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Effects of Lameness, Stage of Lactation and Body Condition Score on Some Blood Parameters in Holstein Cows

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Abstract: Effects of lameness, stage of lactation and body condition score on serum AST and ALT activities as well as serum total protein, triglyceride, cholesterol and albumin concentrations in cows was investigated in the present study. Fifty six pure Holstein cows were included in this study. AST, ALT and cholesterol levels were significantly altered by stages of lactation ($p<0.05$). Total protein, triglyceride, AST, ALT, cholesterol and albumin levels were low at early stages of lactation and dry periods; in the course of time, their concentrations increased. However, in late stages of lactation, serum total protein, triglyceride, AST, ALT, cholesterol and albumin levels declined. No significant alterations were detected in the blood parameters of lame cows. However, AST, ALT and albumin levels were low in cows with a lameness score of 4. Likewise, blood parameters were not affected by body condition. Triglyceride, AST, cholesterol and albumin levels are high in cows with higher body conditions (≥ 2.75).

Key words: Stages of lactation, lameness, body condition, blood parameters

INTRODUCTION

The level of biochemical parameters in the blood is important in determining the health and illness status of animals and in making clinical evaluation. So, it may be understood how normal values turn into abnormal values due to many factors, such as management, feeding and illness (Utlu *et al*., 2004). Furthermore, stages of lactation, milk yield levels, as well as the animals' body conditions and lameness may affect blood values (Ruegg *et al*., 1992; Galip *et al*., 2002; Castello *et al*., 2005; Milinkovic-Tur *et al*., 2005; Parizi and Khalafizadeh, 2006). Lameness took third place after reproduction and udder/mastitis disorders among culling reasons relating to the health of cows in the US (NAHMS, 1996). Lameness in dairy cows is an important disturbance in the economy and animal welfare (Warnick *et al*., 2001). Foot illnesses, which are a very important factor for a cattle farm, may easily be determined by observing cows while they are walking and/or standing (Sprecher *et al*., 1997). Veterinarians and farmers may detect the cows with foot and hoof disorders in early stages with the help of lameness scoring (Hernandez *et al*., 2005). In early lactation, dietary intake is unable to meet the demands of high milk yield. The cows with high milk production must mobilize large amounts of body fat to provide the energy for milk yield and maintenance during early lactation (Jones, 1990). The cow is not capable of consuming the required energy and then, loss of weight and body condition occurs. Thus, dairy cows, in general, have a negative energy balance in early lactation (De Vries and Veerkamp, 2000). One of the indicators of energy balance is the Body Condition Score (BCS). Body

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condition scoring is a subjective method assessing the amount of metabolizable energy stored in body reserves on a live animal (Edmonson *et al*., 1989). Also, body condition scoring can be used as a feed management tool and may also assist in reproductive and health management in dairy farms (Hady *et al*., 1994). Some blood parameters, such as serum cholesterol, triglyceride and total proteins, change during pregnancy and lactation stage (Peterson and Waldern, 1981; Ling *et al*., 2003; Yıldız *et al*., 2005). Most of the dissolved solid matter in blood plasma consists of proteins. Enzymes, which control biochemical reaction rates, play an active role in intermediate metabolism. The presence of enzymes, such as AST (aspartate amino transferase) and ALT (alanine amino transferase) as well as proteins, such as albumin and globulin in various concentrations in serum are generally considered indicators for some pathologic differentiations in tissues and organs (Murray *et al*., 1990; Milinkovic-Tur *et al*., 2005).

The relation between lameness scores and total protein, triglyceride, AST, ALT, cholesterol and albumin levels have not been studied enough, such as relation between BCS with total protein, triglyceride, AST, ALT and albumin levels has not occurred. Hence, the present study was conducted to assess the effects of the stage of lactation, body condition score and lameness score on serum AST and ALT activities as well as total serum protein, triglyceride, cholesterol and albumin concentrations of Holstein cows.

MATERIALS AND METHODS

Environment and Farming System

This study was carried out at a dairy farm (Akar Metal Farm) in the Odemis region, which is located at western part of Eagean Region of Turkey. Fifty six pure Holstein dairy cows were used as animal materials. The herd was housed in free-stall barns and fed a concentrate feed (21% CP, 2700 ME) (7-9 kg per cow/day) and conserved forage (grass and maize silage, 15-20 kg per cow/day). Hay (alfalfa, oat and vetch) was provided *ad libitum*. There was no regular hoof-trimming programme and the animals were exposed to a slurry surface, particularly in the winter. The cows were milked two times daily in milking parlours. In the study, lameness scoring, body condition scoring and the blood sampling of cows were performed on the same day. The mean milk production was 21.7±0.22 kg/cow/day before the study day.

Experimental Procedures

Lameness scoring of cows was done according to a 1-5 scoring system developed by Sprecher *et al*. (1997). According to the scoring system, 1 point indicates normal cows and 5 points indicates severely lame cows. Body condition scoring of the cows was performed according to the method developed by Edmonson *et al*. (1989). In this system, the cows are scored with points between 1 and 5 with 0.25 intervals. The cow with a body condition score of 1 is considered very thin and the cow with a body condition score of 5 is considered very fat. Stage of lactation of each cow was recorded as lactation days and classified as follows for further analysis. (1) 1-70 days, (2) 71-140 days, (3) 141-305 days, (4) 305+ days and (5) dry. The classification according to body condition scores was done as (1) ≤ 2.50 and (2) ≥ 2.75.

Blood was collected from the jugular vein of each animal into serum tubes. Blood samples were allowed to stand 2 h at room temperature to allow proper clotting. The samples were centrifuged at 5000 rpm for 10 min and the serum samples were stored at -20°C until analysis. Total protein, triglyceride, AST, ALT, cholesterol and albumin levels in blood serums were determined via., commercial kits (Spinreact, Santa Coloma, Spain).

Statistical Analysis

The stage of lactation, lameness scores and body condition scores were considered as systematic environmental factors. The following models were used in analyzing data.

$$Y_{ijk} : \mu+a_i+b_j+c_k+e_{ijkl}$$

Where:

Y_{ijk} = The blood values
μ = The general mean
a_i = The effect of lactation days class (i = 1, 2, 3, 4, 5)
b_j = The effect of the Body Condition Score (BCS) class (j = 1, 2)
c_k = The effect of the Lameness Score (LS) class (k = 1, 2, 3, 4)
e_{ijk} = Residual error

The SAS (1999) statistical packet programme was employed in statistical analysis. The significance of differences between groups was determined with Duncan's test. All the values were presented as the least square means and Standart Error of Means (SEM).

RESULTS

Effects of the stage of lactation on serum total protein, triglyceride and albumin were not statistically significant ($p>0.05$). However, total protein level was lower in dry and early stage of lactation than the other stages of lactation (71 to ≥306 day). Serum triglyceride values detected as the lowest level (16.69 mg dL^{-1}) in early lactation stages (1-70 days). While highest value was found as 55.69 mg dL^{-1} and in days 141-305 of lactation. Standard errors of sernm triglyceride levels were very high. Stages of lactation affected serum AST, ALT and cholesterol levels significantly ($p<0.05$). AST (64.23 IU L^{-1}) and cholesterol (100.34 mg dL^{-1}) were low in dry periods. However, their levels were high in days 71-140 of lactation. AST and cholesterol were 80.66 IU L^{-1} and 211.50 mg dL^{-1}, respectively. ALT values were low in dry and early stages of lactation. In the study, only 1 cow had 5 lameness score and it was evaluated with the cows having 4 lameness score (Table 1). Alterations in serum AST and ALT activities as well as total

Table 1: Least-square means and standard errors of total protein, triglyceride, AST, ALT, cholesterol and albumin

Parameters	No.	Total protein (g dL^{-1})	Triglyceride (mg dL^{-1})	AST (IU L^{-1})	ALT (IU L^{-1})	Cholesterol (mg dL^{-1})	Albumin (g dL^{-1})
Lactation stage (day)							
≤70	5	8.39±0.45	16.69±31.21	76.65±5.62^{ba}	23.03±3.86^{b}	146.59±23.68^{bc}	4.10±0.21
71-140	11	8.99±0.33	33.19±22.63	80.66±4.07^{a}	37.10±2.80^{a}	211.50±17.16^{a}	4.19±0.15
141-305	21	8.99±0.23	55.69±15.89	77.26±2.86^{ba}	39.36±1.96^{a}	188.07±12.05^{ba}	4.15±0.11
≥306	7	8.85±0.40	48.74±27.94	67.55±5.03^{ba}	34.93±3.45^{a}	188.54±21.19^{a}	4.16±0.19
Dry	12	8.41±0.30	41.43±20.68	64.23±3.73^{b}	24.90±2.56^{b}	100.34±15.69^{c}	4.03±0.14
p-value		NS	NS	*	*	*	NS
Lameness score							
1	13	8.49±0.27	32.84±18.81	74.02±3.39	31.65±2.32	169.69±14.27	4.13±0.13
2	25	8.80±0.21	48.06±14.48	75.19±2.61	32.66±1.79	184.74±10.98	4.15±0.10
3	9	8.67±0.34	36.70±23.75	72.22±4.28	32.22±2.94	152.91±18.02	4.33±0.16
4	9	8.95±0.32	38.99±22.43	71.65±4.04	30.91±2.77	160.70±17.01	3.90±0.15
p-value		NS	NS	NS	NS	NS	NS
Body condition score							
≤2.50	32	8.93±0.22	33.93±15.15	71.73±2.73	32.99±1.87	159.85±11.49	4.02±0.10
≥2.75	24	8.52±0.24	44.37±16.34	74.81±2.94	30.74±2.09	174.17±12.39	4.23±0.11
p-value		NS	NS	NS	NS	NS	NS

*p<0.05, NS: Non significant; Different superscript letter(s) in the same column indicate statistically significant differences

protein, triglyceride, cholesterol and albumin levels were not significantly affected by lameness scores ($p>0.05$). However, triglyceride, AST, ALT and cholesterol levels were higher in cows with a LS of 2. Total protein was higher in cows with a LS of 4, while albumin was higher in cows with a LS of 3. Body condition scores did not significantly affect the serum total protein, triglyceride, AST, ALT, cholesterol and albumin levels in the cows. Triglyceride, AST, cholesterol and albumin levels were high in the cows with higher conditions (BCS≥ 2.75).

DISCUSSION

The physiological status or lactation stage of the cows significantly can change the serum levels of some blood constituents. For example, serum protein, albumin, AST, ALT and cholesterol levels were affected by the stage of lactation or physiological status (Peterson and Waldern, 1981; Otto *et al*., 2000; El-Sherif and Assad, 2001). However, Yıldız *et al*. (2005) reported that total protein and albumin were not affected in the gestation period, while cholesterol and ALT were decreased toward the end of gestation and in dry periods. Yıldız *et al*. (2005) found that triglyceride and AST increased in dry periods. These results are different than present findings. Castillo *et al*. (2005) reported that the levels of triglyceride, AST and albumin between close-up dry periods and early lactation changed from 46.46 to 38.77 g dL^{-1}, 30.04 to 37.77 IU L^{-1} and 4.24 to 4.32 g dL^{-1}, respectively. Similarly, we found the same tendency between dry periods and early lactation stages for triglyceride (41.43 to 16.69 g dL^{-1}), AST (64.23 to 76.65 IU L^{-1}) and albumin (4.03 to 4.10 g dL^{-1}) levels, respectively.

In the present study, the cholesterol level was high in days 71-140 of lactation. However, it was low in dry period and early lactation stage. Similar results were observed by Arave *et al*. (1975), Peterson and Waldern (1981) and Ling *et al*. (2003). They reported cholesterol level was low in early lactation and it increased gradually to peak at mid lactation and then declined in the dry period. A significant positive relation was found between serum cholesterol level and milk production (Arave *et al*., 1975; Ruegg *et al*., 1992). Also, serum cholesterol levels may be affected by fat content in high ratios during gestation stress and in the early stages of lactation, they may cause an increase in serum cholesterol (Ruegg *et al*., 1992). Increasing weight gain and decreasing in thyroid activity may explain the rise in serum cholesterol during mid lactation; in this stage, cows are generally in a positive energy balance (Arave *et al*., 1975). Decreasing cholesterol level in dry period shows rising nutrient demands of the improving foetus in the uterns (Arave *et al*., 1975). Tissues, such as the corpus luteum and adrenal glands, which use cholesterol for hormone synthesis, may use cholesterol (Pösö *et al*., 2000). Thus, cholesterol levels may change in lactation period.

In this study, the AST and ALT activities changed according to the stages of lactation. Khan *et al*. (2002) determined that AST was no significant differences at the prepartum and postpartum but ALT was significantly higher at the prepartum stage than the postpartum stage. Researcher stated that AST and ALT were higher in the pregnant animals than non-pregnant animals. These enzymes create the structural components of the body of the foetus in the pregnant animals. Therefore, these enzymes may be important for pregnancy. The activities of aminotransferases (such as AST and ALT) in blood are associated with implantation, embryo survival, growth, uterine carbohydrate metabolism, amino acid metabolism and glycogen deposition (Rao and Panda, 1981; Milinkovic-Tur *et al*., 2005). We found that AST and ALT level were low in dry period. Similar results have been reported by some researhers. For example, Ling *et al*. (2003) determined that AST was low in dry periods; however, it increased between day 117 and 151 of lactation. In Holstein mares, Milinkovic-Tur *et al*. (2005) reported that AST activity reduced in the blood plasma during pregnancy and early lactation. On the other hand, El-Sherif and Assad (2001) found that AST and ALT levels were higher in lactating ewes than in dry ewes.

The cows with lameness scores of 1 and 2 are considered healthy and those with scores of 3-5 are considered lame (Sprecher *et al*., 1997). In the present study, there are no significant effects of lameness scores on the blood parameters of cows. Results of the present study (effect of lameness on AST and ALT) are contrary to the results obtained by Stec *et al*. (2006). Researchers reported that ALT and AST values in the cows with developed disorders in the locomotion system were higher than the normative ones. The level of albumin was the highest in cows with a LS of 3, while it was lowest in cows with a LS of 4. This decrease may caused by the severity of the disease. In fact, Whitaker *et al*. (1999) reported that albumin and globulin values are used in evaluating chronic inflammatory diseases as well as long-term protein status and reported that in illness cases, globulin increases while albumin decreases. Any study about the relations between lameness scores and total protein, triglyceride, AST, ALT, cholesterol and albumin levels have not been found in the literature. The reasons for lameness might be different. We can determine a problem in the foot and leg of cows by lameness scoring. However, these disorders can occur by physical injury, heel warts, sole abscess, a stone in the hoof, or even a sore belly (acidosis, displaced abomasum, hardware, etc.) (Robinson and Juarez, 2003). These disorders may affect back posture and stride. Some of these reasons may affect blood parameter levels. But in present study, blood parameters between the lame and non-lame cows did not significantly change. To yield satisfactory results, further experiments regarding lameness are needed.

Cholesterol is a sterol compound existing in animals' tissues most abundantly and is the source of most steroid hormones. Serum cholesterol values are lower in cows with higher losses in body condition scores (Ruegg *et al*., 1992). However, the relation between serum cholesterol density and energy balance has not yet been completely defined (Ruegg *et al*., 1992). In general, dairy cows experience a negative Energy Balance (EB) in early lactation because feed intake cannot support the energy required for milk yield and maintenance. Cows in a negative EB mobilize more body fat reserves and produce glycerol for energy resources leading to increased NEFA concentrations in the blood. These NEFA are taken up by the liver and can be oxidized for additional energy supply or esterified into triglycerides (De Vries and Veerkamp, 2000). Triglycerides are transformed to very low-density lipoproteins, which can be taken up by the udder (Bauman and Currie, 1980). Thus, triglycerides in thinner cows (BCS$\leq$2.50) might be lower. In this study, the effect of BCS on blood metabolites was not significant. Similarly, Lago *et al*. (2001) reported that the body condition score at calving ($\geq$4.0 or $\leq$3.5) did not affect AST. According to the literature, any publication on the relation between BCS with total protein, triglyceride, AST, ALT and albumin levels has not occurred.

Results indicated that the stage of lactation affects AST and ALT activities as well as cholesterol levels statistically. The reasons for these variations may be different physiological periods, milk production, or gestation. Any significant variation was not found in the blood parameters based on lameness scores and body condition scores. Further studies of the relation between lameness scores as well as body condition scores and blood parameters should be performed in larger herds and in more detail.

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