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Changes in Serum Protein and Lipoprotein Fractions During Various Lactation Stages and Dry Period in Dairy Cows

Saime GÜZEL[1] Meltem TANRIVERDİ[2] Ümit POLAT[2]

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Abstract: The aim of this study was to determine the changes in the serum albumin, alpha 1 (α1)-globulin, alpha 2 (α2)-globulin, beta (β)-globulin, gamma (γ)-globulin, and alpha-lipoprotein (high density lipoprotein-HDL), pre-beta-lipoprotein (very low density lipoprotein-VLDL) and beta-lipoprotein (low density lipoprotein-LDL) and total lipid fractions of the cows in dry period and various stages of lactation period. Fourty healthy, four years old, Holstein dairy cows were used as material. Ten of them were in dry period and the rest were in lactation period. Between the days 48-51, 147-161. and 180. and over of lactation period were accepted as first, second and third stage, respectively. There were 10 cows in each lactation stages. Serum protein and lipoprotein fractions were compared between the dry period, first, second and third stages of lactation period. While significant differences were determined for α1-globulin, β-globulin, γ-globulin, HDL, VLDL, LDL and total lipid levels, no significant differences were observed in albumin and α2-globulin between dry period, first, second, third stages of lactation period.

Key Words: Dairy cow, Lipoprotein, Albumin, Globulin

Kuru Dönem ve Laktasyonun Çeşitli Evrelerindeki süt İneklerinde Serum Protein ve Lipoprotein Fraksiyonlarındaki Değişiklikler

Özet; Bu çalışmanın konusu kuru dönem ve laktasyonun çeşitli evrelerindeki ineklerin serum albumin, α1-globulin, α2-globulin, β-globulin, γ-globulin, and α -lipoprotein (HDL), pre- β -lipoprotein (VLDL) and β-lipoprotein (LDL) fraksiyonlarındaki ve total lipid değerlerindeki farklılıkları değerlendirmekti. Materyal olarak Karacabey Doğancı Çiftliğinde bulunan 40 adet sağlıklı 4 yaşlı holstein ırkı süt ineği kullanıldı. İneklerin 10 tanesi kuru dönemde, geri kalanlar ise laktasyon dönemindeydi. Laktasyon döneminin 48-51., 147-161. ve 180. ve sonraki günleri sırasıyla I., II., ve III. evre olarak değerlendirildi. Her laktasyon evresinde 10 inek bulunmaktaydı. Alınan kan örneklerinde kuru dönem ve laktasyonunun çeşitli evrelerindeki protein ve lipoprotein değerleri karşılaştırıldı. Kuru dönem, laktasyonun I., II., ve III. evreleri arasında α1-globulin, β-globulin, γ-globulin, HDL, VLDL, LDL and total lipid düzeylerinde farklılıklar gözlenirken, albumin ve α2-globulin değerlerinde ise herhangi bir farklılık ortaya koyulmadı.

Anahtar Kelimeler: Süt İneği, Lipoprotein, Albumin, Globulin

Introduction

Proteins are composed of amino acids and are important components of all cells and tissues. There are many different kinds of proteins in the body with many different functions. Examples of proteins include enzymes, certain hormones, hemoglobin, low-density lipoprotein ("bad" cholesterol), and others[19]. Serum proteins are classified as albumin or globulins. Albumin is the protein of the highest concentration in the serum. It carries many small molecules, and is important for keeping fluid from leaking out from the blood vessels into the tissues. Globu-

[1] Dr. Uludağ Üniversitesi Veteriner Fakültesi Biyokimya Anabilim Dalı, Görükle kampüsü/BURSA. saime@uludag.edu.tr

[2] Prof. Dr. Uludağ Üniversitesi Veteriner Fakültesi Biyokimya Anabilim Dalı, Görükle kampüsü/BURSA.

lins are divided into α_1, α_2, β, and γ-globulins. In general, α and γ- globulin protein levels increase due to infections, inflammation and nutrional and physiological status (e.g. gestation), and are therefore important health indicators in the body. Serum globulin and immunoglobulin levels can be valuable indicators of ability of animals to perform the activities daily living, and may be useful predictors of subclinical diseases. Lipoprotein particles (Chylomicrons, HDL, VLDL, LDL) is to transports lipids (fats) around the body in the blood. Lipoproteins can also be classified as "alpha" and "beta", based on their migration pattern on serum protein electrophoresis[14,15,19].Serum protein and lipoproteins can be separated by electrophoresis. Electrophoresis is a standart method for evaluating fractions of serum proteins and lipoproteins in clinical biochemistry. The serum is placed on specially treated paper and exposed to an electric current. The proteins in the serum move on the paper to form bands that show the proportion of each protein fraction. A fraction may contain several different types of proteins. Individual proteins, except albumin, are not usually measured. However, protein fractions or groups are measured. The levels of protein fractions can be estimated by measuring the total serum protein and then multiplying that by the relative percentage of each protein fraction[5]. Lipoprotein electrophoresis is a type of protein electrophoresis that determines the amount of complex proteins made up of protein and lipid, lipoproteins such as LDL[15].

In ruminants, plasma lipids do not rise after ingestion of fat. Most of them are the results of de novo synthesis. Still, large amounts of short chain fatty acids are produced in the ruminoreticulum as a result of microbial digestion of carbonhydrates. These are absorbed mainly from the forestomach, which can absorbe fatty acids with chain length up to 12. In conventionally fed ruminants only small amount of the carbonhydrates as glucose. Most of glucose supply is derived from propionate and also from amino acids by gluconeogenesis in the liver[4,20]. Fat is stored as triglyceride and from the depots it is transported as free fatty acids bound to albumin. A considerable part of these acids is taken up by the liver. Therefore, they can be oxidized to CO_2 or to ketone bodies or esterified and combined with phopholipids, cholesterol and apoproteins to form lipopropteins, mainly VLDL. This lipoprotein fraction transports triglycerids to different organs and tissues. After the loss of triglycerids, LDL are formed, and after further metabolism HDL is formed[8,11,16,18,21]. In cattle, HDL is the major fraction comprising more than 80% of the lipoproteins[9] and consisting of about 60 % cholesterol[3]. As the feed for cattle contains very small amounts of cholesterol and there is high level of this substance in plasma, there must be substantial amount of the novo synthesis in the body. Cholesterol can be synthesized in the liver from acetate and it is probably by this way that the cholesterol and the VLDL fraction is formed[17]. There are large variations in fat metabolism during different stages of lactation and pregnancy and the risk of disturbances are also variable. The objective of the present study was to analyse the differences of albumin, α_1-globulin, α_2-globulin, β-globulin, γ-globulin, HDL, VLDL, LDL fractions and total lipid level of the cows between dry period and various stages of lactation periods.

Materials and Methods

The study was performed in fourty healthy, four years old Holstein dairy cows in Karacabey Doğancı Farm. Ten cows were in dry period and the rest were in lactation period. There were 10 cows in each lactation stages. Between the days 48-51, 147-161. and 180. and over of lactation period were accepted as first, second and third stage, respectively. There were 10 cows in each lactation stages. Blood samples were taken from the vena subcutanea abdominis using Vacutainer blood collection tubes and were transported on ice chest to the laboratory for analysis. Serum albumin, α_1-globulin, α_2-globulin, β-globulin, γ-globulin, HDL, VLDL, LDL fractions were measured using agarose gel electrophoresis (REP, Helena-Laboratories). Total lipid levels were measured using a spectrophotometric kit (Labospec). The SPSS statistical software 13.00 was used for statistical analysis (SPSS Inc., Chicago, IL). Differences between groups were evaluated using two-tailed Kruskal-Wallis test.

Results and Discussion

The main objective of this study was to examine the differences of protein and lipoprotein fractions of dry period and first, second and third stages of lactation period in dairy cows. Separation of serum into 5 fractions in the cows (albumin and α_1-, α_2-, β-, and γ-globulins) is usually sufficient for diagnostic purposes. Albumin is the most prominent serum protein in the electrophoretogram of cows, and it constitutes the 39–58% of the total serum protein

content[2]. In this study, serum albumin levels were not stastistically different between dry period and various stages of lactation period (Table 1). Globulins can be divided into three fractions based on their electrophoretic mobility. Most of the α and β-globulins are synthesized by the liver, whereas γ-globulins are produced by lymphocytes and plasma cells in lymphoid tissue. The α fraction migrates as α_1 (fast) and α_2 (slow) fractions, which is in contrast with some reports in the veterinary literature[10] but in accordance with data obtained by cellulose acetate[13] and by agarose gel electrophoresis[11]. These 2 fractions constitute 3.59–7.13% and 5.09–13.96%, respectively, of the total serum proteins. In the present study serum α_1- globulin concentrations were higher in the second stage of lactation than in the dry period ($p<0.01$), first stage of lactation ($p<0.001$) and third stage of lactation ($p<0.05$) (Table 1). There were no statistical differences in α_2-globlin levels between dry period and various stages of lactation periods (Table 1). The β-globulins migrate differently in cows than in goats (as one fraction)[1]. This result is in agreement with that of some authors[10] but is different from the result of some others[12]. The reason for this discrepancy is unclear. The most likely explanation is that the α_2- fraction is considered to be β_1-globulin[10]. In the present study, visual examination of the gel did not allow for separation of the β-globulins into 2 zones. The β-globulins fractions in dairy cows were reported by albergina et al[2] to be 7.38–15.28% of the total serum proteins. Similar to α_1-globulin levels in this study β-globulin fractions were found higher in the second stage of lactation than in the dry period ($p<0.05$), first stage of lactation ($p<0.05$) and third stage of lactation ($p<0.05$) (Table 1). Gama globulin levels were higher in dry period than in second ($p<0.01$) and third stage ($p<0.05$) of lactation and first stage of lactation were higher than second ($p<0.05$) and third stage ($p<0.05$) of lactation (Table 1). Consistent with this, alberghino et al (2) reported that gama globulin levels were 24.2±5.42% of the serum total protein concentration. We found a decrease in serum γ-globulin levels during the second and third stage of lactation period compared to dry period and early lactation period. It could be due to the transfer of immune globulins and amino acids from the bloodstream to the mammary gland for colostrum and milk synthesis. This protein fraction, which provides passive immunity to newborn, accumulates in the bloodstream before moving into the mammary glands where it is fundamental in the colostrum synthesis. The immunoglobulins represent the major protein fraction of colostrum accounting for 70–80% of its total protein content and are the result of selective accumulation from serum Igs that starts before parturition[13].

Table 1. Serum protein fraction levels of dairy cows during dry period and various Lactation stages (n=10)

Tablo 1. Kuru dönem ve laktasyonun çeşitli evrelerindeki süt ineklerinde serum protein fraksiyonlarının düzeyleri (n=10)

Serum Protein Fractions	Dry Period	Lactation Periods		
		Stage 1	Stage 2	Stage 3
	$\bar{X}$ ± S.E.M.	$\bar{X}$ ± S.E.M.	$\bar{X}$ ± S.E.M.	$\bar{X}$ ± S.E.M.
Albumin (%)	43.8±2.205	42.14±2.92	43.72±1.63	49.27±1.85
α_1-globulin (%)	5.13±0.19^a	4.69±0.28^a	6.56±0.40^b	3.90±0.60^a
α_2-globulin (%)	9.44±0.35	8.77±0.20	9.58±±0.30	8.39±0.62
β-globulin (%)	9.05±0.16^a	8.59±0.33^a	14.19±0.81^b	9.70±1.06^a
γ-globulin (%)	32.6±1.85^a	35.42±2.99^a	25.93±1.33^b	26.92±1.80^b

a,b,c,d Means within a row with the same letter are not significantly different

Many-high-yielding dairy cows have a negative energy balance during the first few weeks of lactation. The demand of the mammary gland for glucose often is greater than the glucose available. This imbalance increases fat mobilization, and increases hepatic ketogenesis. An increase in keton-body concentration during early lactation indicates general fat mobilization. This mobilisation of fat is induced by the negative energy balance that occurs during the periparturient period. Hyperketonemia and fatty infiltration of the liver become particularly pronounced during ketosis[3,6,7,9,17]. Bovine ketosis is a common and economically significant disease, and may be primary or secondary. Primary ketosis generally occurs in housed cattle and is seen ten days to six weeks after parturition, with maximum incidence at three weeks[6,7]. In the present study, the HDL levels in dry period were lower than first ($p<0.001$), second ($p<0.001$) and third period ($p<0.01$) of lactation and in second period of lactation were higher than third period ($p<0.001$) of lactation (Table 2). In contrast, VLDL levels in dry period were higher than in first ($p<0.001$), second ($p<0.001$) and third period ($p<0.01$) of lactation and were higher in third period of lactation than in first ($p<0.05$) and second period ($p<0.001$) of lactation (Table 2). Serum LDL levels in dry period were significantly higher than in first ($p<0.001$), second ($p<0.001$) and third period ($p<0.01$) of lactation and were higher in first period of lactation than in second period ($p<0.05$)of lactation (Table 2). Serum total lipid concentrations were

higher in the first stage of lactation than dry period ($p<0.01$) and, second ($p<0.05$) and third period ($p<0.001$) of lactation (Table 2). In the third stage of lactation serum total lipid levels significantly were higher than dry period ($p<0.001$), first ($p<0.001$) and second ($p<0.01$) stages of lactation (Table 2).

Table 2. Serum lipid fraction levels of dairy cows during dry period and various Lactation stages

Tablo 2. Kuru dönem ve laktasyonun çeşitli evrelerindeki süt ineklerinde serum lipoprotein fraksiyonlarının düzeyleri (n=10)

Serum Lipoprotein Fractions	Dry Period	Lactation Periods		
		Stage 1	Stage 2	Stage 3
	$\overline{X}$ ± S.E.M.	$\overline{X}$ ± S.E.M.	$\overline{X}$ ± S.E.M.	$\overline{X}$ ± S.E.M.
HDL (%)	57.56±0.68^{a}	72.24±1.32^{b}	75.24±0.80^{bc}	62.50±6.21^{bd}
VLDL (%)	35.10±0.71^{a}	25.61±1.07^{b}	22.88±0.80^{b}	28.29±0.58^{d}
LDL (%)	6.8±0.38^{a}	2.45±0.24^{b}	1.68±0.22^{c}	1.91±0.40^{bc}
Total lipid (mg/dl)	503.30±11.70^{a}	557.80±7.61^{b}	474.90±27.33^{a}	374.70±9.53^{c}

a,b,c,d Means within a row with the same letter are not significantly different

In conclusion, in this study we determined the differences of α_1-globulin, β-globulin, γ-globulin, HDL, VLDL, LDL fractions and total lipid levels of the cows between dry period and various stages of lactation periods. The determined differences of these parameters, thus can provide unique insight into the metabolic disturbances seen dry period and various stage of lactation.

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