

Alterations of Lipid and Mineral Metabolism during Late Pregnancy and Early Lactation in Holstein–Friesian cows.

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Abstract

The aim of the study was to evaluate changes in blood Lipids and minerals concentrations in Holstein–Friesian dairy cows during late pregnancy, parturition and early lactation. Fifteen clinically healthy dairy cows in good nutritional condition nearly ages, weights and body conditions. Blood samples were taken from 15 cows at 4 and 2 weeks prepartum, day of parturition and at 2 and 4 weeks postpartum. Plasma was separated and analyzed for determination of lipids and lipoproteins profile (total cholesterol, triacylglycerol, Phospholipids, non-esterified fatty acids (NEFA), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), and very low density lipoprotein cholesterol (VLDL-c), β - Hydroxybutyrate (β HBA), phospholipids concentrations and lipoprotein lipase activity. Also, plasma minerals, total proteins and their fraction (albumin, globulin) were determined. The obtained results revealed that, plasma total cholesterol, triacylglycerol, (VLDL-c), phospholipids concentrations and lipoprotein lipase activity were significantly increased at late gestation and early lactation period. Meanwhile, plasma low density lipoprotein cholesterol (LDL-c) and β -HBA concentrations were significantly increased during postpartum as compared with day of parturition. Plasma NEFA and phospholipids were significantly decreased at 4 and 2 weeks prepartum, respectively. Plasma total proteins and albumin concentrations were significantly increased, while total globulin level showed a non-significant during late pregnancy early lactation. A marked increase in plasma calcium, inorganic phosphorus, magnesium, Iron and zinc levels were observed during the late pregnancy and early lactation. While plasma copper and manganese concentrations showed a significant increase at late pregnancy as when compared with the day of parturition. It can be concluded that late pregnancy and onset of lactation were accompanied by marked changes in plasma levels of lipids and lipoprotein profiles, proteins and their fractions and metabolic disturbance in mineral metabolism.

Introduction

The periparturient period during late pregnancy and early lactation is important in terms influence on health of dairy cows which undergo massive changes and great metabolic stress for dairy cows **Piccione et al., (2012)**. During the pregnancy all metabolic pathways are involved in sustaining the foetus growth **(Bell, 2000)**. Instantly after calving a copious milk synthesis and secretion accompanied by alterations in blood metabolite **(Wathes et al., 2009)**. Lipolysis and lipogenesis are regulated to increase lipid reserve during pregnancy and subsequently these reserves are utilized following parturition and the initiation of lactation Moreover, characteristic changes in lipid metabolism were found during late pregnancy and early lactation in most mammals **(Roche et al., 2009)**. Triglyceride accumulates in the liver when its synthesis exceeds disappearance via hydrolysis and lipoprotein export prior to or soon after parturition **Drackley (2003)**. Ruminants have an inherently low capacity for synthesis and secretion of VLDL-c to export triglyceride from the liver but a similar capacity to reconvert NEFA back to triacylglycerol. Moreover, the rate of production of triacylglycerol in the liver is influenced at the time of calving **Litherland et al., (2011)**. The main blood indicators of lipomobilization in ruminants include β HBA, the most important and abundant ketone bodies and NEFA **Cincović et al. (2012)**. NEFA are greatly accumulated as triacylglycerol in the liver, primarily due to a decrease in the VLDL-c synthesis by hepatocytes **Sevinc et al., (2003)**. Lipid mobilization is exacerbated in the dairy cow with the genetic drive for high milk production **Contreras et al, (2009)**. In cattle, increases in blood lipid content are known to induce metabolic diseases such as fatty liver, ketosis and predispose dairy cows to inflammatory based diseases **Herdt (2000)**. The total serum proteins levels weresignificantly affected from the physiological period and increased during lactation if compared to late gestation **(Giuseppe et al., 2012)**. During Late pregnancy and early lactation dairy cows requirements of minerals such as calcium, phosphorus and magnesium indispensable for pregnancy and lactation **(Samardzija et al., 2011)**. Calcium, phosphorus and magnesium are limited to growth and bone formation; high amount of milk minerals is observed in late pregnancy and early lactation **Kupczynski et al., (2011)**. It is also affected by other factors such as stage of lactation and pregnancy status **(Liesegang et al., 2008)**. Trace element copper, zinc, and iron are required for synthesis of many proteins and vast array of enzymes **(Ceylon et al., 2008)**. In addition, zinc is an essential component of over 70 enzymes found in mammalian tissues that require in protein, nucleic acid, carbohydrate, lipid metabolism and immune system **(Spears, 2003)**.

The aim of this study was designed to providing useful information on changes of some biochemical blood parameters in dairy Holstein Friesian cows from late pregnancy to the early lactation period to clarify the metabolic disorders that may occur

in high lactating cows that reflect the guidelines and highlights for the management strategies to reach higher rates of production.

Materials and Methods

Animals:

Fifteen clinically healthy pregnant Holstein Friesian cows of 27 months old and average body weight (685 ± 18.62) kg were selected from private high production dairy farm at Sharkeia province during the late pregnancy (four weeks prior to calving) and early lactation (four weeks after parturition) according to reproductive farm records. Animals were fed on total mixed ration according to NRC (concentrate ration and corn silage) fresh and clean drinking water was supplied ad-libitum. The animals were selected in good health and nutritional condition. During the period of the study the animals were proved to be free from any external, blood and internal parasites.

Sampling:

Individual blood samples were collected before the expected parturition from the jugular vein of each animal five times and periodically at 4 and 2 weeks prepartum, day of parturition and 2 and 4 weeks postpartum.

Blood samples:

Approximately 10 ml of heparinized blood samples were obtained in clean, dry screw capped tubes and plasma was separated by centrifugation at 3000 r.p.m for 10 minutes. The clear plasma was obtained and received in dry sterile sample tube using sterilized pipettes and kept in deep freeze at -20°C until used for subsequent following biochemical parameters.

Biochemical analysis:

Plasma total cholesterol was determined according to the method described by **Ellefson and Caraway, (1976)**, triglycerides **Stein, (1987)**, NEFA and β -Hydroxybutyric acid (BHBA) **Duncombe (1964)**, LDL-c and VLDL - c was estimated according to formula described by **Friedewald, (1975)**. Lipoprotein lipase concentration according to **Karpe, et al., (1992)**. Total protein, **Dumas et al., (1981)**, albumin **Bauer, (1982)**. Plasma globulins were calculated by subtraction of albumin from the total protein **Dumas and Biggs (1972)**, calcium, **Gindler and King (1972)**. Phosphorus, **Goldenberg, (1966)**, magnesium, **Gindler, (1971)**, iron, **Colenbrander and Vink, (1969)**. The levels of plasma copper, zinc, iron and manganese were estimated by atomic absorption spectrophotometer using Atomic Absorption Spectrometer model 2380 (PERKIN-ELEMER).

Statistical analysis:

The obtained data were statistically analyzed using analysis of variance (ANOVA) test and comparative of means were performed according to Duncan Multiple Range test for comparison of Means according to **Snedecor, (1969)** using **SPSS14(2006)**.

Results and Discussion

Table (1): Changes in plasma total cholesterol, Triacylglycerol, HDL-c, LDL-c and VLDL-c concentrations during late pregnancy and early lactation in Holstein–Friesian cows (n=5).

Animal groups	Cholesterol mg/dL	Triacylglycerol mg/dL	HDL-c mg/dL	LDL-c mg/dL	VLDL-c mg/dL
4 weeks prepartum	164.90±4.41 ^a	102.36±3.99 ^a	62.54±3.73 ^a	81.91±0.87 ^a	20.74±0.80 ^a
2 weeks prepartum	161.21±3.15 ^a	101.74±3.70 ^a	60.36±2.55 ^a	79.90± 1.78 ^a	20.35±0.74 ^{ab}
Day of parturition	136.57±5.64 ^b	81.69±2.99 ^c	51.77±4.02 ^a	66.86±4.72 ^b	16.85±0.96 ^c
2 weeks postpartum	155.21±2.75 ^a	90.94±1.68 ^{bc}	54.37±2.90 ^a	82.66±3.64 ^a	18.19±0.34 ^{bc}
4 weeks postpartum	155.38±2.54 ^a	94.32±2.94 ^{ab}	56.87±4.03 ^a	79.65±3.74 ^a	18.87±0.59 ^{abc}

*Data are presented as (means ± S.E)

*Mean values with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

Table (2): Changes in plasma NEFA, β HBA and Phospholipids concentrations and Lipoprotein lipase activity during late pregnancy and early lactation in Holstein–Friesian cows (n=5).

Animal groups	NEFA mg/dl	β HBA mg/dl	Phospholipids mg/dl	lipoprotein lipase U/L
4 weeks prepartum	38.95 ± 1.49 ^c	11.09±.82 ^b	52.39±2.86 ^{bc}	41.07±2.00 ^a
2 weeks prepartum	40.71±1.07 ^{bc}	12.72±.61 ^b	50.61±1.81 ^c	28.79 ±2.03 ^{bc}
Day of parturition	49.37±4.04 ^{ab}	12.91±1.03 ^b	64.15±5.25 ^{ab}	25.62±1.21 ^c
2 weeks postpartum	51.89±4.50 ^a	15.58±1.04 ^a	67.43±5.88 ^a	32.78±2.62 ^b
4 weeks postpartum	58.17±3.58 ^a	16.70±0.79 ^a	75.63±4.66 ^a	34.22±1.79 ^b

*Data are presented as (means ± S.E)

*Mean with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

Table (3): Changes in Plasma total protein, albumin and globulin concentrations and A/G Ratio during late pregnancy and early lactation in Holstein–Friesian cows (n=5).

Animal groups	Total protein g/dl	Albumin g/dl	Globulin g/dl	A/G Ratio
4 weeks prepartum	7.72±0.19 ^a	3.35±0.30 ^a	4.37±0.41 ^a	0.82±0.16 ^a
2 weeks prepartum	7.17±0.11 ^{ab}	2.87±0.24 ^{ab}	4.25±0.15 ^a	0.68±0.079 ^a
Day of parturition	6.09±0.32 ^c	2.33±0.31 ^c	3.66±0.35 ^a	0.64±0.127 ^a
2 weeks postpartum	6.35±0.26 ^{bc}	2.45±0.15 ^c	3.91±0.34 ^a	0.66±0.099 ^a
4 weeks postpartum	7.04±0.39 ^{ab}	2.80±0.16 ^{ab}	4.24±0.54 ^a	0.73±0.130 ^a

*Data are presented as (means ± S.E)

*Mean with different superscript letters in the same column are significantly different at (P ≤ 0.05).

Table (4): Changes in plasma calcium, inorganic phosphorous and magnesium concentrations during late pregnancy and early lactation in Holstein–Friesian cows (n=5).

Animal groups	Calcium mg/dl	Inorganic Phosphorous mg/dl	Magnesium mg/dl
4 weeks prepartum	9.07±0.51 ^a	5.34±0.29 ^a	2.36±0.21 ^a
2 weeks prepartum	7.65±0.41 ^{bc}	4.11±0.31 ^{bc}	1.89±0.08 ^b
Day of parturition	6.62±0.39 ^c	3.90±0.28 ^c	1.67±0.09 ^b
2 weeks postpartum	7.34±0.46 ^{bc}	4.91±0.34 ^{ab}	1.83±0.02 ^b
4 weeks postpartum	8.11±0.32 ^{ab}	4.99±0.22 ^{ab}	2.43±0.17 ^a

*Data are presented as (means ± S.E)

*Mean with different superscripts letters in the same column are significantly different at (P ≤ 0.05).

Table (5): Changes in plasma Copper, Iron, Zinc and manganese concentrations during late pregnancy and early lactation in Holstein–Friesian cow (n=5).

Animal groups	Copper μg/dl	Iron μg/dl	Zinc μg/ dl	Manganese μg/dl
4 weeks prepartum	91 ± 2.7 ^a	115.27 ± 4.43 ^a	84 ± 39 ^a	2.8 ± 0.21 ^a
2 weeks prepartum	85 ± 3.7 ^{ab}	104.74 ± 5.64 ^a	70 ± 2.3 ^b	2.6 ± 0.20 ^a
Day of parturition	78 ± 4.3 ^b	86.46 ± 6.73 ^b	59 ± 4.0 ^c	2.1 ± 0.73 ^b
2 weeks postpartum	81 ± 3 ^{ab}	103.32 ± 3.95 ^a	68 ± 3.6 ^{bc}	2.4 ± 0.14 ^{ab}
4 weeks postpartum	82 ± 3.3 ^{ab}	106.21 ± 4.51 ^a	74 ± 3.7 ^{ab}	2.5 ± 0.17 ^{ab}

*Data are presented as (means ± S.E)

*Mean with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

The obtained data presented in table (1) revealed that, plasma total cholesterol, triacylglycerol and LDL-c concentrations were significantly increased at late gestation and early lactation period. A significant increase in plasma VLDL-c concentration was observed at late gestation followed by a non-significant increase at early lactation period. Meanwhile, plasma HDL-c concentrations were non significantly increased during late pregnancy and early lactation as compared with day of parturition.

Plasma lipoprotein lipase activity was significantly increased at late gestation and early lactation period. Meanwhile, plasma β -HBA concentration exhibited significant increase during postpartum as compared with day of parturition. The mean values of plasma NEFA and phospholipids concentrations were significantly decreased at 4 and 2 weeks prepartum table (2).

Plasma total proteins and albumin concentrations showed a significant increase while total globulin level was non significantly increased during late pregnancy early lactation when compared with day of parturition (Table 3).

The data demonstrated in table (4) showed a marked increase in plasma calcium, inorganic phosphorus and magnesium concentrations during the late pregnancy and early lactation.

The obtained results in table (5) exhibited a significant increase in plasma iron and zinc levels during the late pregnancy and early lactation. Also, plasma copper and manganese concentrations showed a significant increase at late pregnancy and this increase become non-significant at early lactation when compared with the day of parturition.

Physiological fluctuations occur immediately before and after calving in the levels of blood lipid and trace elements. Cholesterol and triacylglycerol associated with the HDL, LDL and VLDL are blood lipid precursors for milk fat synthesis and that the rapid metabolism during lactation is reflected in increases in the lipid concentrations (**De Gariset, al 2010**). Plasma total cholesterol, triacylglycerol and LDL-c concentrations were significantly increased at late gestation and early lactation period. Also, a significant increase in plasma VLDL-c concentration was observed at late gestation in Holstein Friesian cows as compared with day of parturition. Moreover, Plasma lipoprotein lipase activity was significantly increased at late gestation and early lactation period in Holstein Friesian cows. These results are nearly similar to those reported by **Vazquez-Anon et al., (1994)** who observed that, the marked increase in hepatic triglycerides was occurred and its concentration in the liver tissue remains constant or slightly increased during the postpartum transition period. Also, **Douglas et al., (2004)** illustrated that, increased serum lipids concentration were observed during late pregnancy and early lactation. Who added that, plasma LDL showed a direct relationship with milk production. The increase in LDL lipids could be reflective of increased VLDL metabolism because the protein moiety of VLDL moves unidirectionally from VLDL to LDL. Moreover, **Roche et al., (2009)** suggested that, characteristic changes in lipid metabolism were found during pregnancy and lactation in most mammals. This suggestion was supported by findings of **Nazifi, et al., (2002)** who mentioned that, lipoprotein lipase activity increases the uptake of fatty acids by this tissue thereby reducing the availability of fatty acids to the mammary gland of lactating dairy cattle. The increase of cholesterol during pregnancy occurs as a result of decreased activity of LPL and hepatic lipase which responsible for catabolism of lipoproteins. On the other hand, the recorded significant elevation in plasma lipids and lipoproteins profile could be attributed to the changes in lipogenesis and lipolysis regulate adipose tissue triglyceride accumulation. In addition, two major metabolic pathways contribute to fatty acid availability in ruminant adipocytes. The first is acetate conversion into fatty acids, which depends on lipogenic enzyme activities. The second is the activity of lipoprotein lipase (LPL), which is synthesized by adipocytes and migrates in its active form toward the capillary endothelium. There LPL hydrolyzes circulating triglycerides and allows fatty acids to enter the adipose cells. These pathways are under hormonal control (**Faulconnier et al, 1993**). **De Gariset al., (2010)** showed a marked increase in plasma lipids during the late pregnancy because of the rapid metabolism of VLDL in comparison to the LDL. Additionally, the significant increases in triglycerides associated with the VLDL are the major blood lipid precursors used for milk fat synthesis (**Lohrenz et al., 2010**). Another suggestion for the increase in triglyceride associated with the LDL during late pregnancy and early lactation a result of accumulation due to decreased triglyceride metabolism associated with the VLDL in lactating Holstein cows (**Oikawa and Katoh 2002**). It is possible that the metabolism

of VLDL also may be reflected in increased HDL lipids once lactation was established because of similarities of certain peptides between VLDL and HDL (**Tanaka et al., 2011**). Thus, as VLDL is being metabolized rapidly, increases in LDL and HDL lipids could reflect rapid turnover of the VLDL (**Oikawa and Katoh 2002**). Regarding plasma NEFA and phospholipids concentrations a significant decrease were observed at 4 and 2 weeks prepartum. Meanwhile, the mean values of plasma NEFA and β HBA concentrations showed a marked elevation at 2nd and 4th weeks post calving as compared with day of parturition. Phospholipids are incorporated as structural components of the brain and all cell membranes (**Nelson 2000**). Lipid mobilization is exacerbated in the dairy cow with the genetic drive for high milk production (**Contreras et al, 2009**). In cattle, increases in blood lipid content are known to induce metabolic diseases (fatty liver, ketosis) and predispose dairy cows to inflammatory based diseases. A better understanding of how elevated blood lipids may affect dairy cow immunity during the transition period may lead to innovative approaches to control increased disease susceptibility (**Herdts 2000**). The fatty acid composition of the cellular membrane is directly affected by the composition of lipids in blood especially NEFA. Therefore any change in the content of blood NEFA will be reflected directly in the phospholipid membrane of cells (**Sordillo et al, 2009**). NEFA are greatly accumulated as triglyceride in the liver, primarily due to a decrease in the very low density lipoproteins (VLDL) synthesis by hepatocytes (**Sevinc et al., 2003**). Plasma NEFA concentrations decrease rapidly after calving, but concentrations remain higher than they were before calving. The liver is a major site for fatty acid removal from blood (**Bell 1995**). The concentration of NEFA in blood increases around calving or in early lactation, more NEFA are taken up by the liver reported by (**Emery et al., 1992**). The rapid rise in NEFA at calving due to the stress of calving (**Bell, 1995**). The obtained results were in agreement with **Carson, (2008)** who stated that the elevated NEFA concentration was observed in first and second weeks post calving. Higher plasma NEFA concentrations, as showed during the early lactation, are useful for the animals to maximize milk synthesis with lower glucose consumption (**Wheelock et al., 2010**). Moreover, the increase in NEFA concentration reflects the magnitude of mobilization of fat from storage. BHBA indicates the completeness of oxidization of fat in the liver. BHBA is intermediate metabolites of fatty acids oxidation that directly increases the amount of ketone body production (**Herdts, 2000**). Similarly, (**Duffield et al, 2009**) suggested that, elevated β HBA in the first week post calving has more impact on both cow health and milk yield reduction than elevations later in lactation. Who also concluded that, the peak of ketoses incidence was observed after the first week post calving. As confirmed by **Rollin et al., (2010)** who reported that, the elevated concentrations of the ketone bodies acetoacetate, acetone and β -hydroxybutyrate in the blood, milk and urine were observed in early lactation. Plasma total proteins and albumin concentrations were markedly decreased, while total globulin level showed a mild decrease around the parturition,

followed by a gradual increase during postpartum period. The decrease in plasma total protein concentration may be attributed to the fact that the fetus synthesizes its proteins from the amino acids derived from the dam, and growth of the fetus reaching a maximum level during late pregnancy. The variations reflect the maternal requirements of proteins need for milking and providing immunoglobulin (**Mohri et al., 2007**). As confirmed by **Kupczynskir and Chudoba (2002)** who reported that, the change of total protein, albumin and globulin concentrations may be due to the transfer of albumin and immunoglobulins and total protein from blood to the mammary glands. The decrease in plasma protein and their fractions were came in accordance with the results of **Mohri et al., (2007)** who reported that, total serum proteins levels were significantly affected from the physiological period during lactation if compared to late gestation. The variations reflect the maternal requirements of proteins need for milking and providing immunoglobulins. Also, **Janku et al., (2011)** concluded that, serum globulin was decreased during periparturient period. The reduction of globulin level was associated with the production of colostrum in periparturient period. A marked increase in plasma calcium, inorganic phosphorus and magnesium levels were observed during the late pregnancy and early lactation as when compared with the day of parturition. Similarly, **Giuseppe et al., (2002)** reported that, serum calcium, inorganic phosphorus and magnesium levels were markedly higher during all the lactation period. At the beginning of lactation, calcium homeostatic mechanisms have to react to a great increase in demand for calcium mobilization from bone and increased absorption of calcium from the gastrointestinal tract (**Liesegang, 2008**). Furthermore, increasing the milk production, more phosphorus from the ingested amount is transferred to milk and less is excreted with faeces (**Valk et al., 2002**). The passage of calcium across the placenta is unidirectional; back transfer of this element is very limited, so, the mobilization from bone and the increased absorption from the gastrointestinal tract are required to reestablish homeostasis (**Liesegang 2008**). Milk phosphorus and calcium output is directly related to milk yield, as milk phosphorus concentration is constant (**Valk et al., 2002**). Also, it is true that the requirement of calcium and phosphorus depends on the physiological status and the animal's productivity (**Brezezinska and Krawczyk 2009**). Additionally, **Meglia et al., (2001)** illustrated that, the decrease in blood levels of calcium and phosphorous is expected to decrease at calving due to the large demand of colostrum and milk production. Finally, the lowered plasma calcium, inorganic phosphorous and magnesium concentrations observed in the present study in early postpartum due to increased demand such minerals for synthesis of milk coupled with the relatively slow response in regulating absorption from the intestinal tract. Trace minerals play key roles in an immune response, udder and cow health and have a positive effect on milk production maintaining a healthy cow. The obtained results showed a gradual decrease in plasma copper, iron, zinc and manganese concentrations around the calving, followed by a mild increase during the early lactation. This decrease

may be due to the rapid need for zinc in synthesis of colostrum may explain why zinc concentration is lowered in blood of cows on day of calving (House and Bell, 1993). The obtained results are coincide with Meglia et al., (2001) who mentioned that, serum copper and zinc concentrations were significantly lowered at calving. Who add that, the concentration of serum copper and zinc were markedly increased one month after calving compared with day of parturition. Also, Kincaid (2000) mentioned placental transfer of copper from the cow to the fetus and lowered absorption from the intestinal tract. The obtained decrease in plasma manganese concentration at parturition may be due to the faetal growth. Manganese regulates cellular differentiation during early embryonic development (Hostetler et al., 2003). Furthermore, manganese plays crucial roles in enzymatic and metabolic pathways which are critical for embryo development during pregnancy in cattle (Griffiths et al., 2007). A significant decreased iron level showed towards parturition meanwhile a significant increase noticed during pre and postpartum period these results agree with Weiss et al., (2010) who concluded that during the close-up period, Fe blood volume increases. Moreover, Shahzad et al., (2001) who observed that the highest concentration of serum iron observed at 2 monthes pre and post partum mainwhile the lowest level recorded near to parturation. The decrease may be due to the inceased domaned of iron storage during normal pregnancy.

Conclusion

In conclusion, biochemical changes suggested that the late pregnancy and early lactation periods of Holstein Friesian cows had metabolic disturbances in plasma lipid profile and LPL activity may have a key role in evaluating the metabolic status. The results can also be regarded as the starting material for studying the dynamics of metabolism development of some macro- and microelements. The knowledge to be obtained can be used both in preventive diagnostics and justification of animal nutrition which affect in production and reproduction of health condition of high producing lactating cow.

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