



Investigation of the effect of feeding TMR (Total Mixed Ration) with different protein and energy contents on milk yield, average feed consumption and blood antioxidant status in Holstein dairy cows during early lactation period

[Investigação do efeito da alimentação com Ração Total Misturada (RTM) com diferentes teores de proteína e energia sobre a produção de leite, consumo médio de ração e estado antioxidante sanguíneo em vacas leiteiras da raça Holandesa durante o início do período de lactação]

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ABSTRACT

This study aimed to evaluate the effects of Total Mixed Rations (TMRs) formulated with different energy and protein levels on milk yield and antioxidant status in Holstein dairy cows during early lactation. Twenty multiparous Holstein cows (2nd–3rd lactation) were grouped based on milk yield and assigned to two dietary treatments: R1 (low energy-protein) and R2 (high energy-protein), with 10 cows per group. The trial lasted 45 days at a certified dairy farm in Ankara, under housing and management conditions appropriate to animal welfare. Turkey. All animals were fed ad libitum with TMRs prepared according to NRC (2000) standards. Daily milk yield and group-based feed intake were recorded, and blood samples were collected at the end of the trial to assess antioxidant parameters. Results showed that cows in the R2 group exhibited significantly higher milk yields on days 10, 20, 30, and 45, indicating improved lactation performance with high energy-protein TMR. Additionally, superoxide dismutase (SOD) and albumin levels were significantly elevated in the R2 group, suggesting enhanced antioxidant capacity. Other antioxidant parameters remained unaffected. In conclusion, feeding high-energy and protein TMRs to dairy cows during early lactation positively affected milk production and protected against oxidative stress.

Keywords: antioxidant status, energy, milk yield, protein, TMR

RESUMO

Este estudo teve como objetivo avaliar os efeitos de rações mistas totais (RMTs) formuladas com diferentes níveis de energia e proteína sobre a produção de leite e o estado antioxidante em vacas leiteiras da raça Holandesa durante o início da lactação. Vinte vacas multíparas da raça Holandesa (2^a–3^a lactações) foram agrupadas com base na produção de leite e distribuídas em dois tratamentos dietéticos: R1 (baixo valor energético-proteico) e R2 (alto valor energético-proteico), com 10 vacas por grupo. O experimento durou 45 dias, em uma fazenda leiteira certificada em Ancara (Turquia), sob condições de alojamento e manejo adequadas ao bem-estar animal. Todos os animais foram alimentados ad libitum com RMTs preparadas de acordo com os padrões do NRC (2000). A produção diária de leite e o consumo de ração por grupo foram registrados, e amostras de sangue foram coletadas ao final do experimento para avaliar os parâmetros antioxidantes. Os resultados mostraram que as vacas do grupo R2 apresentaram produções de leite significativamente maiores nos dias 10, 20, 30 e 45, indicando

melhor desempenho na lactação com RMTs de alto valor energético-proteico. Além disso, os níveis de superóxido dismutase (SOD) e albumina foram substancialmente elevados no grupo R2, sugerindo maior capacidade antioxidante. Outros parâmetros antioxidantes permaneceram inalterados. Em conclusão, a alimentação de vacas leiteiras com TMRs de alta energia e proteína durante o início da lactação afetou positivamente a produção de leite e protegeu contra o estresse oxidativo.

Palavras-chave: estado antioxidante, energia, produção de leite, proteína, TMR

INTRODUCTION

Global population growth and awareness of healthy nutrition have further highlighted the strategic position of milk and dairy products in the food chain. Accordingly, both herd size and average yield are being increased in farms to increase milk production efficiency (Çetin and Uçar, 2018). This global population growth has made meeting humanity's basic nutritional needs even more crucial. In this context, the scientific community is constantly conducting research aimed at increasing productivity, particularly in animal husbandry and agriculture. Genetic selection has led to noticeable improvements in the milk and meat production capacities of animals. However, for this increased production to be sustainable, animal care and feeding conditions must also be improved at the same rate (Oltenacu, and Algers, 2005). In modern dairy farming systems, the expected production and reproductive performance of animals is quite high (Vires and Marcondes, 2020). Today's dairy cows are expected to produce a high volume of high-quality milk, in addition to giving birth to one calf per year. For example, during the peak lactation period, a cow should produce 30–40 liters of milk per day; this milk should contain 3–5% milk fat and 3–3.2% milk protein (Doran *et al.*, 2022). Milk production involves a production cycle that includes a lactation period lasting approximately 305 days and a dry period of 60 days. This process, combined with an 80-day insemination period and a 285-day gestation period, results in a highly intensive production and reproductive cycle that pushes the physiological limits of the dairy cow (Maltz, 2020). In today's intensive dairy production systems, a balanced diet planned for the different phases of the production cycle is critical for maintaining animal health and biological functionality. Especially during the peripartum period, dairy cows frequently experience negative energy balance (NEB), and to address this energy deficit, they mobilize their body fat reserves and release non-esterified fatty acids

(NEFAs) into the bloodstream. Although homeostatic mechanisms attempt to maintain blood parameters within physiological limits, increased metabolic demands in early lactation lead to significant changes in metabolite and hormone levels. While these biochemical changes are not direct indicators of disease, they disrupt the physiological balance of animals, making them more susceptible to metabolic diseases (Monteverde *et al.*, 2017). Recent research has demonstrated that nutritional strategies implemented during this period not only improve animal health and welfare but also play a decisive role in increasing milk yield and reproductive performance (Abbate *et al.*, 2020).

In today's high-yield dairy production systems, the production and reproductive performance expected of cows makes them more susceptible to various metabolic diseases. Therefore, care and feeding strategies appropriate to the animals' physiological needs must be meticulously implemented. While oxidative stress, one of the stress factors encountered throughout the production cycle, is not directly defined as a metabolic disease, it is closely related to the biochemical mechanisms underlying many metabolic disorders (Ingvarsen and Moyes, 2013). In this context, oxidative stress is considered both a trigger of diseases and an accompanying process (Tashla *et al.*, 2021). According to studies, Total Mixed Rations (TMR) did not exist in 1917 and were primarily developed in the second half of the past century. As milk yield increased, herd sizes grew, and modern milking technologies advanced, producers gradually shifted toward complete feeding systems. The first full research article on TMR was published in 1966, marking its formal introduction into dairy science. The TMR system ensures that every bite of feed contains a balanced and complete nutrient profile, minimizing issues like feed separation, selective eating, and digestive disorders. Today, TMR is widely adopted in large-scale dairy operations due to its efficiency, improved feed intake

consistency, and positive effects on cow health and milk production (Schingoethe, 2017).

This study addresses the role of nutritional strategies in managing metabolic stresses, particularly oxidative stress, encountered in modern dairy production systems, providing important and timely information for both researchers and dairy farmers. Increasing milk yield and intensive production cycles strain animals' physiological limits, increasing the risk of metabolic problems such as negative energy balance (NEB) and oxidative stress, particularly during early lactation. In this context, optimizing the energy and protein content of the Total Mixed Ration (TMR) system has the potential to not only increase milk yield but also improve the antioxidant capacity and overall health of animals. This study evaluates the effects of different energy and protein levels of TMR in early lactation, offering a holistic approach that addresses both production performance and metabolic health indicators. In this respect, the study contributes to the development of applicable feeding protocols at the farm level and fills a gap in the academic literature regarding nutritional management of oxidative stress. Monitoring blood antioxidant levels and correlating them with milk yield offers a new perspective for both field applications and advanced research. In line with this information, the aim of this study was to investigate the effects of different energy and protein contents of Total Mixed Rations (TMR) formulated to meet the requirements of dairy cows on milk yield, oxidative stress coping capacity and blood antioxidant levels in early lactation.

ETHICAL STATEMENT

This research was submitted to and approved by the Ankara University Animal Experiments Local Ethics Committee (AUHADYK) under document number 2010-5-48.

MATERIALS AND METHOD

In the study, 20 Holstein breed dairy cows in the first period of lactation (all animals are in their 2nd-3rd lactation on average) were used as animal material. All animals have the same care and feeding standards except for the ration change.

Animals were divided into groups according to their milk yield approximately 10 days after birth. Holstein dairy cows used in the study were grouped to have an average daily milk yield of 32.07kg. The study was organized in two groups, each consisting of 10 dairy cows. Group 1 (R1) was fed with a ration with low protein and energy content, while group 2 (R2) was fed with a ration with high protein and energy content. Dairy cows were housed at Özce Mining Kesikköprü Farm in Bala district of Ankara during the trial period. The study lasted a total of 45 days. In the study, alfalfa hay, corn hanging silage were given as roughage material, while commercial mixed feed, barley and corn paste were given as concentrated feed. All feeds were mixed in the TMR vehicle and given to the animals. Feeding TMR (total mixed ration) fresh rations were prepared in two meals at 08:00 and 18:00. Milking was simultaneous with the prepared TMR protocol, and the animals were milked under sterile conditions with a separate milking room and an automatic milking machine. The enterprise is a certified dairy enterprise (ISO 22000, ISO 9001 etc.). Feed amounts, dry matter, energy and other nutrient needs of the animals were calculated according to NRC (Nutrient Requirements of Dairy Cattle, 2001) standards, and the animals were fed ad libitum. Daily access to fresh and clean water was provided. This study evaluated the contents of Total Mixed Rations (TMRs) formulated with different energy and protein levels to meet the physiological needs of dairy cows. Relevant feed formulations are presented in Table 1, and the chemical analysis results of these rations are presented in Table 2.

Table 1. The composition of the TMR (Total Mix Ration) (kg)

Feed for animals	R1	R2
Alfalfa Hay	5	6,55
Straw	-	0,2
Corn Grain Silage	16	15
Concentrate Feed	8	10,5
Barley	1,5	2
Corn Crushed	2	2,7

Group 1 (R1) (Holstein breed dairy cows) was fed with a ration with low protein and energy content, Group 2 (R2) (Holstein breed dairy cows) was fed with a ration with high protein and energy content.

Table 2. Analysis of TMR (Total Mix Ration) composition

	R1	R2
CP, %	15.7	16.4
Starch, %	27.4	27.8
ME, Kcal/kg	2115	2608

CP: Crude Protein,

Group 1 (R1) (Holstein breed dairy cows) was fed with a ration with low protein and energy content, Group 2 (R2) (Holstein breed dairy cows) was fed with a ration with high protein and energy content.

Daily milk yield was recorded for each animal. Daily feed consumption was determined by subtracting the feed left over from the feed given the previous day. Since the animals were fed in groups, feed consumption was determined on a group basis rather than individually. Therefore, feed utilization rate was not calculated. Nutritional analyses of the feeds used in the study were carried out according to the method reported by AOAC (Association of Official Methods of Analysis, 2000).

At the end of the experiment, blood samples taken from the animals' tail vein, the *vena caudalis*, were placed in anticoagulant (EDTA) tubes. After separating some whole blood from the blood samples, the remaining blood was centrifuged at 3000 rpm for 15 minutes to obtain plasma. Samples were stored at -20°C until analysis. Antioxidant enzymes superoxide

Table 3. Average feed consumption (kg) of Holstein dairy cows in group feeding

Days	Group	Feed consumption
10 th day	R1	22.86
	R2	35.064
20 th day	R1	24.14
	R2	35.28
30 th day	R1	24.622
	R2	35.51
45 th day	R1	25.291
	R2	35.65

Group 1 (R1) (Holstein breed dairy cows) was fed with a ration with low protein and energy content. Group 2 (R2) (Holstein breed dairy cows) was fed with a ration with high protein and energy content.

dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) activities were determined using commercial kits (Cayman Chemical Company, USA) and an ELISA device (Epoch, Biotek, USA). Whole blood glutathione (GSH) analysis was determined colorimetrically (Epoch, Biotek, USA) (Beutler *et al.*, 1963). Malondialdehyde (MDA) value and ceruloplasmin values in plasma were determined by specific methods, respectively (Yoshioka *et al.*, 1979; Colombo and Richterich, 1964). A commercial test kit (Biolabo, Maizy, France) was used to determine albumin and total protein values. The globulin value was calculated by subtracting albumin from total protein (Doumas, 1971).

Statistically, the homogeneity of variances was examined with the Levene test. The significance of the difference between the control and experimental groups for each variable was examined with the Independent Sample T test. SPSS package program was used for statistical analyses.

RESULTS

The animals in the study were group-fed rather than individually fed. Therefore, individual dry matter intakes cannot be calculated. Instead, the animals' average dry matter intake was calculated and is presented in Table 3.

In our study, individual daily milk yields of the animals were recorded. Milk yields of the animals on days 0, 10, 20, 30, and 45 are shown in Table 4. Except for initial milk yield, yields on these days were found to be higher in the R2 group, with significance levels of P: 0.000, P: 0.001, P: 0.001, and P: 0.001, respectively. TMR feeding with high energy-protein content in the R2 group had a positive effect on milk yield.

In our study, the blood antioxidant status of the animals at the end of the trial is shown in Table 5. SOD and albumin values were significantly affected and were found to be higher in the R2 group (P: 0.040, P: 0.033, respectively). Other parameters were not affected by feeding TMR with different energy and protein levels.

Table 4. Daily milk yield of Holstein dairy cows (L)

Daily milk yield	Group	$\bar{x}$	S $\bar{x}$	P
0 th day	R1	31.95	3.38	0.672
	R2	32.20	3.24	
10 th day	R1	25.75 ^b	2.04	0.000*
	R2	40.32 ^a	1.69	
20 th day	R1	28.70 ^b	2.55	0.001*
	R2	40.66 ^a	1.64	
30 th day	R1	29.14 ^b	2.16	0.001*
	R2	40.23 ^a	1.62	
45 th day	R1	29.67 ^b	2.05	0.001*
	R2	40.20 ^a	1.68	

Mean ($\bar{x}$) and standard error (S $\bar{x}$) values of each group (n:10). Statistically significant (P<0.05)*

Group 1 (R1) (Holstein breed dairy cows) was fed with a ration with low protein and energy content,

Group 2 (R2) (Holstein breed dairy cows) was fed with a ration with high protein and energy content.

Table 5. Influence of fed with TMR with different energy and protein levels on antioxidant statu

Blood Parameters	Groups				Significance
	R1		R2		
	$\bar{x}$	S $\bar{x}$	$\bar{x}$	S $\bar{x}$	P
MDA ($\mu\text{mol/L}$)	3.01	0.20	2.77	0.15	0.159
GSH (mg/dL)	67.37	2.08	69.16	1.83	0.385
SOD (U/mL)	186.65	3.87	203.51	8.72	0.040*
CAT (nmol/min/mL)	25.01	1.22	28.41	1.5	0.403
GPx (nmol/min/mL)	0.32	0.02	0.31	0.01	0.523
Ceruloplasmin (mg/dL)	16.51	0.84	16.73	0.64	0.412
Albumin (g/dL)	3.18	0.07	3.19	0.03	0.033*
Total protein (g/dL)	6.76	0.20	6.79	0.15	0.675
Globulin (g/dL)	3.57	0.20	3.59	0.15	0.798

Mean ($\bar{x}$) and standard error (S $\bar{x}$) values of each group (n:10). Statistically significant (P<0.05).

Group 1 (R1) (Holstein breed dairy cows) was fed with a ration with low protein and energy content,

Group 2 (R2) (Holstein breed dairy cows) was fed with a ration with high protein and energy content.

DISCUSSION

Feeding with TMR, which has a higher energy and protein content, had a positive impact on the animals' milk yield. In dairy cattle, the early lactation period generally covers the first 70 days after calving and is a critical phase in terms of both production and physiological stress. During this period, milk production increases rapidly with the advancement of milking days, while dry matter intake does not increase at the same rate. This imbalance creates an energy deficit in the animals, known as negative energy balance (NEB). Similarly, inadequate dry matter intake during this period creates a protein deficit,

leading to negative protein balance (NPB). The primary priority in feeding dairy cattle is to provide nutrients sufficient to meet the animal's vital functions and productivity potential. These requirements are examined under two main headings: Maintenance allowance: nutrients necessary to meet the animal's basic physiological needs. Yield allowance: nutrients necessary for performance-based activities such as milk production. Accurately calculating these two requirements is crucial for both increasing productivity and protecting animal health. Inaccurate calculations can lead to either under- or over-supply of nutrients, which can negatively impact the animal's health. Nutrients are

generally classified into four main groups: Energy sources, Proteins, Vitamins, and Minerals. The balanced inclusion of these components in the ration, in line with the animal's determined dry matter intake, is essential for creating an effective and sustainable feeding program (Winnicki *et al.*, 2010). According to NRC (Nutrient Requirements of Dairy Cattle, 2001) data, the daily metabolizable protein (MP) requirement for Holstein dairy cattle and heifers during advanced gestation is approximately 900 grams. However, this value does not include specific physiological processes such as the development of mammary tissue. Studies by Bell *et al.* (2000) indicated an additional 120 grams/day of MP is required for mammary gland development. Furthermore, NRC (Nutrient Requirements of Dairy Cattle, 2001) reported that approximately 44,8 grams of MP is required to produce 1 kg of milk with a 3% real protein content. This value is directly related to milk yield and is an important parameter to consider in ration formulations. In dairy cattle, the late stages of pregnancy and lactation are critical stages during which energy requirements increase significantly. Especially during the last month of pregnancy, the animal's daily energy needs exceed its basic vital needs by approximately 20%. This increase is associated with the rapid growth of the developing fetus and the metabolic burden of preparation for birth. During lactation, energy needs increase even further with milk production. For example, a cow producing 30 kg of milk per day requires approximately 26 megacalories (Mcal) of additional net energy to maintain this production. This amount is important for both milk synthesis and maintaining body condition (Coşkun *et al.*, 1997). Dairy cows in the postpartum period require high levels of energy to maintain vital functions and support milk production. During this period, the animal's total daily energy requirement to meet physiological needs such as maintenance, growth, and milk yield is approximately 25.1 megacalories of net lactation energy (NEL). This energy requirement is critical for reducing the risk of negative energy balance (NEB) and preventing metabolic diseases, especially in the early stages of lactation. This value should be considered in ration formulations, and feeding strategies should be developed to support animal performance and health (Bauman and Currie, 1980).

In our study, milk yields during the study, except for the initial milk yield, were found to be statistically significant in the R2 group. Dietary energy levels are shown to be a determining factor in the severity of negative energy balance and the resulting metabolic disorders, particularly in early lactation (Gong, 2002). In addition to these findings, a study by Broderick (2003) demonstrated that increasing the energy density of the diet by reducing the proportion of roughage had positive effects on milk yield. Similarly, Ballard *et al.* (2001) reported that adding small amounts of energy sources such as sugar beet pulp, molasses, propylene glycol, and calcium propionate to the diet during the postpartum period supported milk yield by increasing the energy content of the diet. In a study designed with ration energy levels of 1.51 NEL (Mcal/kg, low energy), 1.56 NEL (Mcal/kg, medium energy) and 1.61 NEL (Mcal/kg, high energy), milk yield of dairy cows in the early lactation period was positively affected in favor of the group with the highest energy (Kovanlıkaya, and Türkmen, 2023). In a study, energy-corrected milk (ECM) output relative to dry matter intake (DMI). Cows fed sunflower meal yielded the lowest ECM per kilogram of DMI (1.54 kg), indicating inferior energy efficiency. In contrast, soybean meal supported the highest ECM production (1.69 kg/kg DMI), followed closely by RSM (1.67 kg) and DDGS (1.63kg). This highlights that while all diets were equalized for net energy concentration, the metabolic utilization of that energy varied notably with protein source. Sunflower meal exhibited reduced conversion efficiency, suggesting its limited suitability for energy-optimized milk production compared to SBM, RSM, and DDGS (Yıldız *et al.*, 2015). The same study also assessed the effects of four different protein sources on milk yield and composition in lactating Holstein cows were evaluated: sunflower meal (SFM), soybean meal (SBM), rapeseed meal of canola type (RSM), and dry distillers' grains with soluble derived from maize (DDGS). Cows receiving sunflower meal showed the lowest milk production, averaging 30.1kg/day. In contrast, soybean meal resulted in the highest milk yield (33.2kg/day), along with superior energy efficiency and economic return. Diets containing rapeseed meal (RSM) and DDGS produced intermediate outcomes, not reaching the effectiveness of SBM but outperforming SFM. These findings suggest that

sunflower meal may be a less suitable protein source for maximizing milk production in dairy rations (Yıldız *et al.*, 2015). Feeding TMR, which has a high energy and protein content, increased milk yield by reducing the negative energy and protein balance in early lactation. Dietary energy density and protein source were determinants of milk production and energy use efficiency. These results demonstrate that careful selection of energy levels and protein sources in ration formulation is critical for production and animal health.

As dairy cattle go through physiological stages such as reproduction, pregnancy, fetal development, parturition and lactation, they are significantly affected by hormonal changes that occur in the first year after adulthood (Blanc *et al.*, 2006). Many common diseases in dairy cows, such as mastitis, metritis, fetal membrane retention, and udder edema during the peripartum and early lactation, cause oxidative stress. Furthermore, the increased milk production following parturition, resulting from genetic selection for high milk yields in cows, also creates oxidative stress (Kılıçel and Tepeli, 2023). During normal physiological and metabolic processes, the production of free radicals such as reactive oxygen species (ROS) occurs continuously in tissues. Water is formed by the reduction of oxygen used for ATP synthesis in mitochondria; however, when oxygen is not fully reduced, intermediate oxygen compounds are formed. The most common free radical in cells is superoxide radical, generated by the electron transport chain in mitochondria (Indo *et al.*, 2007). Free radicals formed during oxidative metabolism led to the formation of fatty acid hydroperoxides. These peroxides react with fatty acids, triggering a chain reaction that produces new free radicals. Under stressful conditions, macrophages also play a role in free radical production. ROS are also synthesized by immune cells (Victor *et al.*, 2004). In addition to free radicals and ROS, other oxidative molecules such as reactive nitrogen species (RNS) and reactive chlorine species are also formed during metabolic activities (Phaniendra *et al.*, 2015). In our study, when the blood antioxidant status of the animals was examined at the end of the trial, SOD and albumin values were significantly affected by feeding with TMR containing different levels of energy and protein and were found to be higher in the R2 group. The increase

in SOD activity in our study indicates that the organism activates its antioxidant systems to combat oxidative stress. Albumin, in particular, can neutralize reactive oxygen species (ROS) through its sulfhydryl (-SH) group. This group neutralizes free radicals by donating electrons during oxidative stress (Roche *et al.*, 2008). In a study showed that supplementing low- and high-energy diets prepared for transition and early-lactation dairy cows could improve antioxidant status and reduce plasma β -hydroxybutyrate, ultimately improving lactation performance. Furthermore, the response to antioxidant supplementation was more pronounced in the high-energy diet (Wang *et al.*, 2010). In another study the combination of pasture-based feeding with a total mixed ration (TMR) offered ad libitum indoors can be considered an effective strategy for improving milk composition. Cows grazed for eight hours per day and subsequently supplemented with TMR show significant increases in milk fatty acid profile (especially vaccenic acid, conjugated linoleic acid, and omega-3 fatty acids), lipophilic antioxidant levels (α -tocopherol, retinol, β -carotene), and total antioxidant capacity (TAC). This feeding regimen also allows for the maintenance of TMR intake, thus optimizing milk quality through rational feed management (Şanta *et al.*, 2022). In a separate study in Holstein cows fed ad libitum TMR, plasma antioxidant capacity was unaffected, but decreases in MDA were observed (Prommachart *et al.*, 2021). On the other hand, In a study in which early lactation Holstein cows were fed with four different feeding regimens consisting of different ratios of total mixed ration (TMR) and 100% TMR (T0), 75:25 (T1), 50:50 (T2) and 25:75 (T3) pasture, milk antioxidant quality was negatively affected as TMR increased (Salado *et al.*, 2018). Feeding strategies play a fundamental role in managing oxidative stress and shaping milk quality in dairy cows. Factors such as ration energy density, grazing duration, and feed composition can significantly influence both metabolic responses and milk components. Balanced and rich feeding protocols, particularly during the transition and early lactation periods, support antioxidant defenses, reduce lipid peroxidation, and can positively impact milk productivity. However, differences in milk composition are observed between the use of TMR alone and the combination of grazing-based systems. This suggests that feed management should be

optimized not only in terms of quantity but also in terms of source and timing. Therefore, rational and seasonally appropriate feeding plans are critical for both protecting animal health and enhancing the functional value of dairy products.

CONCLUSION

This study evaluated the effects of feeding TMR with different energy and protein levels on milk yield and antioxidant parameters. Although individual dry matter intake could not be calculated due to group-based feeding, evaluations based on average consumption values indicated that milk yield in the R2 group, which has a higher energy and protein content, increased significantly throughout the experiment. Furthermore, statistically significant increases were detected in superoxide dismutase (SOD) and albumin levels in the R2 group, indicating that the ration provided a positive biochemical response in combating oxidative stress. No significant changes were observed in other antioxidant parameters. Overall, feeding TMR with high energy and protein content improved lactation performance and supported certain antioxidant indicators.

ACKNOWLEDGEMENTS

We would like to thank Veterinarian İbrahim Teker for her support throughout our study.

FINANCIAL SUPPORT

This study has not been financially supported by any person or institution.

CONTRIBUTORS

O Durna, G Yıldız, D Kocamaz and K Onk executed the experiment; O Durna completed the manuscript; G Yıldız, O Durna, D Kocamaz and O Merhan were in charge of laboratory analyses. O Durna was responsible for supervision and writing of the manuscript.

DATA AVAILABILITY STATEMENT

Data-in-article – the research data are available within the article itself

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