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Research Article

COMPARATIVE EVALUATION OF NEFA, ALBUMIN, GLUCOSE AND TAG LEVELS IN NON-PREGNANT COWS (LESSER THAN ONE MONTH AND UP TO A MONTH AFTER PARTURITION) AND PREGNANT COWS (LESSER THAN 8 MONTHS AND UP TO 8 MONTHS)

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ABSTRACT
The animals attempt to supply the needs for milk production by drawing on body fat reserves. Thus, the animal experience a period of negative energy balance and it has to mobilize tissue lipid and protein in order to sustain productive function. For this research totally 100 Holstein cows of Tabriz city were studied, 50 dairy cows were pregnant and 50 cows were non-pregnant. After blood clotting, all samples centrifuged by 1000 rpm for 20 minute until blood serum was obtained. For Glucose analyzing we used GOD-POP method, for three glyceride analysis (TAG) used calorimetric method, for albumin used biuret and for NEFA analyzing used enzymatic test. Results showed significant differences between all groups on studied biochemical parameters and NEB between different groups has significant effect ($P<0.05$) on TAG, Glucose, NEFA and Albumin parameters of studied cows.

Keywords: *Dairy Cow, Biochemical Parameters, Pregnant, Non-Pregnant*

INTRODUCTION
Dairy cows are at increased risk factors for much kind of disease and disorders during the peripartituent period. At this time there is incising milk production, but a lag in feed intake. These combinations create negative energy balance in animal body (Contreras *et al.,* 2009). Energy balance is one of the most critical nutritional factors impacting on animal health and immunity, lactation and milk production, meat production and also in genital and reproductive performance (Herdt, 2000; Sordillo *et al.*, 2009; Sordillo, 2008). The animals attempt to supply the needs for milk production by drawing on body fat reserves. Thus, the animal experience a period of negative energy balance and it has to mobilize tissue lipid and protein in order to sustain productive function (Waldron and Overton, 2006). Plasma glucose and non-esterified fatty acids (NEFA) decline with age and are lower during 5 to 8 week in early weaned calves (Quigley *et al.*, 1991). This mobilization of fatty acids results in the production of major ketone bodies, acetone, acetoacetate and β-hydroxybutirate (BHB). These compounds are important as a source of energy when carbohydrate levels are reduced. However, the accumulation of these compounds can lead to ketosis. Ketosis has been shown in dairy herds with significantly reduced milk yield (Leslie *et al.*, 2003). Marked decrease in dry matter intake immediately before calving is extremely important in the development of excessive negative energy balance (Duffield, 2004). Metabolic disorders are highly multifactorial and a wide range of animal, managerial and fed factors may lead to such problems (Stengärde *et al.*, 2008). Energy metabolism was analyzed by the measurement of blood concentrations of glucose, acetoacetate and NEFA (Gergácz and Szücs, 2009). Glucose is the primary metabolic fuel, and is absolutely required for vital organ function, fetal growth and milk production (LeBlanc, 2006). Glucose represents an overriding metabolic demand during the transition period because of the requirements of the mammary gland for lactose synthesis (Overton, 2003). The homeoeretic mechanisms have role to maintain the high level of milk production, via mobilization of body fat store, which results in elevated levels of circulating ketone bodies in early lactation. Ketone bodies are intermediate metabolic products. They provide available energy to peripheral tissues when carbohydrates levels are limited (Leslie *et al.*, 2001). Cows with precalving energy deficit would start mobilizing energy reserves in the last week before parturition. This could be measured via serum or plasma NEFA (Duffield, 2000). The NEFA could

undergo a variety of different metabolic paths within the liver. Liver becomes infiltrated with fat during ketosis, although a significant fraction of assimilated NEFA could be re-esterified. Also, substantial portions of NEFA are converted in ketone bodies. Evidence from other species indicates that the rate of hepatic ketogenesis from NEFA is determined both by the rate of supply of NEFA and by carbohydrate status of liver (Baird, 1982). The estimation of NEFA/BHB ratio elucidates the relative significance of lipolysis and ketogenesis (Sato *et al.*, 1998). The "gold standard" test for subclinical ketosis is determination of blood BHB. This ketone body is more stable in blood than acetone and acetoacetate. Clinical ketosis generally involves much higher levels of BHB (Sato *et al.*, 1998). Hepatic ketogenesis may be augmented by diminishing carbohydrate status (Baird, 1982). Blood parameters that may reflect nutrition status of the cow, such as glucose, NEFA, BHB, and total cholesterol, also enzymes and proteins that reveal liver status are of interest to be included in the transition period profiles (Stengärde *et al.*, 2008). Clinical ketosis typically occurs spontaneously in susceptible high yielding dairy cows between the 2nd and 7th week of lactation (Baird, 1982). The more obvious biochemical features of the condition are hyperketonemia, with minimum blood acetoacetate concentration, hypoglycemia, and elevated concentration of NEFA in the blood, fatty infiltration of the liver and loss of liver glycogen (Baird, 1982). The aim of present study was to investigating the changes of NEFA, TAG, Glucose and Albumin of dairy cowsin non-pregnant cows (Lesser than one monthand up toa month after parturition) and pregnant cows (lesser than 8 months and up to 8 month) and compare with each other to find out metabolic changes and disorders of this research groups.

MATERIALS AND METHODS

Sampling

For this research totally 100 Holstein cows of Tabriz city were studied, 50 dairy cows were pregnant and 50 cows were non-pregnant. 25 cows of non-pregnant samples were up to a month after parturition and other 25 cows were lesser than one month after parturition. In pregnant group we had 25 cows lesser than 8 months after pregnancy and 25 cows up to 8 months after pregnancy.

For this study we took blood samples by using jugular vein. Samples were kept in 24 degree Celsius until blood clots were observed. After blood clotting, all samples centrifuged by 1000 rpm for 20 minute until blood serum was obtained.

Blood Serum Analyzing

For blood serum analyzing we were used Pars Azmoon company kits. For Glucose analyzing we used GOD-POP method, for three glyceride analysis (TAG) usedcalorimetric method, for albumin used biuret and for NEFA analyzing used enzymatic test.

Statistical Analysis

For statistical analyzing we used SPSS version 19 and ANOVA test (POSTHOC=DUNCAN ALPHA). Ranges of significant difference were data with P< 0.05.

RESULTS AND DISCUSSION

Results of studied parameters presented in table 1. There were significant differences between studied groups in investigated parameters (P<0.05). In this table non-pregnant groups less than 1 month (-1), up to a month (+1) also pregnant cows group less than 8 month (-8) and up to 8 months (+8) were presented.

Table 1: Results of Blood Biochemical Parameters of Studied Groups

Parameters	**Non-Pregnant**		**Pregnant**	
	-1	**+1**	**-8**	**+8**
NEFA	699.20 ± 245.35^{a}	662.92 ± 205.48^{b}	562.64 ± 70.07^{b}	531.60 ± 143.34^{c}
Albumin	3.158 ± 0.52^{a}	3.32 ± 0.46^{ab}	3.37 ± 0.51^{ab}	3.58 ± 0.33^{b}
Glucose	38.52 ± 11.30^{a}	41.84 ± 7.13^{a}	48.24 ± 8.67^{b}	54.16 ± 9.20^{c}
TAG	52.76 ± 13.77^{a}	63.80 ± 18.27^{ab}	57.12 ± 18.82^{a}	75.00 ± 29.36^{b}

Calving and the onset of lactation lead to a sudden increase in the energy requirements of the dairy cow. This time also is characterized by a drop in feed intake, therefore lowering the amount of energy provided by feed. The imbalance between the energy that the cow consumes and the energy needed for production demands is termed negative energy balance (NEB) (Contreras *et al.*, 2009). Cows adapt to NEB periods by moving body fat reserves through a process known as lipid mobilization. Although this is a normal adjustment common among mammals, lipid mobilization is exacerbated in the dairy cow with the genetic drive for high milk production (Duffield, 2004).

Research in humans has linked elevation of blood lipids to metabolic diseases such as type-2 diabetes, metabolic syndrome, and to inflammatory diseases such as cardiac, hypertension, asthma, and Alzheimer's disease. In cattle, increases in blood lipid content are known to induce metabolic diseases (fatty liver, ketosis) and predispose dairy cows to inflammatory-based diseases (mastitis, metritis, and lameness) affecting animal welfare and profitability (Waldron and Overton, 2006). 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 (Duffield, 2004).

Changes in the concentration and composition of plasma non-esterified fatty acids (NEFA) directly affect white blood cell function. A first way of altering immune function is by changing the composition of the cellular membrane of blood cells. In animal cells, this membrane is composed of phospholipids. These molecules are formed by different kinds of fatty acids including saturated, monounsaturated and polyunsaturated. Fatty acids together with some proteins form a bilayer that surrounds and protects the cells (Waldron and Overton, 2006). 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 white blood cells. Fatty acids from the cellular membrane are involved in the inflammatory process as they are transformed into lipid mediators. Some examples of lipid mediators are prostaglandins and prostacyclins. These fatty acid-derived molecules can cause damage by inducing changes in blood vessels and altering white blood cell function. A second way of altering immune function is by altering the internal communication of white blood cells. Saturated fatty acids directly affect cell behavior, making a cell more likely to promote rather than fight inflammation. Therefore, changing the composition of fatty acids within the cellular membrane of white blood cells may be an effective way of altering how a cow responds to infectious and metabolic diseases during the transition period (Sordillo *et al.*, 2009).

Glucose is the primary metabolic fuel, and is absolutely required for vital organ function, fetal growth and milk production (LeBlanc, 2006). Glucose represents an overriding metabolic demand during the transition period because of the requirements of the mammary gland for lactose synthesis (Overton, 2003).

Many published results confirmed that the selection and measures to increase milk productivity significantly affect the increase in the intensity of metabolic processes in cows in peripartal period (Van Saun, 2008). In turn, negative energy balance is associated with elevated plasma NEFA and hepatic lipid concentrations (Colazo *et al.*, 2009). Ketosis is a disorder of carbohydrate and fat metabolism characterized by increased concentrations of ketone bodies in blood (ketonemia), urine (ketonuria), milk (ketolactia), and other body fluids (Geishauser *et al.*, 1998). Subclinical ketosis is important common condition early lactation dairy cattle. It is associated with losses in milk production and increased risk of periparturient disease (Duffield, 2000). Regulation of the metabolism between carbohydrates and lipids occurs between adipose tissue, liver, intestine and udder, and this is essential for the animal's adaptation to negative energy balance. Adaptation of the animals appeared before calving, as the physiological process of incoming lactation. In dairy cows, the massive energy demand to support milk production is partly met through gluconeogenesis (LeBlanc, 2006). Glucose concentration is under tight homeostatic control. Some dairy cows in early lactation cannot provide balanced relationship in the metabolic processes and regulatory mechanisms can induce pathological condition. Results showed significant differences between all groups on studied biochemical parameters and NEB on different groups has significant effect ($P<0.05$) on TAG, Glucose, NEFA and Albumin parameters of studied cows.

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