Ankara Üniv Vet Fak Derg, **67**, 235-241, 2020
DOI: 10.33988/auvfd.590696

The effect of sodium bentonite on growth performance and some blood parameters in post-weaning Tuj breed lambs

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Received date: 11.07.2019 - Accepted date: 03.01.2020

Abstract: The purpose of this study was to investigate the effect of sodium bentonite supplementation on fattening performance and some blood parameters in Tuj lambs. In the study, 18 male lambs were divided into 3 groups. While the control group was fed with basal ration, the experimental groups were fed with sodium bentonite supplementation 1% and 2% dose in addition to the basal ration. On the 0th, 15th, and 30th days of the study, there was no statistically significant difference between groups in terms of mean live weights, live weight gain, daily live weight gain, daily feed consumption and feed conversion ratio (P>0.05). On the 45th day of the study, the difference between the groups in terms of live weight gain, daily live weight gain and feed conversion ratio were statistically found significant (P<0.05). The difference between glutathione peroxidase (GPx), glutathione (GSH), ceruloplasmin, albumin, total protein and globulin values of Tuj breed lambs were not statistically significant (P>0.05).The use of sodium bentonite in Tuj breed lambs was statistically found significant in terms of malondialdehyde (MDA), superoxide dismutase (SOD) and catalase enzyme activities (CAT) (P <0.05). In conclusion, the use of sodium bentonite in Tuj lambs positively affected fattening performance. When the blood antioxidant parameters were examined, increase in SOD and CAT values was protective against oxidative damage in lambs used sodium bentonite. However, the increase in MDA value has doubted the safety sodium bentonite use in lambs.
Keywords: Blood parameters, lamb, performance, sodium bentonite, Tuj

Sütten kesilmiş Tuj ırkı kuzularda sodyum bentonit kullanımının besi performansı ve bazı kan parametreleri üzerine etkisi

Özet: Bu çalışmanın amacı sütten kesilmiş Tuj ırkı kuzularda sodyum bentonit kullanımının performans ve bazı kan parametreleri üzerine etkisinin araştırılmasıdır. Çalışmada 18 erkek kuzu 3 gruba ayrılmıştır. Kontrol grubu temel rasyonla beslenirken deneme gruplarına temel rasyona ek olarak %1 sodyum bentonit ve % 2 sodyum bentonit dozlarında ticari ürün olan sodyum bentonit verilmiştir. Çalışmada sütten kesilmiş Tuj ırkı kuzularda sodyum bentonitin farklı dozlarda kullanımının çalışmanın 0., 15. ve 30. günlerinde canlı ağırlık artışı, günlük canlı ağırlık artışı, günlük yem tüketimi ve yemden yararlanma oranını etkilemediği görülmüştür (P>0,05). Çalışmanın 45. gününde gruplar arası fark canlı ağırlık artışı, günlük canlı ağırlık artışı ve yemden yararlanma oranını yönünden istatistiksel olarak anlamlı bulunmuştur (P<0,05). Çalışmada rasyonuna sodyum bentonit ilavesi glutatyon (GSH), glutatyon peroksidaz (GPx), seruloplazmin, albümin, total protein ve globulin değerlerinin üzerinde önemli bir farklılık oluşturmadığı saptanmıştır (P>0,05). Tuj kuzularında sodyum bentonit kullanımı malondialdehit (MDA), süperoksit dismutaz (SOD) ve katalaz (CAT) değerlerine etkisi istatistiksel olarak anlamlı bulunmuştur (P<0,05). Sonuç olarak, Tuj kuzularında sodium bentonit kullanımı besi performansını olumlu etkilemiştir. Kan antioksidan parametreleri incelendiğinde SOD ve CAT değerlerindeki artış sodium bentonitin oksidatif hasara karşı koruyucu etkisinin olduğu saptanmıştır. Fakat MDA değerindeki artış kuzu rasyonlarında sodium bentonit kullanımının güvenirliliğini sorgulatmıştır.
Anahtar sözcükler: Kan parametreleri, kuzu, performans, sodyum bentonit, Tuj.

Introduction

Clays are natural materials with large surface areas and are used in industry due to their absorption and adsorption properties (34). Clay minerals mainly hydrate include aluminum silicate (brucite), kaolinite, montmorillonite, illite, chlorite, sepiolite, bentonite, zeolite and hydrate sodium calcium aluminosilicate (16). Bentonite (dioctahedral montmorillonite) is an approved additive for reducing mycotoxin contamination in feeds for all animal species. (13). Bentonite is a naturally

occurring clay mineral. Therefore, the use of bentonite as an additive in animal nutrition is not expected to adversely affect the environment. The clay is currently authorized as a binder, as an anticaking agent and as an anticoagulant. Bentonite is not genotoxic. Therefore it is approved as a food additive without restriction. Like other clays, bentonite is not measurably absorbed to any measurable extent. Consequently, bentonite is unlikely to expose the consumer to harmful residues of any chemical component in animal product consumption. Food products obtained from animals fed diets containing bentonite additives seem to be safe for those who consume them (14). Clay-based antimicrobials include a known antimicrobial drug and metal nanoparticle. In addition, clay minerals can intercalate many inorganic and organic ions, replacing K^+, Ca^{2+}, etc. in interlayers sites (ion-exchange capacity (2, 43). Bentonite is a clay with a cation exchange capacity of 0.72 meq / g (26).

Bentonite is mainly composed of clay minerals of the smectite (montmorillonite) group and has a wide variety of industrial uses, including the treatment of edible mineral oils, paints, cosmetics and medicines (8). Research has shown that bentonite concentrations of 20 000 mg/kg complete feed have no negative effects on any animal species (14).

Due to their adsorption and pellet binding abilities, clays are used in concentrate feeds to prevent or delay mold and fungus growth. In addition, it has been determined that clays can reduce the toxic effects of heavy metals and prevent their toxicological and pathological effects by binding to mycotoxins in the digestive tract (32). The most important effect of clays in ruminant rations deals with urea metabolism. Clay has the capacity to adsorb ammonia formed in the rumen. With high adsorption power of clay, it can protect the animal against ammonia accumulation at toxic level by keeping excessive ammonia in the rumen (10). Due to its high adsorption properties, bentonite has being used in animal feeds to improve animal health. Clays added to the ration can bind and immobilize toxic substances in the gastrointestinal tracts of animals and thus reduce their toxicity (41). Bentonite provides a buffer power advantage in the rumen. It can help to adjust the pH value in the rumen or intestine and to neutralize the acidity in the digestive system. Due to the digestive-regulating effects of bentonite, it contributes to the health and growth performance of ruminants (20). Sodium bentonite supplementation to feeds containing undegradable protein reduces the concentration of ammonia in the rumen and increases the passage of feed protein and bacterial protein to the small intestine, due to the cation exchange capacity of bentonite (42).

Prohibition of the use of antibiotics and other growth factor chemicals for residual release and resistance to bacteria has led to the search for alternative feed additives (5, 39). In recent years, several studies were conducted that were focused on the clay minerals (33, 36). However, no study was found in the literature that focused on the use of sodium bentonite in the Tuj breed of lambs. In our study, sodium bentonite was preferred because of features such as toxin binding, preventing excessive nitrogen loss in ruminants, increasing performance, leaving residue, being natural and not causing environmental pollution. Therefore, the aim of this study was to investigate the effect of sodium bentonite on fattening performance and some blood parameters such as glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx) catalase (CAT) enzyme activities, ceruloplasmin, albumin, total protein and globulin in Tuj breed lambs.

Material and Methods

Animals, experimental design and feed: In the study, Tuj breed lambs, raised in Kars, Ardahan and Igdir provinces, which is a local sheep breed in Turkey were used as animal material. This study was carried out with the permission of the Kafkas University Animal Experiments Local Ethics Committee (KAU-HAYDEK /2019-26) report. In the study, 18 male lambs aged between 5.5 and 6 months were used. The lambs were randomly divided into 3 groups. The experiment was conducted in Kafkas University Faculty of Veterinary Medicine, Prof. Dr. Ali Rıza AKSOY Training, Research and Implementation Farm. The experiment was continued for a total of 45 days. During the study, the animals were housed in individual boxes (180 cm × 150 cm × 120 cm; height, length, width, respectively) equipped with feeders dispensing pasture grass and concentrate feed separately. During the study, the animals were fed with pasture grass and lamb grower feed. The lamb grower feed used in this study was obtained from a feed factory.

Dry matter, energy and other nutrient requirements of animals were calculated according to NRC (28) standards in the study. The daily amount of concentrate feed provided to the lambs was 750 g per animal. Pasture grass and water was supplied *ad libitum* during the trial. Animals were fed mainly with roughage. The content of the concentrated feed is given in Table 1. Animals were accustomed to experimental diets for 10 days. All lambs were treated against internal and external parasites. In the study, while the control group (C) was fed with basal ration, the experimental groups were fed with sodium bentonite (KARBEN®) at 1% (B1) and 2% (B2) levels respectively in addition to the basal ration. Sodium bentonite used in this study was given to experimental groups together with lamb grower feed. Sodium bentonite used in this study were obtained from the company named KarBen Bentonite Industrial Mining Chemical R & D

Nano Technologies Industry and Trade Inc. in Turkey. The physical and chemical properties of the sodium bentonite used in the study are presented in Table 2.

Table 1. Ingredient composition of concentrate feed

Ingredients	Amount, %
Wheat	3.75
Barley	17.5
Corn	12.5
Soybean meal	2.5
DDGS	2.5
Sunflower seed meal (28-30 CP)	2.5
Sunflower seed meal (36 CP)	2.5
Safflower Meal	6.25
Cotton seed meal (28-30 CP)	4.6
Wheat Bran	20
Corn Bran	6.3
Leaf (Cotton) (25 CP)	6.25
Molasses	9
Marble powder	3.25
Salt	0.5
Vitamin mineral premix	0.1

[1]CP: Crude Protein, [2]DDGS: Dried Distillers Grains with Solubles, [3]The vitamin & mineral premix provided the following (per kg): 4.000.000 IU vitamin A, 800.000 IU vitamin D3, 5.000 IU vitamin E, 400 mg vitamin B2, 2 mg vitamin B12, 5.000 mg vitamin PP, 1.000 mg D-pantothenic acid, 20.000 mg choline, 50 mg Co, 5.400 mg Fe, 185 mg I, 6.900 mg Mn, 800 mg Cu, 6.400 mg Zn, 14 mg Se.

Tablo 2. Physical and chemical properties of sodium bentonite

Physical properties	
Moisture	% 10-12
pH	7.5-8.5
Chemical properties	**%**
SiO_2	56.87
Al_2O_3	17.73
Fe_2O_3	3.84
CaO	2.85
MgO	1.85
Na_2O	2.09
K_2O	0.95

Feed Analysis: Nutrient analysis of the feeds were determined according to the method reported in AOAC (1); while NDF (Neutral Detergent Fiber) and ADF (Acid Detergent Fiber) analyses for pasture grass were determined according to the method reported by Goering and Van Soest (18).

Determination of performance parameters: Animals were weighed before morning feeding in the beginning and on the 0^{th}, 15^{th}, 30^{th} and 45^{th} days of the trial. At the end of the trial, daily feed consumption and feed conversion ratios of each group were calculated. Feed conversion ratio was calculated as the proportion of daily feed consumption (pasture grass and concentrated feed dry matter) to daily weight gain (kg/kg).

Biochemical analyses: The end of the experiment, blood samples were taken from the *v.brachialis* of the animals with anticoagulant (EDTA) tubes, after separating a fair amount of the blood samples as whole blood, plasma of the remaining blood was obtained. Samples taken were centrifuged at 3000 rpm for 15 minutes, and stored at -20 ^{o}C until the analyses were carried out. SOD, GPx and CAT antioxidant enzyme activities in plasma were determined by ELISA device (Epoch, Biotek, USA) using commercial kits (Cayman Chemical Company, USA). Whole blood reduced GSH analysis was determined colorimetrically (Epoch, Biotek, USA) according to the method issued by Beutler et al. (7), while MDA in plasma by Yoshoiko et al. (50), ceruloplasmin by Colombo and Ricterich (9), and albumin and total protein levels by commercial test kit (Biolabo, France). The globulin was determined by subtraction of the albumin from the total protein according to Doumas et al. (11).

Statistical analysis: For the significance of the differences between the statistical calculations belonging to the groups and the mean values of the groups, one-way analysis of variance (ANOVA) method was used, and for the significance control of the difference between the groups, the Tukey test was applied. For this purpose, SPSS packaged software was used (SPSS Inc., Chicago, IL, USA).

Results

The amounts of nutrients and metabolizable energy values of concentrated feed and pasture grass are shown in Table 3. The performance parameters of the study are given in Table 4. There was no statistically significant difference among experiment groups in terms of mean live weights, live weight gain, daily live weight gain, daily feed consumption and feed conversion ratio on the 0^{th}, 15^{th}, and 30^{th} days of the study ($P>0.05$). On the 45^{th} day of the study, the difference among the groups in terms of live weight gain, daily live weight gain and feed conversion ratio were found to be statistically significant ($P<0.05$).

Blood parameters are given at the end of the study in Table 5. The difference between GPx, GSH, ceruloplasmin, albumin, total protein and globulin values of Tuj breed lambs were not statistically significant ($P>0.05$). The use of sodium bentonite significantly influced MDA, SOD and CAT levels in Tuj breed ($P<0.001$).

Table 3. Nutrient and energy levels of the feeds (%).

Feeds	DM	CP	EE	CF	CA	Calcium	Phosphorus	ADF	NDF	ME
Concentrate	87.36	16.25	2.36	11.03	7.99	0.64	0.42	-	-	2531
Pasture grass	92.1	9.13	-	-	7.6	0.65	0.16	38.70	62.60	1767

DM: Dry matter, CP: Crude protein, EE: Ether extract, CF: Crude fiber, CA: Crude ash; ADF: Acid detergent fiber, NDF: Neutral detergent fiber, ME: Metabolic energy (kcal/kg)

Table 4. The effect of sodium bentonite on the live weight, live weight gain, daily live weight gain, daily feed consumptions, feed consumptions and feed conversion ratio of lambs

Traits	Control	B1	B2	P
Live weight, kg, days				
0	39.50±0.56	38.66±0.42	38.83±0.65	0.545
15	43.08±0.80	42.08±0.81	42.66±0.79	0.682
30	45.50±0.97	45.58±0.63	45.83±1.13	0.966
45	48.41±0.58	48.66±0.47	48.41±0.94	0.958
Live weight gain, kg, days				
0-15	3.58±0.27	3.41±0.65	3.83±0.27	0.798
15-30	2.41±0.39	3.50±0.40	3.16±0.58	0.281
30-45	2.91±0.56	3.08±0.41	2.58±0.66	0.815
0-45	8.91^{b}±0.08	10.00^{a}±1.12	9.58^{ab}±0.35	0.012
Daily live weight gain, g, days				
0-15	238.88±18.08	227.77±43.39	255.55±18.59	0.798
15-30	161.11±26.41	233.33±27.21	211.11±39.12	0.281
30-45	194.44±37.92	205.55±27.77	172.22±44.23	0.815
0-45	198.14^{b}±1.85	222.22^{a}±2.86	212.96^{ab}±7.81	0.012
Daily feed consumption (dry matter), g, days				
0-45	1925.12±7.16	1932.18±4.29	1933.87±9.09	0.662
Feed conversion ratio, days				
0-45	9.71^{a}±0.08	8.70^{b}±0.10	9.13^{ab}±0.31	0.008

C: control, B1: 1% sodium bentonite, B2: 2% sodium bentonite. All values are given as mean ± standard error of mean (SEM). (n=6). $^{a.b}$; The differences between the mean values with a different letter in the same row were statistically significant (P<0.05)

Table 5. The effect of sodium bentonite on MDA, GSH, SOD, CAT, GPx, ceruloplasmin, albumin, total protein and globulin

Traits	Control	B1	B2	P
MDA (µmol/L)	2.64^{b} ±0.06	3.13^{a} ±0.12	3.24^{a} ±0.06	0.000
GSH (mg/dL)	37.11±3.31	40.22±4.97	41.02±6.13	0.376
SOD (U/mL)	1.11^{b} ±0.15	1.84^{a} ±0.12	1.91^{a} ±0.05	0.000
CAT (nmol/min/mL)	25.31^{b} ±5.25	34.67^{a} ±6.13	39.46^{a} ±3.58	0.001
GPx (nmol/min/mL)	287.35±17.84	297.20±5.33	300.81±10.03	0.177
Ceruloplasmin (mg/dL)	19.01±2.85	19.13±1.60	19.16±1.57	0.991
Albumin (g/dL)	2.89±0.06	2.93±0.10	2.91±0.06	0.623
Total protein (g/dL)	7.12±0.08	7.10±0.09	7.13±0.04	0.720
Globulin (g/dL)	4.23±0.09	4.16±0.17	4.21±0.09	0.617

C: control, B1: 1% sodium bentonite, B2: 2% sodium bentonite. All values are given as mean ± standard error of mean (SEM). (n=6). $^{a.b}$; The differences between the mean values with a different letter in the same row were statistically significant (P<0.05). MDA: malondialdehyde, GSH: glutathione, SOD: superoxide dismutase, CAT: catalase enzyme activities, GPx: glutathione peroxidase.

Discussion and Conclusion

Some clay species used as feed additives in farm animals, to improve digestibility of nutrients and growth performance, reduce diarrhea formation, to bind mycotoxins, and to minimize adverse effects such as odor and other gaseous emissions such as NH_3 and H_2S (6). Improvements in growth performance by using clay in ration are explained by the fact that clay increases the digestibility of nutrients (47).

There are some recent studies using bentonite and other clay groups in animal feeding. Sherwood et al. (36) reported that the addition of 1.2% clinoptilolite to cattle rations did not affect body weight gain and dry matter intake. The ash content of meat in the group containing 3% zeolite was significantly higher compared to the group containing 1.5% bentonite and control group (33). The addition of 2% sepiolite to daily calf rations had a positive effect on body weight gain and diarrhea prevention (15).

No scientific data were found regarding the use of sodium bentonite in Tuj lambs. However, there are current studies using sodium bentonite and other clays in animals of different species. In addition, there are recent studies using sodium bentonite to demonstrate its ability to blind aflatoxin and heavy metal. However, the results of this study compared to the current research involving clay minerals, some similarities and differences were observed. Dietary supplementation of 1.5% and 3% bentonite improved live weight gain, daily live weight gain and feed conversion ratio of Zandi lamb exposed to lead; however, no significant differences were observed live weight and feed consumption (4). This study is consistent with our results.

Previous studies revealed that dietary use of zeolite positively influenced live weight and live weight gain of lambs (27, 38). In addition, Walz et al. (42) reported that the addition of 0.75% sodium bentonite to the concentrate was positively affected by the live gain in Suffolk lambs. The use of 2% sodium bentonite as a pellet binder in broiler rations had a positive impact on live weight gain, feed conversion ratio and feed consumption (25). In another study using different clay minerals, feed conversion ratio was positively affected (46). On the other hand, the addition of bentonite (3%) and / or vermiculite (3%) together or separately to the Merino lambs' ration had no significant effect on the feed conversion ratio (29). Ortiz et al. (30) reported that 1% and 2% kaolinite supplementation to the concentrated diets of Holstein beef cattle increased the live weight at the end of the fattening period but did not affect the live weight gain. Moreover, Khadem et al. (23) reported that the addition of bentonite to ration increased the feed consumption of the growing lambs. Addition of sepiolite to egg laying rations did not influence feed consumption, egg production, egg weight and feed conversion ratio (45). The difference between the results obtained can be explained by the type, quality, chemical composition of the clay mineral used and the differences in maintenance and feeding conditions.

Hematological values are very important for the assessment of the physiological statuses of the animals, management conditions, the nutrition and the diagnosis of the health statuses of the animals (24). Oxidation events occur continuously in live metabolism and reactive oxygen substances taken from outside accelerate these oxidation events. Antioxidants are substances that rapidly react to radicals and prevent the progression of autoxidation / peroxidation (12). Endogenous antioxidants and enzymatic antioxidants include SOD, CAT, GPx and GR while nonenzymatic antioxidants are glutathione, albumin and ceruloplasmin (3, 35).

Lipid peroxidation is the largest indicator of oxidative stress. MDA measurement is the most well-known and simple test of lipid peroxidation under oxidative stress and is most useful in clinical applications. In the case of oxidative stress, MDA, which is one of the main by-product aldehydes of lipid peroxidation, accumulates in tissues and peripheral circulation (31,49). The SOD enzyme is the first stage of the antioxidant defense system. It plays a critical role in eliminating superoxide radicals (21). CAT which plays an important role in the cell redox balance catalyzes the destruction of hydrogen peroxide to water and oxygen in order to maintain the cellular redox balance and reduce the toxic effect of hydrogen peroxide (19). In our study, the use of sodium bentonite in lamb rations increased the MDA, SOD and CAT values in the experimental groups as compared to the control group.

The literature conducted research, it showed that there have not been any studies conducted to investigate the effects of sodium bentonite on blood plasma antioxidant parameters in lambs. However, although there are recent studies investigating the antioxidant parameters of different clay minerals in animals, they are quite limited. Zhao et al. (51) reported that the use of montmorillonite in swine diets increased serum SOD and MDA levels. Addition of palygorskite composites, a naturally available hydrated magnesium-rich aluminium silicate (44) to broiler diets improved the antioxidant status of broilers increased serum CAT activity on day 21 of the study and decreased serum MDA value on day 42 of the study (48). Jiao et al. (22) reported that the use of copper-loaded / zinc-loaded montmorillonite in piglets rations increased SOD and decreased MDA in the jejunum and ileum. The addition of modified palygorskite to broiler rations increased jejunal total superoxide dismutase activity (T-SOD) on days 21st and 42nd of the study. On the 21st day of the study, the ileal MDA level and on the 42nd day of the study, the jejunal MDA accumulation decreased. The addition of modified

palygorskite to broiler rations had a positive effect on antioxidant capacity (40). These studies are consistent with our research findings in terms of SOD and CAT values. On the other hand, in a different study, the effects of adding sodium bentonite to broiler rations on SOD, CAT and GPx activities and lipid peroxide (LPO) levels in the liver and the kidney were investigated. Significant decreases in GPx and CAT activities, as well as an increase in the LPO level were detected in liver tissues (17). In another study, the use of palygorskite composites in broiler rations decreased the MDA content of the duodenal mucosa on the 21st day of the study, whereas the SOD value was not affected (44). The differences in the data may be associated with different conditions, such as the use of the clay group in different doses and structures, alone or in combination with substances such as aflatoxin. New studies should be supported to understand the effects of sodium bentonite on blood antioxidant parameters in animals.

In this study, dietary supplementation of 1% and 2% sodium bentonite significantly improved daily weight gain and feed conversion ratio of Tuj lambs. At the end of the study, MDA, SOD and CAT values increased. As a result, the use of sodium bentonite in Tuj lambs, especially at 2% dose, had a positive effect on fattening performance. Increase in SOD and CAT values of blood antioxidant parameters was protective against oxidative damage. However, the increase in MDA value is doubted the reliability of sodium bentonite use in lambs. In the light of the data obtained from this study, it has been clarified that dietary use of sodium bentonite, which is a natural product, influenced the performance and blood antioxidant parameters in Tuj breed lambs. Therefore, it is concluded that our study will shed light on the parameters that were investigated and will be a good source of literature.

Acknowledgement

We would like to thank Taner AYDIN for his support on grammar correction.

Financial Support

We would like to extend our deepest thanks to Emrah Cebeci from KarBen Bentonite Industrial Mining Chemical R & D Nano Technologies Industry and Trade Inc. who provided the sodium bentonite material.

Ethical Statement

This study was approved by the Kafkas University Animal Experiments Local Ethics Committee (KAU-HAYDEK /2019-26)

Conflict of Interest

The authors declare that they have no conflict of interest.

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