P<0.05).

**Table (9):** Mean values ( $\pm$ SE) Serum total proteins, Albumin and globulins in transition dairy cows:

Group	Total Proteins (g/dl)	Albumin (g/dl)	Globulins (g/dl)
1	6.24 $\pm$ 0.18b	3.16 $\pm$ 0.10b	3.08 $\pm$ 0.22b
2	5.40 $\pm$ 0.19c	2.99 $\pm$ 0.20c	2.41 $\pm$ 0.16c
3	7.60 $\pm$ 0.19a	4.06 $\pm$ 0.20a	3.54 $\pm$ 0.16a

Means bearing different letters are significantly differed at (P<0.05).

**Table (10):** Mean values ( $\pm$ SE) of serum glucose (mg/dl), ALT (u/l) and AST (u/l) in Transition dairy cows:

Group	Glucose (mg/dl)	ALT (u/l)	AST (u/l)
1	55.65 $\pm$ 1.83a	24.66 $\pm$ 1.55a	65.93 $\pm$ 5.70a
2	42.48 $\pm$ 1.41c	22.40 $\pm$ 1.35a	81.66 $\pm$ 3.63a
3	52.27 $\pm$ 1.49b	26.27 $\pm$ 1.91a	49.53 $\pm$ 4.20a

Means bearing different letters are significantly differed at (P<0.05).

**Table (11):** Mean values ( $\pm$ SE) of Blood urea and serum creatinine levels in transition dairy cows:

Group	Urea (mg/dl)	Creatinine (mg/dl)
1	35.22 $\pm$ 1.07b	1.02 $\pm$ 0.02a
2	36.49 $\pm$ 0.90b	1.12 $\pm$ 0.05a
3	39.44 $\pm$ 1.09a	1.00 $\pm$ 0.03a

Means bearing different letters are significantly differed at (P<0.05).

#### 4. DISCUSSION:

Transition period is one of the most critical periods during life of dairy cows. Where cows become undergo physiological changes to prepare themselves for the onset of lactation till reach the peak of milk production. Clinical examination of cows during periparturient period showed some significant alteration in accordance with Ghanem, *et al.* (2012) including the increase in respiratory and pulse rate which may be related to stress of pregnancy and parturition Kelly (1984). The ruminal atony or decrease in ruminal movements near parturition could be attributed to increased intra-abdominal pressure by gravid uterus on rumen during advanced pregnancy

and somewhat reduced calcium level during early post parturient stage Goff (2008). Transition stage not only affect clinical situation of dairy cows but also affect significantly internal hemogram including reduced RBCS count , Hb.content , MCV, PCV,MCH and MCHC values in early lactation period when compared with late pregnant one, these obtained results coincides with that observed by Gävan, *et al.* (2010) and Ghanem, *et al.* (2012), the reduction in RBCS count, MCV, and MCH returned to deficiency of iron during peri-parturient periods, that resulted in a decreased MCV (microcytosis) because cells undergo an extra division due to inadequate hemoglobin concentration (Roy *et al.*, 2010). PCV% and Hb content (gm/dl) showed a significant decrease

during peri-parturient periods, that was attributed to anemia due to stress of pregnancy and parturition (Daramola *et al.*, 2004). Elevation of serum cortisol around parturition (Preisler *et al.*, (2000) and Kim *et al.*, (2005), resulting in a significant decrease in total leucocytic count, lymphocyte % and neutrophils % in the early lactating group compared with late pregnant one. The significant decrease of serum calcium level after birth was coincides with that observed by Zeinab (2007) and Hagawane *et al* (2009) whose suggested that deficiency may be returned to improper food metabolite absorption from gastrointestinal tract, insufficient mobilization of Ca from the skeleton, excessive Ca loss in urine and draining of high percent of Ca and phosphorus Hassan(1988) in colostrum during excessive milking soon after birth. The significant changes of magnesium was in agreement with Crnkic(2010) who concluded that these changes may be due to insufficient food intake or poor bioavailability of Mg in the digestive tract but, these results disagreed with Bajora *et al*(1994) who found no significant changes in serum magnesium level in pre- and post-parturient cows. Na and Cl in pre-partum were significantly higher than those at calving and post-partum due to their secretion in colostrum and milk Daramola *et al.*, (2004) and Ghanem *et al.*, (2012). On other hand potassium level not changed in three groups, Serum total proteins as parturition approach usually decreased may be due to the fetus synthesizes all its proteins from the amino acids derived from the dam and fetal muscle growth reaching its maximum size at late pregnancy (Jainudee, and Hafez, 1994). Serum globulins showed a significant decrease during transition period, which associated mainly with the production of colostrum that rich in antibodies that derived from  $\gamma$ -globulins and other immunological changes occurred around parturition (Janku *et al.*, 2011). Serum total protein, albumin and urea are indicators of diet protein intake (Toharmat *et al.*, 1999). Urea is a good indicator of long term intake of dietary protein (Toharmat *et al.*, 1999 and Kida, 2003). On contrary to albumin which is not a long indicator of protein intake due to its short half-life in blood. The significant increase of serum urea level at 3<sup>rd</sup> week after calving might be returned to excess dietary protein intake that enhance the hepatic microsomal activity which favors the transformation of alimentary ammonia into urea (Giuseppe *et al.*, 2006). On the other hand the non-significant changes in creatinine level disagree with Ghanem *et al.*, (2012) who reported elevated

creatinine level near calving. Creatinine level rises around parturition due to fetal maternal circulation and the load of organic waste of the newborn Ferrell (1991). During transition period there is no liver cell injury (Castaneda-Gutierrez *et al.*, 2009), so the levels of ALT and AST not-significantly changed in periparturient period. Our data findings concerning glucose are compatible with those obtained by Herdt (2000) who reported that, the prepartum increase of some hormones as cortisol and estrogen resulting in raising of glucose value. While the transient fall in glucose levels in the early lactation can be returned to withdrawal of large amount of blood glucose by the mammary gland for the synthesis of milk lactose Chandra *et al.*, (2013) and Neama, 2015). Serum total cholesterol concentration was minimum following calving and got build up as the lactation progresses Grummer (1993) and Zeinab (2007). The higher level of cholesterol with advancement of lactation was a physiological adjustment to meet the lactation requirements as those reported by Neama (2015). The hormonal level of estrogen along with thyroxin play a vital role in reducing the cholesterol levels during pregnancy (Hagawane *et al.*, 2009). Triglycerides mean values revealed significant decrease in the early lactating group compared with late pregnancy group. As reported previously by Ghada *et al.*, (2015), Triglycerides concentration significantly decreased at calving and remained at the lowest concentration until the 8<sup>th</sup> week of lactation. The reasons for such disturbances are probably due to triglycerides accumulation in the liver (Bremmer *et al.*, 2000 and Turk *et al.*, 2004); and triglycerides taking up by the mammary gland for milk fat synthesis and secretion (Bernard *et al.*, 2008 and Mantovani *et al.*, 2010). TAC concentration was significantly lower before calving (Castillo *et al.*, 2003; Bozukluhan *et al.*, 2013 and Mousa and Galal 2013) and the highest concentration of TAC was observed 8 weeks post-partum (Castillo *et al.*, 2006). The lowest mean value of TAC (2 weeks before calving and at parturition) was coincident with the absence of vitamins and mineral supplementation, absorption along with the stress of heavy pregnancy and onset of parturition and lactation. Increased MDA and NO concentration just before and after calving were due to initiation of lactation and colostrum secretion. But these changes did not last long as animal adapted itself, therefore the postpartum fatty liver and ketosis did not occur and this reflected in the level of BHBA, this explain the

non-significant changes that noticed in BHBA during transition periods in cows under investigation El-Maghraby and Mahmoud (2016).

## CONCLUSION

Results of our study indicate the occurrence of significant changes of hematological, serum biochemical and indicators of oxidant-antioxidant status of dairy cows during the per-partum and post-partum periods. Dairy cows seemed to have more oxidative stress and low antioxidant defense capacity during early lactation stage, which may contribute to the incidence of many metabolic diseases. Therefore, further studies are needed to clarify correlation between increased oxidative stress and incidence of postpartum production diseases of transition dairy cows.

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