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Relationships between the Postpartum Body Condition Score, Colostral IgG Content, Liver Lipid Metabolism and Oxidative Stress in Dairy Cows

This study aimed to evaluate the relationships between the postpartum body condition score (BCS) and colostral IgG, lipid metabolism, liver functions and oxidative stress parameters in dairy cows with BCSs of ≤ 3.5 and > 3.5 .

This investigation included 60 dairy cows of the same breed, that were 3-5 years old and had given two or more births in a dairy farm. Care was taken to ensure that the BCS changes of the cows were < 0.5 from the dry period to calving.

In the 30 cows with a BCS of ≤ 3.5 within 24 hours of birth, the mean BCS level was 3.24 ± 0.24 and the mean serum β -hydroxybutyric acid (BHBA) level was 0.36 ± 0.07 . In the 30 cows with a BCS of > 3.5 within 24 hours of birth, the mean BCS level was 3.96 ± 0.14 and the mean serum BHBA level was 0.38 ± 0.07 .

Compared to the values determined in cows with a BCS > 3.5 , serum non-esterified fatty acids (NEFA), high-density lipoprotein (HDL), paraoxonase-1 (PON-1) and malondialdehyde (MDA) levels were significantly lower, and triglyceride, albumin, SOD and colostral IgG levels were significantly higher in cows with a BCS of ≤ 3.5 .

It was concluded that a BCS change of < 0.5 from the drying period to parturition and a postpartum BCS of ≤ 3.5 in dairy cows would be beneficial for better management of the metabolic process, oxidative stress and high colostral IgG level.

Key Words: Body condition score, oxidative stress, metabolites, colostral IgG

Sütçü İneklerde Doğum Sonrası Vücut Kondüsyon Skoru, Kolostral IgG Konsantrasyonu, Karaciğer Lipid Metabolizması ve Oksidatif Stres Arasındaki İlişkiler

Bu çalışmada, vücut kondüsyon skorları (VKS) ≤ 3.5 ve > 3.5 olan süt ineklerinde postpartum VKS ile kolostral IgG, lipid metabolizması, karaciğer fonksiyonları ve oksidatif stres parametreleri arasındaki ilişkilerin değerlendirilmesi amaçlandı.

Bu araştırma, bir süt çiftliğinde iki veya daha fazla doğum yapmış, aynı ırktan, 3-5 yaşlarında 60 süt ineğini içermektedir. İneklerin VKS değişikliklerinin kuru dönemden buzağılamaya kadar < 0.5 olmasına özen gösterildi.

Doğumdan sonraki 24 saat içinde VKS'si ≤ 3.5 olan 30 inekte ortalama VKS seviyesi 3.24 ± 0.24 ve ortalama serum β -hidroksibütirik asit (BHBA) seviyesi 0.36 ± 0.07 idi. Doğumdan sonraki 24 saat içinde VKS'si > 3.5 olan 30 inekte ortalama VKS seviyesi 3.96 ± 0.14 ve ortalama serum BHBA seviyesi 0.38 ± 0.07 idi.

VKS'si > 3.5 olan ineklerde belirlenen değerlerle karşılaştırıldığında; VKS'si ≤ 3.5 olan ineklerde serum non-ester yağ asidi (NEFA), yüksek yoğunluklu lipoprotein (HDL), paraoksanaz-1 (PON-1) ve malondialdehit (MDA) düzeyleri anlamlı olarak daha düşüktü ve trigliserit, albümin, SOD ve kolostral IgG düzeyleri anlamlı olarak yüksekti.

Sonuç olarak, süt ineklerinde lipid metabolizmasının ve oksidatif stresin daha iyi yönetimi ve daha yüksek kolostral IgG seviyesinin sağlanabilmesi için, ineklerdeki VKS değişikliğinin kuru dönemden doğuma kadarki dönemde < 0.5 ve doğum sonrası VKS'nin ≤ 3.5 olmasının yararlı olduğu kanısına varıldı.

Anahtar Kelimeler: Vücut kondüsyon skoru, oksidatif stress, metabolitler, kolostral IgG

Introduction

Determination and monitoring of body condition scores (BCS) in dairy cattle farms is a very effective and practical method that can be used to evaluate nutritional status, milk yield, reproductive performance, metabolic processes and welfare status. BCS is a subjective method based on the determination of the thickness of the subcutaneous fat in the back, waist and sacrum regions of cows and their relationship with the bone protrusions in the pelvic region by inspection and/or the palpation method. In addition, BCS is a method that effectively shows the usable energy reserve for the metabolism of cows. Negative energy balance (NEB), which generally occurs in cows with high milk yield, negatively affects metabolic processes, oxidative stress and colostrum quality, and this leads to changes in the BCS of cows (1-3).

The energy deficiency that occurs as a result of the metabolic energy provided by nutrition not being at a level high enough to meet the requirement for normal bodily functions, is compensated with the energy obtained by the destruction of the fat tissues on the outer surface of the body. Physical changes in the fat tissues around the back, waist, ribs, coccyx and mammary glands are taken into account in the evaluation of BCS (4, 5).

In dairy cows, NEB occurs most prominently during parturition and the onset of colostrum-milk production. Monitoring BCS changes in cows in these periods is very important in terms of evaluating the developmental status of NEB. NEB first causes an initial rise of blood serum non-esterified fatty acids (NEFA), which is an indication of the stimulation of fat mobilization in the body. That, in turn, causes accumulation of β -hydroxybutyric acid (BHBA) in blood serum. Changes of the energy balance in the postpartum period in cows with high milk yield are classified as a type of postpartum adaptation, which is a normal physiological process. However, metabolic disorders and infectious diseases that may occur in cows during this period cause deficiencies in this physiological adaptation (6, 7).

It has been reported that the probability of the occurrence of disorders related to the reproductive system and milk production is increased in cows with a birth BCS of ≤ 3.0 and > 3.5 , whereas a decrease in colostrum IgG levels has been observed in cows with a BCS of > 3.5 (4, 5, 8). In addition to containing many important substances that are important for calf health, colostrum IgG concentration is considered to be a distinguishing feature to evaluate colostrum quality (8).

Serum high density lipoprotein (HDL), triglyceride and total cholesterol values are the main parameters that should be checked to monitor postpartum lipid metabolism (9-11). Serum total protein, albumin, glucose, aspartate amino transaminase (AST) levels are determined in the evaluation of liver functions in dairy cows in which NEB develops (12-14). In addition, albumin, a significant part of which is synthesized in the liver, is an important element of the extracellular antioxidant system, as it protects thiol groups against oxidative stress (15).

Metabolic adaptation in cows begins in the last days of pregnancy and continues postpartum. The adaptation process is characterized by a change in the antioxidant/oxidant ratio and an increase in fat mobilization activity (16-18). The increase in fat mobilization leads to an increase in free radicals, which is the cause of oxidative stress. If this situation cannot be compensated by antioxidants, postpartum diseases and metabolic disorders are likely to crop up (15, 19, 20). One of the parameters used for the determination of oxidative stress is SOD, which functions in the natural elimination of superoxide; the other is malondialdehyde (MDA), which occurs as a by-product of lipid peroxidation and is used in the evaluation of oxidative degeneration of lipids (21). It is stated that the serum paraoxsanaz-1 (PON-1) level may show the levels of

HDL oxidation inhibited by PON and oxidative modification of HDL, and this may be related to the development of inflammation in the body (22-24). It has been reported that changes in the serum PON-1 level in dairy cows are associated with the level of oxidative stress and lipid metabolism disorders that occur in relation to reproductive stress and the degree of metabolic adaptation (25-27).

During the process by which dairy cows give birth, physiological functions such as the formation of colostrum and initiation of lactation, in addition to maximizing the physiological, metabolic and hormonal recovery in the body, and organ regeneration activity, collectively cause oxidative stress. It is stated that the importance of monitoring the levels of postpartum BCS, NEB, BHBA, serum oxidative stress, liver and lipid metabolism parameters is crucial in the successful management of this process (28-34). Evaluation of blood serum AST, total protein, glucose and bilirubin levels provide important data on the status of lipid metabolism and liver functions, which are stated to change in relation to the degree of NEB (35-37).

In this study, it was aimed to evaluate the relationships between postpartum lipid metabolism, liver functions and oxidative stress parameters and colostrum IgG and BCS values in dairy cows whose BCS values were determined within 24 hours after birth and with normal postpartum serum BHBA levels.

Materials and Methods

This study was granted ethical approval by the Ankara University Animal Experiments Local Ethics Committee (Decision No: 2020-11-86).

This study evaluated 60 dairy cows of the same breed that were 3-5 years of age, had two or more births, and were used in a dairy farm. Care was taken to ensure that the BCS changes of the cows were < 0.5 from the dry period to the calving. This procedure was determined by considering BCS evaluations during routine clinical examination. BCSs were calculated during the dry period (2 months before calving) and within 24 hours after calving. The BCSs in these 60 cows were determined by a body condition score system method (38), progressing in quartiles (0.25 points) and having a total BCS of 5.

The first group was comprised of 30 cows that had a BCS of ≤ 3.5 within 24 hours of birth. The 30 cows of the second group were determined to have had a BCS of > 3.5 within 24 hours of birth.

Serum was extracted from the blood samples, and from each specimen 6 ml was collected in each tube without anticoagulant after birth. This took place within one hour of birth, and afterward the serum samples were stored at $-80\text{ }^{\circ}\text{C}$ until the analysis.

A portable device (VET-TD-4235 Beta Keton Monitoring System, TalDoc Technology Corporation, Taiwan, ROC) was used to determine the level of BHBA. Blood serum albumin, total protein, total cholesterol,

triglyceride, HDL, glucose, urea, creatinine and aspartate amino transaminase (AST) were measured using an automated biochemistry analyzer (Mindray BS120). Blood serum NEFA, PON-1 and colostral IgG levels were measured using respective ELISA test kits (Sun Red Biotechnology Company China, Cat No: BIO K 420/1, Rel Assay Diagnostics Cat No: RL0031, Turkey, Cat No: 201-04-0186, Bio-X Diagnostics, Belgium). The blood serum superoxide dismutase (SOD) level was measured using the Cayman 706002 commercial test kit. The blood serum malondialdehyde (MDA) level was measured using a commercial ELISA test kit (Sun Red Biotechnology Company, Cat No: 2.01-04-0255, China).

For statistical analysis, prior to evaluating statistical significance all data were tested for parametric test assumptions, namely normality with the Shapiro-Wilk test, and homogeneity with the Levene test. The differences among the two groups were analyzed using the Student's t-test when parametric assumptions were met and the Mann-Whitney U test showed otherwise. All statistical analyses were examined with a 5% margin of error. Data were analyzed using SPSS software package v.14.01.

Results

In the group of 30 cows that had a BCS of ≤ 3.5 within 24 hours of birth, the mean BCS level was 3.24 ± 0.24 and the mean serum BHBA level was 0.36 ± 0.07 . In the group of 30 cows with a BCS of > 3.5 within 24 hours of birth, the mean BCS level was

3.96 ± 0.14 and the mean serum BHBA level was 0.38 ± 0.07 .

Blood serum glucose, albumin, total protein, AST, total cholesterol, triglyceride, HDL and NEFA levels in cows with BCS ≤ 3.5 and BCS > 3.5 are shown in Table 1.

Compared to the values in the cows with a BCS of 3.24 ± 0.24 , in the cows with a BCS of 3.96 ± 0.14 , the blood serum HDL and NEFA levels were found to be significantly higher ($P < 0.05$) and the blood serum albumin and triglyceride levels were significantly lower ($P < 0.05$). These results indicate that lipid mobilization due to postpartum NEB development was significant in the cows with a BCS of > 3.5 .

Blood serum urea, creatinine, BHBA, PON-1, SOD, MDA and colostral IgG levels of cows with BCS ≤ 3.5 and BCS > 3.5 are shown in Table 2.

Compared to the value in the cows with a BCS of 3.24 ± 0.24 , in the cows with a BCS of 3.96 ± 0.14 , while the blood serum SOD level was found to be significantly lower ($P < 0.001$), the MDA level ($P < 0.001$) and the PON-1 level ($P < 0.009$) were found to be much higher. These results show that cows with > 3.5 BCS are under more severe oxidative stress than the cows with < 3.5 BCS.

The colostral IgG level in the cows with a BCS of 3.96 ± 0.14 was lower ($P < 0.001$) than that in the cows with a BCS of 3.24 ± 0.24 . This indicates that the level of colostral IgG content in cows with > 3.5 BCS was negatively affected by the postpartum lipid metabolism and oxidative stress load.

Table 1. Blood serum glucose, albumin, total protein, total cholesterol, triglyceride, HDL, AST and NEFA levels in cows with BCS ≤ 3.5 and BCS > 3.5

Groups by BCS	Biochemical Parameters							
	Glucose (mg/dL)	Albumin (g/dL)	Total Protein (g/dL)	Total Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	AST (U/L)	NEFA ($\mu\text{mol/mL}$)
BCS ≤ 3.5 (3.24 ± 0.24)	63.5 ± 6.82	$4.06 \pm 0.1^*$	7.47 ± 0.25	74.36 ± 2.24	$9.53 \pm 0.78^*$	$42.24 \pm 1.67^*$	82.77 ± 3.09	$35.97 \pm 1.35^*$
BCS > 3.5 (3.96 ± 0.14)	82.87 ± 9.22	$3.88 \pm 0.1^*$	6.99 ± 0.22	79.09 ± 5.06	$7.15 \pm 0.39^*$	$49.94 \pm 2.1^*$	90 ± 4.26	$40 \pm 2.58^*$
P		< 0.05			< 0.05	< 0.05		< 0.05

The results are presented as arithmetic mean \pm standard deviation

* A statistically significant differences were found between albumin, triglyceride, HDL and NEFA levels in the two groups ($P < 0.05$)

Table 2. Blood serum urea, creatinine, BHBA, PON-1, SOD, MDA and Colostral IgG levels of cows with BCS ≤ 3.5 and BCS > 3.5

Groups by BCS	Biochemical Parameters						
	Urea (mg/dL)	Creatinine (mg/L)	BHBA (mmol/L)	PON-1 (U/L)	SOD (U/g protein)	MDA (nmol/g protein)	Colostral IgG (mg/ml)
BCS ≤ 3.5 (3.24 ± 0.24)	35.97 ± 1.35	1.58 ± 0.05	0.36 ± 0.07	309 ± 18.65	24.26 ± 1.1	2.63 ± 0.23	122.067 ± 10.8911
BCS > 3.5 (3.96 ± 0.14)	40 ± 2.58	1.74 ± 0.06	0.38 ± 0.07	$357.53 \pm 24.23^*$	$20.29 \pm 0.45^{**}$	$3.33 \pm 0.30^{**}$	$100.86 \pm 13.73^{**}$
P				< 0.05	< 0.001	< 0.001	< 0.001

The results are presented as arithmetic mean \pm standard deviation.

* A statistically significant differences were found between PON-1 levels in the two groups ($P < 0.05$)

** The SOD and MDA and colostral IgG levels in the two groups were significantly different ($P < 0.001$)

Discussion

In addition to the increased energy requirement during and after calving in dairy cows, the initiation of colostrum-milk production causes aggravation of NEB.

The body condition score measurement is used to determine the NEB status of cows under field conditions (5, 39, 40). It is stated that there are important relationships between BCS changes and the management of metabolic processes, milk yield, fertility and general health status in dairy cattle (41, 42). Dairy cows with a body condition score of $2.5 \leq 3.5$ were considered to be in good condition, and those with a body condition score of >3.5 were considered to be a high body condition (43, 5). The dairy cows we used in our study were divided into two groups as those with a BCS of ≤ 3.5 (good condition) and as those with a BCS of >3.5 (high condition) in accordance with the limitations of BCS values stated by the researchers. In this study, we aimed to evaluate the relationships between the values of BCS, colostral IgG content, liver, lipid metabolism and oxidative stress parameters in the cows of both groups.

Roche et al. (5), have stated that it is possible that the metabolic process can be successfully managed by evaluating the changes in the levels of parameters related to energy metabolism in the postpartum period in dairy cows. Similarly, in their study evaluating liver and lipid metabolism, Çolakoğlu and Küplülü (28) point out that the postpartum metabolic process in cows can be successfully monitored by taking into account the changes in BCS, NEFA and BHBA values. Some authors found these values to be at the lowest level in the cows with a birth BCS of 3.0-3.25. Podpečan et al. (44) concluded that it is important to examine liver and lipid-related parameters and metabolic factors in evaluating the metabolic profile of cattle on a herd basis. Zhao et al. (29), stated that blood serum NEFA, BHBA, MDA and SOD values determined in the cows with a birth BCS of 4.5-5.0 were higher than the values in the cows with a birth BCS of 3.0-4.25. Turk et al. (45) and Drackey et al. (46) point out that postpartum lipid mobilization and oxidative stress are the part of a complex series of metabolic adaptation parameters. They have evaluated the blood serum levels of triglyceride, total cholesterol, HDL, urea, creatinine, glucose, total protein, AST and PON-1. As a result, they determined that in the transition period, the blood serum triglyceride, total cholesterol, HDL-C, BHB, FFA, TAS and PON1 levels were significantly affected. Aviram et al. (23) and Trevisi et al. (24) indicated that liver function markers are closely related with the blood serum albumin, bilirubin, cholesterol and PON-1 levels.

Bernabucci et al. (15) and Gheise et al. (32), stated that cows with a higher calving BCS are more susceptible to oxidative stress due to postpartum body reserve losses and changes in metabolic processes. Folnožic et al. (33), evaluated the birth BCS of 3.35-3.75 in the cows as the ideal body condition, while a BCS of >4.0 as adiposity. In addition, they found blood serum triglyceride, HDL, cholesterol and NEFA levels to be

higher in the cows they evaluated as having an ideal body condition, compared to the values they determined in the cows scored as adiposity. But they have not found significant differences in the case of blood serum BHBA concentration during the transition period in both groups. O'Boyle et al. (34), pointed out that there is an increase in oxidative stress and a decrease in serum NEFA in cows with high postpartum BCS, but that negative energy balance did not occur in these cows. Çolakoğlu et al. (35), found the blood serum MDA level in the cows with a birth BCS of 2.75-3.0 to be higher than that of in the cows with birth BCS of 3.25-3.75. Same authors stated that the incidence of oxidative stress and metabolic diseases increased in the cows with low birth BCS values. They also found that the cows with low BCS values had lower glucose concentrations than those with adequate values. In our study, it has been evaluated that the decrease in the serum SOD level and the increase in the MDA level in cows with $BCS > 3.5$ may have occurred as a result of lipid peroxidation and superoxide dismutation due to the increase in oxidative stress. We evaluated that cows with high birth BCSs were more susceptible to oxidative stress due to loss of body reserve and changes in metabolic processes. In addition, no significant difference was observed in blood BHBA concentrations between the two groups. In our study, serum glucose and BHBA levels were found to be lower in cows with a BCS of ≤ 3.5 than in cows with a BCS of >3.5 . Blood serum urea, creatinine, total protein and total cholesterol levels were found to be higher in cows with a BCS of ≤ 3.5 than in cows with a BCS of >3.5 . However, the changes in these parameters, which were not statistically significant, were found to be compatible with the results of various investigators (35, literature).

Sevinç et al (36), reported after evaluating serum GGT, AST and bilirubin levels that they provide important data regarding lipid metabolism and liver functions which change depending on NEB. Concerning the development of NEB, the liver is the organ which is the main provider and location of the energy conversion in which lipid metabolism also plays a role (47). Zhou et al. (37), have evaluated the liver-specific plasma protein albumin and the liver/intestinal lipoprotein cholesterol in cows 3 and 28 days after birth, and they noted that there were significant correlations between the parameters related to milk yield and the parameters indicative of liver function. In our study, when compared with the values in the cows with a BCS of 3.96 ± 0.14 ; in the cows with a BCS of 3.24 ± 0.24 serum NEFA, HDL, PON-1 and MDA values were found to be lower and serum triglyceride, albumin, SOD, and colostral IgG values were found to be higher. The statistically significant increase in serum PON-1, NEFA and HDL levels and a rather major decrease in serum triglyceride and albumin levels in cows with a BCS of 3.96 ± 0.14 may be associated with lipid mobilization resulting from the development of NEB. The low serum protein, albumin and colostral IgG levels in cows with a BCS of 3.96 ± 0.14 compared to the values in the other group suggest that the immune system might be suppressed in cows with a BCS value of >3.5 . These results show that the increase in oxidative stress

activity resulting from the development of postpartum NEB in cows with BCSs of >3.5 causes more severe disruption in the energy metabolism balance and leads to the emergence of higher levels of oxidative stress products. Immune system suppression, which is thought to occur in direct proportion to the severity of NEB developing in dairy cows with a high postpartum BCS value, leads to a decrease in colostral IgG levels. Conneely et al. (8) stated that there is a significant inverse correlation between colostral IgG levels and BCS value in dairy cows. Shearer et al. (48) reported that there is a relationship between the increase in the BCS and the amount of colostral IgG in cows in the dry period. Contrary to the opinions above, Immler et al. (49) stated that they could not find a relationship between BCS and colostrum quality in their study, in which they evaluated colostrum quality with a Brix refractometer. Abdullağolu et al. (50), determined that the increase in colostral IgG value in cows with a BCS of 3.5 was not statistically significant compared to the values determined in cows with BCSs of 2.5-3.0 and 4.0. Soufleri et al (51), while they could not find a significant difference between BCSs and colostrum total solid contents, they determined a significant negative

relationship between colostrum yield and BCS values and reported that they could obtain less colostrum from cows with BCSs of >3.50. In our study, colostral IgG level was found to be significantly lower in cows with BCSs of >3.5 compared to cows with BCSs of ≤3.5. This result can be interpreted as the suppressive effect of oxidative stress and increase in lipid metabolism metabolites on the immune response in cows with BCSs of >3.5.

When blood serum BHBA, NEB, liver and lipid metabolism parameters, oxidative stress markers and colostral IgG levels were evaluated together with BCS values in postpartum dairy cows, it was concluded that a BCS change of <0.5 from dry period to calving and a postpartum BCS of ≤3.5 are beneficial for better management of lipid metabolism and oxidative stress and higher colostral IgG level in cows.

Conflict of Interest

The authors declared that there is no conflict of interest.

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