Effects of silymarin (*Silybum marianum*) supplementation on milk and blood parameters of dairy cattle

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Abstract

The present study was conducted to investigate the effect of dietary silymarin on milk yield, milk fat, and certain blood parameters of dairy cattle. Silymarin is a naturally accepted hepatoprotectant that is used in the treatment of liver diseases in human beings, and has been tested in dairy cows during peripartum. Animals are subject to subclinical fatty liver. In the first part of the study, the silymarin (20 g/head/day) was supplemented to dairy cattle rations in the last 21 days (peripartum) of pregnancy. In the second part of study, silymarin was added to the rations of Holstein dairy cows for three weeks postpartum. A total of 40 Holstein dairy cows at 2nd lactation with 550-600 kg live weight and average body condition score of 3.5 were used. Dairy cows were randomly separated into two treatment groups (20 cows in each). The first group was control (no addition) and the second group was treatment (silymarin supplemented) group. Treatments significantly increased milk yield, but decreased milk protein. Postpartum bodyweight loss was significantly less in the silymarin group than in the control group. Differences in postprandial plasma triglyceride (TRG) and total cholesterol (TC) levels were found to be significant. Plasma alanine aminotransferase (ALT) and total protein (TP) values of the groups were also significantly different. As a result, it was observed that silymarin supplementation of the ration did not have side effects, and peak milk yields could be achieved earlier with silymarin treatment. Application of silymarin is believed to be useful. It was also observed that silymarin treatments speeded up the metabolic adaptation process of the dairy cows at the beginning of lactation. It was suggested that silymarin should be used in transition periods of dairy cattle.

Keywords: Body condition score, carsil, milk thistle, milk yield, peripartum,
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Introduction

Various metabolic problems are experienced through periparturient periods of dairy cattle. Dry matter (DM) intakes decrease during the last period of pregnancy and at the beginning of lactation. Together with negative energy balance, intense mobilization is experienced in body building scores (Drackley, 1999; Bobe *et al*., 2004; Oltenacu & Broom, 2010). The energy balance of high-yield cows is disrupted when the required energy is not supplied with their rations. Then, as a result of maladaptation to this negative energy balance, hepatic lipidosis (fatty liver) and ketosis-like metabolic diseases occur (Herdt, 2000; Mann *et al*., 2015).

While the survival rate energy requirements of animals with different milk yields are the same, yield rate requirements may be different. This difference points out the significance of energy balance being provided through feeding in high-yield animals (Capper *et al*., 2008). In hepatic lipidosis cases, non-esterified fatty acid quantities increase and they are esterified to triglyceride through oxidation of hepatic fatty acids. Hepatic lipidosis increases the risk of ketosis, abomasum displacement, metritis, and immune system suppression, and reduces reproductive performance (Bobe *et al*., 2004). During the peripartum period, in addition to reduced dry matter intake and negative energy balance, intense metabolization of body building stones is experienced. In that period, hepatoprotective agents are used to prevent fat accumulation in the liver. Methionine, choline, propylene glycol, and glycerol were used as preservatives in the cattle during the studies and their therapeutic properties were discovered (MoaUem *et al*., 2007; Grummer,1993; Bertics & Grummer, 1999; Goff *et al*.,1996). Silymarin is a hepatoprotective agent that contains liver-protecting substances. It is a standard extract that is taken from *Silybum marianum L.* (milk thistle) seeds and is used in the treatment of human liver diseases with various aetiologies (Saller *et al*., 2001). Silymarin extract is a

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complex structure composed of several flavonolignan isomers (Skottova *et al*., 2003). Silymarin has been used in herbal treatments for 2000 years.

Today, it is commonly used in the protection and treatment of the liver (Morazzoni & Bombardelli, 1995; Tamayo & Diamond, 2007). Improvements and increased functionality were observed in damaged livers with silymarin treatments (Leng, 1996; Fraschini *et al*., 2002). Silymarin influences cell permeability and has an antioxidant characteristic that prevents lipid peroxidation and membrane breakdown (Muriel & Mourelle, 1990; Mira *et al*., 1994; Fuchs *et al*., 1997). Such impacts of silymarin were reported by other researchers (Campose *et al*., 1989; Muriel & Mourelle, 1990; Mira *et al*., 1994). Skottova *et al*. (2003) reported that the polyphenol fraction of silymarin had positive effects on the lipoprotein profile of plasma and prevented fatty liver development in rats. Silymarin also significantly reduced serum gamma glutamyltranspeptidase, alanine transaminase (AST) and the aspartate transaminase levels of rats with liver damage (Wang *et al*., 1996).

The present study was therefore conducted to investigate the effects of silymarin supplementation to dairy cattle rations in preventing negative energy balance-induced liver damage on serum blood parameters [calcium (Ca), glucose (GL), triglyceride (TRG), cholesterol (TC), phosphorus (P), ALT and AST, total protein (TP)], milk yield, and components of fat, protein, lactose.

Materials and Methods

A commercial dairy herd with 1700 lactating Holstein Friesian dairy cows located in Kayseri Province, Turkey, was used. The herd was housed in a free-stall barn, fed on total mixed rations (TMR), and milked twice daily. Silymarin was supplemented to rations of second parity Holstein dairy cows with three weeks to calving. In the second part of study, silymarin was added to the rations of Holstein dairy cows for three weeks postpartum. The cows had live weights between 555 and 600 kg. The cows were selected from those with an average body condition score (BCS) of 3.5. Two groups were formed with 20 cows each (a total of 40 dairy cows were used). The first group was fed a regular ration (control). Together with the regular ration, silymarin extract was supplied to the second group orally, immediately after mixing with water to have 20 g/head/day silymarin consumption.

Animal feeds were supplied based on DM intake to have 20.77 kg feed per animal. Rations were prepared in accordance with the norms specified by National Research Council (NRC, 2001) for Holstein breed dairy cows with an average daily milk yield of 35 litres to meet their daily nutritional needs. Nutritional and chemical composition of the feeds supplied to animals are provided in Table 1 and the components of concentrate feed are listed in Table 2. The chemical composition of concentrate feed is provided in Table 3.

Table1 Components and chemical composition of TMR

Ingredients	**Naturally/kg**	**DM kg**	**Ration DM%**
Corn silage	13.00	4.02	19.34
Wheat straw	2.70	2.57	12.38
Dry alfalfa	1.50	1.38	6.65
Beet pulp	1.00	0.19	0.91
Cattle milk feed (concentrated feed)	13.50	12.61	60.73

Parameters	**Dry alfalfa**	**Corn silage**	**Beet pulp**	**Wheat straw**
CP%	25.10	8.93	11.00	4.96
CA%	9.08	4.28	4.48	7.56
DM%	92.00	30.90	18.80	95.20
CC%	1.98	3.22	2.00	1.63
ADF%	28.60	28.00	23.30	49.10
NDF%	39.20	45.20	45.50	73.20
ME (Mcal/kg)	2.040	2.320	2.65	1.52

Naturally (added to the ration), CP: crude protein (%of DM), CA: crude ash (% of DM), DM: dry matter (% of fresh), crude fat (% of DM), ADF: acid detergent fibre (% of DM), NDF: neutral detergent fibre (% of DM), ME: metabolizable energy (Mcal/kg DM)

Table 2 Components of concentrate feed supplied to animals

Concentrated feed components	Naturally, kg	DM, kg	Ration DM%
Grain barley	2.73	2.67	22.06
Soybean meal (46% CP)	3.48	3.24	27.00
Soybean full-fat	0.65	0.6	5.00
Kernel corn	1.56	1.48	12.23
Kernel wheat	0.65	0.61	5.04
Corn bran	0.32	0.30	2.47
Wheat bran	0.65	0.60	5.00
Molasses	0.45	0.39	3.22
Cottonseed meal (30% CP)	0.26	0.24	2.00
Bypass oil	0.32	0.31	2.56
Marble powder	0.27	0.27	2.23
Sunflower seed meal (37% CP)	1.17	1.10	9.09
Salt	0.23	0.21	1.73
DCP (dicalcium phosphate)	0.07	0.07	0.06
Premixed*	0.01	0.01	0.08

Naturally (added to the ration): vitamin-mineral premixed two kg of eating a ton. Mineral and vitamin content: vitamin A 12,000,000 UI; vitamin D3 2,400,000 UI; vitamin E 50000 mg; vitamin K3 1000 mg; vitamin B1 600 mg; vitamin B2 2500 mg; vitamin B6 150 mg; vitamin C 2000 mg; niacin 200000 mg; folic acid 2000 mg; biotin 200 mg; choline chloride 100000mg; d l methionine 330 mg; iron 80000 mg; copper 15000 mg; manganese 50000 mg; cobalt 150 mg; zinc 150000 mg; iodine 800 mg; selenium 150 mg

Table 3 Nutrient composition of concentrate feed (milk feed) (DM basis)

Nutrients	Calculated results (Declaration of manufacturer)	Analysed results
CP%	22	22.45
DM%	88	93.42
CA%	8	8.22
CS%	12	8.74
CF%	-	3.64
ME	2750	2785

CP: crude protein (%of DM), DM: dry matter (% of fresh), CA: crude ash (% of DM), CC: crude cellulose (% of DM), CF: crude fat (% of DM), ME: metabolizable energy (Mcal/kg DM)

Analyses of DM, CA, CP, CC, and CF contents of feed sources were carried out in accordance with AOAC (1998). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) analyses were carried out in accordance with Van Soest *et al*. (1991), and metabolic energy (ME) was calculated by the equation proposed by Robinson *et al*. (2004):

$$ME = 14.03-(0.01386 \times CF\%)-(0.1018 \times CA\%).$$

From the beginning of the experiment, bodyweights were measured weekly with a digital balance (± 500 g).

Daily milk yields of each animal were recorded to a herd management programme separately from the Dairymaster milking system. Then, milk yield per animal was taken from the system. A total of 40 milk samples were taken and subjected to Foss MilkoScan FT1 device (Milkoscan, Foss, Denmark) without waiting for chemical analysis.

At the beginning and end of the study, 80 blood samples were taken from the vena jugularis four hours after feeding. The blood samples were placed 10 ml tubes containing heparin, and centrifuged at 3000 rpm

for 10 minutes to obtain blood plasma. The resultant plasma samples were kept in a deep freezer at -20 °C until the time of analysis.

Plasma samples were then thawed at room temperature and subjected to ALT, AST, TP, glucose, triglyceride, and cholesterol analyses at the central laboratories of Erciyes University Medical Faculty. For metabolite analysis of the plasma samples, an autoanalyser with commercial kits (Abbott Diagnostics, Architect, USA) was used.

The following mathematical model was used in experiments:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

Where: Y_{ij} = observation value for j^{th} replication of i^{th} treatment
μ = general population mean
α_i = effect of i^{th} treatment
e_{ij} = random error for j^{th} replication of i^{th} treatment

Significance level was set as P <0.05. Results were expressed as mean ± standard error. All analyses were carried out with the GLM module of SAS software.

Results

The differences in prenatal and postnatal first-week bodyweights were not found to be significant (Table 4). However, bodyweights of the groups were significant in the second and third week (P <0.05).

Table 4 Body weights at the beginning and the end of experiment and milk components

Parameters	Control	Silymarin	SEM	P
Prenatal BW	564.15	564.95	2.624	0.881
First week BW	518.85	519.95	2.426	0.824
Second week BW	493.00	514.55	2.901	<0.001
Third week BW	488.00	514.55	3.142	<0.001
Milk yield,%	27.30	30.50	0.492	<0.001
Milk fat,%	3.54	3.46	0.013	0.003
Milk protein,%	3.72	3.45	0.027	<0.001
Milk lactose,%	4.94	4.94	0.013	0.939

SEM: standard error of mean; P: probability; BW: bodyweight (kg)

There was less postnatal weight loss in the silymarin treated cows than the control group. There were significant differences in the milk yields of the control and the treatment group (P <0.001). The silymarin group had higher milk yield than the control group. On the other hand, milk fat and protein contents were higher in the control group than in the treatment group. The differences in milk fat and protein contents were found to be significant, but the differences in milk lactose contents were not (P = 0.939).

The values obtained from the blood samples in the initial stage of the study are provided in Table 5. The differences among the blood parameters of the groups were not found to be significant.

Compared with prenatal blood samples, the glucose concentration of postnatal samples decreased. This decrease was lower in the silymarin group. The differences in plasma glucose contents of the groups were not found to be significant (P = 0.036). While there were significant differences in the concentrations of plasma TRG (P = 1.000) and plasma cholesterol (P = 0.680), the silymarin group had higher TRG and cholesterol concentrations than the control treatment. However, it was not statistically significant. While the effects of silymarin treatments on AST enzyme activity were not significant (P = 0.416), (P = 0.397), increasing ALT enzyme activities were observed with silymarin treatments (P <0.001). The prenatal treatment group had lower protein concentrations than the control group, but these differences were not found to be significant (P = 0.085). However, postnatal plasma protein concentrations were higher in the treated group (P <0.001). The differences in prenatal Ca and P concentrations of plasma samples of the silymarin group were not significant. On the other hand, lower decreases were observed in the Ca and P

concentrations of postnatal plasma samples of the treatment group, but the differences were not found to be significant (P = 0.844), (P = 0.283).

Table 5 Biochemical blood parameters of experimental cows at the beginning and end of experiment

Parameters	Prenatal		SEM	P
	Control	Silymarin		
GL (mg/dL)	53.40	46.65	1.627	0.036
TRG (mg/dL)	31.65	31.65	0.533	1.000
TC (mg/dL)	101.55	99.65	2.264	0.680
AST (U/l)	63.25	60.15	1.878	0.416
ALT (U/l)	27.45	28.00	1.013	0.790
TP (mg/dL)	7.51	7.11	0.117	0.085
Ca (mg/dL)	14.75	13.55	1.274	0.644
P (mg/dL)	7.33	6.80	0.614	0.675
Parameters	**Postnatal**		**SEM**	**P**
	Control	**Silymarin**		
GL (mg/dL)	41.15	45.65	1.236	0.068
TRG (mg/dL)	14.15	26.65	1.100	<0.001
TC (mg/dL)	78.55	98.15	2.333	<0.001
AST (U/l)	59.75	56.50	1.890	0.397
ALT(U/l)	30.45	38.00	1.179	<0.001
TP (mg/dL)	6.26	7.11	0.132	<0.001
Ca (mg/dL)	12.65	13.15	1.244	0.844
P (mg/dL)	5.25	6.60	0.622	0.283

SEM: standard error of means; P: probability; GL: glucose (mg/dL); TRG: triglyceride (mg/dL); TC: total cholesterol (mg/dL); AST: aspartate amino transferase enzyme (U/l); ALT: alanine amino transferase enzyme (U/l); TP: total protein (g/dL); Ca: calcium (mg/dL); P: phosphorus (mg/dL)

Discussion

Experiments were conducted in two phases, namely prenatal and postnatal. In comparison with the control treatment, bodyweight, milk yield, differences in parameters were observed in serum blood TRG, TC, ALT, AST, and TP. There were no problems with regard to health of animals in both prenatal and postnatal periods. Bodyweight losses were higher in the control group than in the treatment group (Gerloff *et al*., 1986). Body weight measurements at the end of the third week revealed that weight loss was higher in the control group than in treatment group. Previous studies also reported body weight losses in early lactation period (Ingvartsen *et al*., 2003; Meijer, 2010). However, weight loss was lower in the silymarin-treated group.

Higher weight losses in the control group than the treatment group could be related to hepatic lipidosis and resultant malfunction of the liver (Gerloff *et al*., 1986). Lucy *et al*. (2001) indicated that total milk yield could be improved with proper feeding throughout the transition period. Silymarin reduced the negative conditions experienced in transition to metabolic adaptation at the beginning of lactation and thus improved the milk yields. Although there are not any concrete evidences that silymarin treatments improved milk yields, similar findings were reported in previous studies (Garavaglia *et al*., 2015). Besides antioxidant impacts, silymarin was reported to have hepatoprotective effects, and to prevent fat accumulation in the liver (Tedesco *et al*., 2004). In terms of the plasma glucose levels, significant differences were not observed between prenatal and postnatal values. The decrease in plasma glucose levels was lower in silymarin group than in control group.

The decrease in insulin concentration might have resulted in reduced serum glucose concentration. With silymarin treatments, plasma insulin concentration may increase fatty acid oxidation in the pancreas. Stimulation of insulin segregation from pancreas β-cells by glucose depends on the availability of fatty acids (Stein *et al*., 1996). Fatty acid oxidation in β-cells may increase in cows fed with silymarin-supplemented

rations and reduced availability of fatty acids may cause an increase in insulin segregation. On the other hand, silymarin supplementation may alter peripheral glucose metabolism and insulin sensitivity, and thus influence glucose metabolism. It was observed that silymarin influenced not only β-oxidation of fatty acids, but also glucose metabolism. Silymarin treatments increased glucose oxidation and storage in patients with type II diabetes (Mingrona *et al*., 1999; Tedesco *et al*., 2004). Present plasma glucose quantities comply with those of earlier reports (Mehta & Gangwar, 1985; Karagül *et al*., 1999; Polat *et al*., 2002).

It has been reported in previous studies that silymarin reduces triglyceride synthesis in liver (Skottova & Kreeman, 1998) and activates fatty acid β-oxidation (Valenzuela & Garrido, 1994). However, in another study, less reduction was reported in the TRG quantities of the silymarin group than the control group. Current plasma triglyceride levels do not comply with those of earlier reports (Mourelle *et al*., 1989; Valenzuela & Garrido, 1994; Skottova & Kreeman, 1998).

Although there were no significant differences in TC and TRG concentrations, there was a numerical decrease in TC concentrations of treatment group than the control.

Plasma cholesterol levels were similar to earlier findings (Haddad *et al*., 2011). Haddad *et al*. (2011) reported that silibinin extracts (200 ml/kg) in a high-fat liquid diet decreased the cholesterol levels of rats, but the differences were not significant. Sobolova *et al*. (2006) reported increased serum cholesterol levels of rats with silymarin extract treatments. In another similar study, silymarin significantly reduced liver cholesterol levels (Skottova *et al*., 2003; Sobolova *et al*., 2006). The ALT and AST exhibit quite high activity in acute or chronic liver damage, and such activity can be identified in serum (Turgut, 2000). Fatty liver syndrome in lactating cows may reduce the appetite (Cebra *et al*., 1997), and ketosis at early lactation may increase AST enzyme activity (Steen, 2001). This may be used as a bio-indicator for possible liver damage (Kauppinen, 1984; Meyer & Harvey, 1988). Contrary to AST activity, ruminants do not have high ALT activity. Changes in serum ALT activities thus cannot be clearly identified in liver damage (Forenbacher, 1993). There are distinctive irregular and slight changes in AST and gamma glutamyl transferase enzyme activities in pregnancy and early lactation periods. On the other hand, ALT activities decrease distinctively during the seventh and eighth months of pregnancy and at the beginning of lactation (Tainturier *et al*., 1984). In the present study, differences in AST activities of treatment and control group were not found to be significant. The decrease in AST activity may be related to silymarin supplementation. As indicated, an increase in serum AST activity may be used for sub-clinic diagnosis of possible liver damage (Kauppinen, 1984; Meyer & Harvey, 1988). Therefore, different silymarin levels may be experimented with for various periods to elucidate these effects. The present findings may also provide significant contributions for researchers that are studying the protective effects of silymarin on liver. In the present study, significant differences were observed only in postnatal ALT activities. These differences had resulted from transition from feed adaptation to lactation periods. Stojević *et al*. (2005), reported significant increases in serum ALT activities in early lactation compared with the control treatment. In the present study, silymarin supplementation increased ALT activities, but such changes were not considered sufficient to be related to liver damage.

Conclusion

It was concluded that silymarin supplementation to feed rations did not have negative side effects. It was also observed that silymarin supplementations might speed up the transition to metabolic adaptation of dairy cows at the beginning of lactation, and might result in earlier achievement of peak milk yield. Various silymarin doses could be applied to observe the impacts of silymarin supplementation on tissues, especially on liver. In further studies, the effects of silymarin treatments on the metabolic profile could be studied in detail. The present findings might contribute to those future studies on silymarin supplementation.

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Authors' Contributions

İÜ designed the project, collected the data and did the statistical analysis. ACO did the laboratory analysis. TA wrote and edited the manuscript until it was submitted to the journal to be considered for publication.

Conflict of Interest Declaration

The authors declare that there is no conflict of interest.

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