

Flaxseed oil supplementation in Holstein dairy cows fed total mixed rations

Uso de aceite de linaza con ración total mezclada en vacas lecheras Holstein

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ABSTRACT

The effectiveness of flaxseed oil in managing increased oxidative stress during early lactation through feeding strategies prompted this study. The aim of this study was to investigate the effects of oral flaxseed oil supplementation on milk yield and blood antioxidant status in early-lactation Holstein dairy cows fed a Total Mixed Ration. Thirty multiparous Holstein cows (2nd–3rd lactation) were divided into two diets, 15 cows in each group. The control group was fed the Total Mixed Ration diet prepared as the control group, while the experimental group received an oral dose of 5 ml of flaxseed oil per day. The experiment lasted 28 days. Results. Milk yields of the animals on days 0, 14, 28, and 0–28 were not statistically affected by flaxseed oil supplementation. At the end of the experiment, Glutathione, superoxide dismutase, catalase, and glutathione peroxidase values were significantly affected and were higher in experimental group. Other parameters were not statistically affected by the addition of flaxseed oil to the Total Mixed Ration. Oral administration of flaxseed oil in addition to Total Mixed Ration to dairy cows during early lactation provided protection against oxidative stress.

Key words: Antioxidant status; flaxseed oil; milk yield; total mixed ration

RESUMEN

El uso del aceite de semilla de lino en el control del estrés oxidativo en la lactancia temprana a través de estrategias nutricionales, ha venido proponiéndose para evitar el efecto de balance negativo energético en vacas de alto nivel de producción. De allí que el objetivo de este estudio fue investigar los efectos de la suplementación oral con aceite de linaza sobre la producción de leche y el estado antioxidante sanguíneo en vacas lecheras Holstein al inicio de la lactancia, alimentadas con una ración completa mezclada. Treinta vacas Holstein multíparas (2.^a–3.^a lactancia) se dividieron en dos grupos de 15 vacas cada uno. El grupo control recibió una dieta de ración completa mezclada, mientras que el grupo experimental recibió una dosis oral de 5 ml de aceite de linaza al día. El experimento duró 28 días. La producción de leche de los animales en los días 0, 14, 28 y entre los días 0 y 28 no se vio afectada estadísticamente por la suplementación con aceite de linaza. Al final del experimento, los valores de glutatión, superóxido dismutasa, catalasa y glutatión peroxidasa se vieron significativamente afectados y fueron mayores en el grupo experimental. Los demás parámetros no se vieron afectados estadísticamente por la adición de aceite de linaza a la dieta de ración completa mezclada. La administración oral de aceite de linaza a vacas lecheras, además de la ración total mezclada, durante el inicio de la lactancia, proporcionó protección contra el estrés oxidativo.

Palabras clave: Estado antioxidante, aceite de linaza, producción de leche, ración completa mezclada

INTRODUCTION

Dairy cows (*Bos taurus*) are under the pressure of oxidative stress, particularly during the transition and early lactation periods, due to increased metabolic load, hormonal changes, and environmental factors [1]. This leads to adverse effects at the biomolecular level, such as lipid peroxidation, protein oxidation, and DNA damage, due to the inadequacy of antioxidant defense systems despite increased reactive oxygen species (ROS) production [2].

Plant extracts, thanks to their polyphenols, flavonoids, and other natural compounds, have significant potential in mitigating the negative effects of oxidative stress. For example, a study using a polyphenol extract containing green tea, capsicum, and fenugreek showed that supplementation increased feed intake and milk yield in dairy cows and also improved the Nrf2-mediated (Nuclear factor erythroid 2-related factor 2) oxidative stress response [3].

Similarly, the use of rumen bypass chicory extract in cows fed a high-concentrate diet has been reported to increase milk yield, reduce oxidative stress in mammary tissue, and increase superoxide dismutase (SOD) and catalase (CAT) activities [4].

Therefore, the combined use of plant extracts and oil sources rich in polyunsaturated fatty acids (PUFAs), such as flaxseed oil, in conjunction with a Total Mixed Ration (TMR) may be effective in reducing the negative effects of oxidative stress by supporting the blood antioxidant defense system (e.g., SOD, CAT, glutathione[GSH]) in dairy cows, as well as affecting milk yield and feed intake [4].

Flaxseed (*Linum usitatissimum* L.) is the only economically valuable species in the Linaceae family, which contains a total of 13 genera and approximately 300 species [5]. The term “usitatissimum” means “most useful” [5]. The plant is cultivated for fiber production as fiber flax or for oil production as oil flax [6]. Flaxseed contains 35–45% oil [7]. Flaxseed oil is rich in omega-3 (ω -3, alpha-linolenic acid), an important component of human nutrition, with omega-3 making up approximately 50–55% of total fatty acids [8].

In light of this information, the use of herbal extracts to control oxidative stress due to increased metabolic load in early lactation is considered a promising approach for both protecting animal health and optimizing milk yield. The aim of this study was to investigate the effects of oral flaxseed oil supplementation in TMR diets on milk yield and blood antioxidant status in early-lactation Holstein dairy cows.

MATERIALS AND METHODS

Animals, experimental design and feed

In the study, 30 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 34.89 kg.

The study was conducted in two groups, each consisting of 15 dairy cows. While control group (C) was fed with the TMR diet prepared as the control group, trial group (F) received flaxseed oil at an oral dose of 5 ml·day⁻¹TMR. When administering flaxseed oil orally, sterile disposable syringes were used daily. Dairy cows were housed at Özce Mining Kesikköprü Farm in Bala district of Ankara during the trial period.

The study lasted a total of 28 days (d). In the study, alfalfa (*Medicago sativa*) hay, corn (*Zea mays*) hanging silage were given as roughage material, while commercial mixed feed, barley (*Hordeum vulgare*) and corn paste were given as concentrated feed. All feeds were mixed in the TMR vehicle and given to the animals.

Feeding 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 [9] standards and the animals were fed *ad libitum*. Daily access to fresh and clean water was provided. Because animals were group-fed, individual feed intake could not be determined. All animals in the study were fed 40 kg·d⁻¹ of TMR per animal throughout the experiment. Therefore, feed conversion ratios were not calculated.

The formulations of the relevant TMR are presented in TABLE I, and some nutrient analysis results of this ration are presented in TABLE II. Plant oil has been obtained from Botalife commercial company based in Isparta, Türkiye. The fatty acid composition of the flaxseed oil applied have been given in TABLE III.

TABLE I
The composition of the total mixed ration (kilograms)

Feed for animals	Amount
Alfalfa Hay	6.5
Straw	0.2
Corn Grain Silage	17
Concentrate Feed	13
Barley	1.0
Soybean	0.7
Corn Crushed	2.5
Water	2.0
Yeast	0.10

TABLE II
Analysis values of total mixed ration composition

Analysis Values	
CP*, %	16.4
Starch, %	27.8
ME*, Kcal·kg ⁻¹	2608.0

*CP:Crude Protein, ME; Metabolizable energy

TABLE III
Flaxseed oil fatty acid composition information used in this study

Flaxseed oil*	%
Palmitic acid	5.30
Palmitoleic acid	0.30
Oleic acid	20.40
Linoleic acid	16.2
Linolenic acid	54.2
Stearic acid	3.10
*Botalife	

Milk yield

Daily milk yield was recorded for each animal.

Nutritional analysis

Raw nutrient analyses of the feeds used in the study were performed according to Association of Official Analytical Chemists [10].

Blood antioxidant status

Blood samples taken from the caudal vein (tail vein) were placed in anticoagulant Ethylenediaminetetraacetic acid tubes. One portion was separated for whole blood. The other portion was centrifuged (Heraeus Christ, Germany) for blood plasma at 1200 G for 15 min. All samples were stored (So-Low Environmental Equipment Company, USA) at -20°C until analyzed. SOD, glutathione peroxidase (GPx), and CAT activities were measured using commercial kits (Cayman Chemical Company, USA) and an ELISA device (Epoch, Biotek, USA). GSH levels were determined colorimetrically (Epoch, Biotek, USA) [11]. Malondialdehyde (MDA) and ceruloplasmin values were determined according to specific methods, respectively [12, 13].

Albumin and total protein values were determined using a commercial test kit (Biolabo, Maizy, France). The globulin value was calculated mathematically by subtracting albumin from total protein [14].

Statistical analysis

Levene's test was used for statistical homogeneity of variances. The significance of the differences between groups for each variable was examined using the Independent Samples T-test. The SPSS software package was used for statistical analyses [15].

RESULTS AND DISCUSSION

In this study, individual daily milk yields of the animals were recorded. Milk yields of the animals on d 0, 14, 28, and 0–28 are shown in TABLE IV. Milk yield for all d was not statistically affected by the addition of flaxseed oil.

The milk yield-enhancing effects of herbal extracts occur primarily through improved rumen fermentation, increased feed utilization efficiency, and support for energy metabolism. Extracts

TABLE IV
Daily milk yield of Holstein dairy cows (Liters)

Days	Groups				Significance
	C		F		
	\bar{x}	S \bar{x}	\bar{x}	S \bar{x}	
0 th day	33.85	0.69	35.93	1.33	0.178
14 th day	35.04	0.67	35.77	1.02	0.557
28 th day	34.97	0.70	36.23	1.16	0.362
0–28 th day	976.43	17.38	1001.89	27.38	0.439

¹Mean (\bar{x}) and standard error (S \bar{x}) values of each group. Statistically significant ($P < 0.05$). ²C: Control group (C) basal ration; only Total Mixed Ration (TMR) and trial group (F); 5 ml·d⁻¹ flaxseed oil was given orally in addition to TMR

containing essential oils and phenolic compounds, in particular, promote volatile fatty acid production by regulating the balance of the rumen microbiota. This contributes to increased propionate concentration and, consequently, increased glucose synthesis.

Because glucose is the primary building block of milk lactose, this process may directly affect milk volume [16, 17, 18]. In this study, milk yield for d 0, 14, 28, and 0–28 were not statistically affected by the addition of flaxseed oil. Dietary fat supplementation in high-yielding dairy cows can have varying effects on milk yield and milk fat composition. The use of linoleic acid-rich oil sources tended to reduce milk fat yield and concentration, while palm oil supplementation was effective in increasing milk yield [19].

In one study, the inclusion of extruded flaxseed in the dairy cow diet improved milk yield, milk composition, and milk Omega-6: Omega-3 ratio [20]. In a study by Benchaar *et al.* [21], the use of flaxseed and flaxseed oil separately improved milk yield in dairy cows.

Studies investigating the synergistic effects of flaxseed oil, either alone or with different plant extracts, found that flaxseed increased milk yield in dairy cows [22, 23]. High levels of fat in the diets of lactating cows may negatively affect feed intake and fiber digestibility, which may lead to a feeling of rumen fullness [24]. Differences in findings may be due to variables such as the form of fat used, the type of supplemented compounds, the composition of the basal ration, and the stage of lactation.

Furthermore, high fat content in the diet of lactating cows can negatively impact feed intake and fiber digestibility, leading to a feeling of rumen fullness, which may have a limiting effect on milk yield.

In this study, the blood antioxidant status of the animals at the end of the trial is shown in TABLE V. GSH, SOD, CAT and GPx values were significantly affected and were higher in the F group ($P: 0.007$; $P: 0.000$, $P: 0.007$, $P: 0.000$, respectively). Other parameters were not statistically affected by the addition of flaxseed oil to the TMR.

Recently, plant extracts have emerged as an effective nutritional strategy for reducing oxidative stress. Phenolic compounds, flavonoids, tannins, and essential oils derived from plants provide physiological benefits by scavenging free radicals and promoting antioxidant enzyme activity. Natural extracts such as grape seed extract, green tea polyphenols, rosemary and thyme have been

TABLE V
Effect of feeding with flaxseed oil and Total Mixed Ration on antioxidant status

Blood Parameters	Groups				Significance
	C		F		
	\bar{x}	S \bar{x}	\bar{x}	S \bar{x}	P
MDA ($\mu\text{mol}\cdot\text{L}^{-1}$)	2.13	0.03	1.82	0.09	0.007*
GSH ($\text{mg}\cdot\text{dL}^{-1}$)	60.26	2.30	87.31	5.55	0.000*
SOD ($\text{U}\cdot\text{mL}^{-1}$)	194.61	5.35	224.31	8.46	0.007*
CAT ($\text{nmol}\cdot\text{min}^{-1}\cdot\text{mL}^{-1}$)	26.79	1.55	31.28	2.32	0.120
GPx ($\text{nmol}\cdot\text{min}^{-1}\cdot\text{mL}^{-1}$)	0.36	0.01	0.54	0.03	0.000*
Ceruloplasmin ($\text{mg}\cdot\text{dL}^{-1}$)	14.42	0.87	14.34	0.63	0.944
Albumin ($\text{g}\cdot\text{dL}^{-1}$)	3.34	0.02	3.29	0.03	0.331
Total protein ($\text{g}\cdot\text{dL}^{-1}$)	6.86	0.08	6.80	0.13	0.743
Globulin ($\text{g}\cdot\text{dL}^{-1}$)	3.52	0.08	3.51	0.14	0.974

¹Mean (\bar{x}) and standard error (S \bar{x}) values of each group. Statistically significant ($P < 0.05$)*

²C: Control group (C) basal ration; only Total Mixed Ration (TMR) and trial group (F); 5 ml.day⁻¹ flaxseed oil was given orally in addition to TMR MDA: Malondialdehyde, GSH: Glutathione, SOD: Superoxide dismutase, GPx: Glutathione peroxidase, CAT: catalase

shown to improve biomarkers related to oxidative stress and positively affect metabolic adaptation processes in dairy cows [25].

Flaxseed and its products are the richest sources of PUFAs. Flaxseed, a good source of omega-3 fatty acids, contains high levels of alpha-linolenic acid (ALA), which constitutes 18% of the total seed (DM basis) and approximately 52–58% of the total fatty acids of the seed or oil [26]. Flax products are rich in plant lignans, which have powerful antioxidant properties [27].

Antioxidants react very quickly with radicals, preventing the progression of auto-oxidation/peroxidation [28]. Endogenous antioxidants are non-enzymatic antioxidants consisting of glutathione, albumin, and ceruloplasmin, while SOD, CAT, GPx, and glutathione reductase are enzymatic antioxidants that form the enzymatic defense line [29, 30]. The SOD enzyme is the first line of the antioxidant defense system. It plays a critical role in the elimination of superoxide radicals [31].

Glutathione peroxidase is found in the cytoplasm of cells and protects cells against oxidative damage caused by hydrogen peroxide (H_2O_2) [32]. Lipid peroxidation has become the biggest indicator of oxidative stress. MDA measurement is the best-known and simplest test of lipid peroxidation under oxidative stress and is very useful in clinical applications. In the case of oxidative stress, MDA, one of the main byproduct aldehydes of lipid peroxidation, accumulates in tissues and peripheral circulation [32].

The SOD enzyme is known to play a critical role in scavenging superoxide radicals, as well as being the first step of the antioxidant defense system [31]. CAT, which plays an important role in cellular redox balance, contributes to maintaining cellular redox balance and reducing the toxic effect of H_2O_2 by catalyzing the breakdown of H_2O_2 into water and oxygen [33]. Dietary antioxidants can be used to correct redox imbalances. Important dietary antioxidants include vitamins, trace elements, some fatty acids, and phytonutrients such as beta-carotene, polyphenols, flavonoids, among others. [34].

In this study, when the blood antioxidant status of the animals was examined at the end of the trial, it was seen that GSH, SOD, GPx and CAT values were significantly affected and were higher in group F.

In a study using flaxseed in dairy cows, there was a significant increase in the level of PUFA (conjugated linoleic acid and alpha-linolenic acid) in serum, a significant decrease in GSH-Px and CAT activity, and an increase in lipid peroxide (MDA) concentration in the fat groups compared to the control group [35]. In another study using flaxseed in dairy cows, flaxseed supplementation increased GPx activity and decreased MDA concentration in milk [36].

In a study in which green tea polyphenols were supplemented as 0.2 g.kg⁻¹ dry matter to cows in the transition period, plasma ROS, MDA and H_2O_2 concentrations were lower, while SOD, GPx and total antioxidant capacity activities were higher [37].

In a study in which oregano oil was added to Tuj breed lamb (*Ovis aries*) rations, SOD and GPx values were significantly affected while MDA, GSH and CAT values were not affected [38]. MDA, GSH, SOD and GPx values were significantly affected by the addition of chia oil to quail diets [39].

In the study where plant extract oil, peppermint oil, juniper oil, rosemary oil and thyme oil were added to the drinking water of quail breeders, CAT values were not affected, but MDA, GSH, SOD and GPx values were significantly affected [40]. In the study where an essential oil mixture (peppermint oil (*Mentha x piperita*), juniper (*Juniperus communis*) oil, rosemary (*Salvia rosmarinus*) oil, thyme (*Thymus vulgaris*) oil) was given to Turkish native geese (*Anser anser*), it was observed that GSH values were statistically affected [41].

In the study using safflower (*Carthamus tinctorius*) oil in quails (*Aquila chrysaetos*), blood MDA, GSH, SOD, GPx and CAT values were statistically affected by the addition of safflower oil [42].

When evaluating the effects of rations supplemented with plant extracts and flux products on the antioxidant defense system, the high SOD, GPx, CAT, and GSH levels observed in group F in this study indicate that enzymatic and non-enzymatic antioxidant lines were activated holistically.

The increase in SOD activity reveals that superoxide radicals were removed more effectively in the first step, while the subsequent increases in GPx and CAT indicate that the resulting H_2O_2 and lipid hydroperoxides were rapidly detoxified. The increase in GSH levels suggests that intracellular redox capacity was maintained despite the increased activity of GPx, and that polyphenolic compounds likely supported the GSH cycle.

This coordinated enzymatic response resulted in the suppression of MDA, the end product of lipid peroxidation, and demonstrated a significant reduction in oxidative stress load. Although some studies in the literature have reported that flaxseed increases oxidative stress depending on its PUFA content, while others have indicated that lignan-derived antioxidant effects are dominant, the results obtained in this study point to a metabolic adaptation pattern where phenolic compounds balance the PUFA-induced oxidative load and the SOD-GPx-CAT-GSH axis is synergistically strengthened.

CONCLUSION

The use of flaxseed oil in Holstein dairy cows during early lactation did not affect milk yield and had a protective effect against oxidative damage by increasing blood GSH, SOD, CAT and GPx levels. Based on the data obtained from this study, it is believed that flaxseed oil can be safely used as a feed additive and to protect against oxidative stress in dairy cows.

Ethics statement

This study was conducted with approval number AU-HADYEK /2019–5–46 from the Ankara University Animal Experimentation Local Ethics Committee.

Author contributions

OD and GY designed and conducted the experiment; OD undertook the manuscript writing; and GY and OD were responsible for the laboratory analyses. All authors read and approved the final version of the article.

Conflict of interest

We declare that there is no conflict of interest among the authors involved in the study.

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