Sepiolite as an effective supplement for low-protein diets with the constant energy-protein ratio in broilers

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Abstract
The present study aimed to examine the influence of sepiolite on growth performance, meat quality, intestinal health, some blood parameters, and digestibility of nutrients in broilers fed low-protein diets with the constant energy-protein ratio. A total of 252, daily male broiler chicks were allocated to four treatment groups further divided into 9 replicates each containing 7 chicks. Low-protein diets having a constant energy-protein ratio were formulated by lowering protein and energy levels of the control group diet by 5%. Sepiolite was used at the level of 1% in the diets. After 42 days of trial, total feed consumption, total body weight gain, total feed conversion ratio, and carcass yield were not influenced by reducing protein, sepiolite supplementation, and interaction between low-protein-low-energy diet and sepiolite. Reducing protein in the diets led to reducing the digestibility of nutrients, increasing ileal viscosity, decreasing villus height, villus surface area in duodenum and jejunum, and increasing abdominal fat and ether extract, cooking losses, total oxidant status, and oxidative status index in breast meat. Sepiolite supplementation to low-protein diets increased crude protein digestibility, reduced viscosity, increased villus height/crypt depth values and reduced cooking losses, and increased water holding capacity in breast meat. Blood serum biochemical parameters and minerals were not affected by sepiolite supplementation to low-protein diets. Therefore, it is concluded that sepiolite can be added as a beneficial supplement in broiler diets as well as in low-protein diets with a constant energy-protein ratio.

Keywords Broiler · Digestibility · Intestinal histomorphology · Low protein-low energy diet · Meat quality · Performance · Sepiolite

Introduction
Diet plays an important role in maintaining poultry health, growth performance, and production. Protein is the most expensive component and the second important nutrient in the diets of poultry. It is important to reduce the protein content of the diets in the poultry production industry to reduce the environmental pollution from nitrogen wastes to maintain sustainability in production. Environmental pollution from nitrogen excretion is a major problem in poultry houses and its results have adverse effects on the health of workers and poultry, soil, and groundwater (Kim et al. 2014; Chalova et al. 2016; Attia et al. 2020). Reducing protein in the diets decreases the undigested protein reaching the hindgut and thereby decreases the substrate available for infectious bacteria to proliferate. However, low-protein diets with suboptimal amino acid supply have negative effects on performance in poultry (Aftab et al. 2006; Hilliar et al. 2020). Thus, some strategies can be used to improve diet utilization, performance, and meat quality and decrease abdominal and subcutaneous fat in low-protein diets (Suartika et al. 2014; Ndazigaruye et al. 2019; Gharib-Naseri et al. 2021). One of them may be sepiolite supplementation.

Sepiolite, a hydrated magnesium silicate \( \text{Si}_{12}\text{Mg}_8\text{O}_{30}(\text{OH}_2)_4(\text{OH})_4 \) in the group of phyllosilicates (Sardi et al. 2004), is a feed additive in animal nutrition. The wide range of applications of sepiolite is based on its high absorption and adsorption capacity (Murray, 2000). It
improves the performance of broilers and laying hens (Torruetero 1982; Ouhida et al. 2000; Ayed et al. 2011; Eser et al. 2012; Yalcın et al. 2017). Sepiolite supplementation decreased the ileal (Yalcın et al. 2017) and jejunal (Ouhida et al. 2000) digesta viscosity and may reduce the antinutritional effects of diets having high viscosity which in turn improved the digestibility of nutrients, especially in fat and nitrogen balance (Yalcın et al. 2017). Pancreatic enzymes can be adsorbed to the surface of sepiolite forming active complexes at different digestive pH ranges (Cabezás et al. 1991). The enzyme-sepiolite complexes are resistant to proteolysis and in this way increase the active digestive enzymes in the gut. Cabezás et al. (1991) concluded that poultry fed diets containing sepiolite will benefit from the feed more efficiently than the diets having no sepiolite. The most important point in terms of the welfare and nutrition of broilers is to reduce intestinal viscosity. This may be more beneficial under poor hygiene conditions or when fed low-digestible diets. Sepiolite can improve gut health by promoting the activity of enzymes and/or binding harmful substances throughout the gut. However, no research was seen to investigate the sepiolite effects on broilers fed diets having low protein. Thus, this research was planned to investigate the effects of sepiolite supplementation to the low-protein diets with a constant energy-protein ratio on the growth performance, digestibility of nutrients, carcass characteristics, meat quality, and blood parameters in broilers.

Materials and method

Experimental design and diets

Experimental procedures were approved by the Animal Ethics Committee of Ankara University (2013–5–39). This study involved 252 male 1-day-old broiler chicks (Ross 308) which were allocated to 4 treatment groups that consisted of 9 replicates of 7 chicks. Each replicate was housed in pens (90×80×80 cm). As a litter, wood shavings were used in each pen. Water and mash feed were provided ad libitum for 6 weeks. The ingredients and chemical composition of experimental diets are shown in Table 1. Normal energy and normal protein (NPNE) diets were designed according to the recommendation of NRC (1994), and all chicks were fed starter diets from 0 to 21 and grower diets from 22 to 42 days of age. Low-protein-low-energy diets (LPLE) were formulated by reducing protein and energy levels of NPNE diets by 5%. Therefore, a constant energy-protein ratio was obtained. Sepiolite at the level of 1% was included in the diets of NPNE and LPLE to obtain the diets of sepiolite with NPNE (SNPNE) and sepiolite with LPLE (SLPLE) as shown in Table 1. Sepiolite was obtained from a private company (sepiolite, Exal TH, Tolsa Turkey, Eskişehir). Sepiolite consisted of 65% sepiolite, 9% attapulgite, 18% dolomite, and 8% calcite. Sepiolite had 8.23% moisture and 89.87% ash. It had 2.6 mg/kg As, 1.16 mg/kg Pb, 0.02 mg/kg Hg, and less than 1 mg/kg Cd. Water holding capacity was higher than 150%. A 5% of sepiolite particles were larger than 600 µ, 40% is smaller than 250 µ, 70% is smaller than 125 µ, and 7% is larger than 38 µ in diameter.

Traits measured

The nutritional analysis of the diets was made according to the AOAC (2000). Metabolizable energy (ME) values of diets were estimated (Carpenter and Clegg 1956).

At the beginning of the experiment and every week, the chicks were individually weighed. Body weight gains were determined from the differences between the weights of subgroups. Feed consumption was calculated weekly as a subgroup. Feed conversion ratio (FCR) was determined as kilogram feed consumed per kilogram weight gain.

On the 32nd day, one pen from each group was placed in the individual pen. Chromic oxide, as an indigestible indicator, at the level of 0.3% to the diets was added. Feces were collected during a week. Feed and fecal samples were analyzed according to Williams et al. (1962) for the analysis of chromic oxide and then, the content of chromium was determined using an optical emission spectrometer with inductively coupled plasma (ICP-OES, Varian Vista MPX CCD Simultaneous ICP-OES). Dry matter (procedure 930.15), crude protein using the Kjeldahl procedure (procedure 968.06) ash, and organic matter (procedure 942.05) were determined according to the AOAC (2000). Organic matter was calculated. The following formula was used to calculate the apparent total tract digestibility of nutrients:

\[
\text{NATTD} = \left\{1 - \frac{[\text{DC} \times \text{FN}]}{\text{FC} \times \text{DN}}\right\} \times 100
\]

where NATTD is the apparent total tract digestibility of nutrient (%), DC is the content of chromium in the diet (%), FN is the nutrient content of the feces (%), FC is the chromium content of the feces (%), and DN is the nutrient content of the diet (%).

These broilers were slaughtered after a week and ileum digesta was collected. Digesta was homogenized and centrifuged at 12,000 × g for 10 min. The supernatant was used to determine the viscosity. The viscosity as centipoises (cPs) was measured (Graham et al. 1993) using a viscometer (model LVDV-I, Brookfield Digital Viscometer, Brookfield Engineering Laboratories Inc., Stoughton, MA).

One bird per replicate was evaluated for histomorphological analysis on day 42 (Onbaşlar et al. 2017). Duodenum, jejunum, and ileum segments of adequate length were removed, flushed with physiological saline solution, and submerged in a 10% formalin solution. Villus height (VH), villus width (VW), and crypt depth (CD) were measured using a microscope (Olympus BX51-DP71, Tokyo, Japan).
using Cellsens programs (CS-ST-V1.8) (Luna 1968; Xu et al. 2003). A total of 10 well-oriented villus-crypt units/intestinal samples were randomly selected and measured. The ratio of villus height to crypt depth (VH/CD) and villus surface area (VSA = 2π(VW/2) × VH) were calculated (Sakamoto et al. 2000; Çalık et al. 2019).

At the 42nd day of the trial, a total of 18 chicks from each group (2 broilers were randomly taken from each subgroup) were slaughtered. After slaughtering, hot carcass, liver, heart, kidney, spleen, bursa Fabricius, gizzard, and abdominal fat were weighed and percentages were determined by dividing carcass and organ weights by slaughter weights. Left and right breast meat samples from each carcass were sliced properly. Right breast meat samples after 1-day storage at +4 °C were used to determine water holding capacity (WHC) and cooking losses (CL).

WHC of meat samples was measured by centrifugation technique. The samples were cut into cubes of 10 × 10 × 10 mm, placed in a tube with filter paper at the bottom, and then centrifuged at 1000 × g at 4 °C for 15 min. WHC was calculated by the following formula:

\[ \text{WHC} (%) = \frac{(Wt_2/Wt_1) \times 100}{1} \]

where Wt1 is the sample weight (g) prior to centrifugation and Wt2 is the sample weight (g) after centrifugation (adopted from Hashizawa et al. 2013). For the determination of CL, meat samples were weighed and individually placed into plastic bags. These bags were immersed in a water bath (80 °C) for 20 min, cooled on absorbent paper at room temperature, and weighed. CL was calculated as the ratio of the difference in weight between the raw and cooked meat relative to the weight of the raw meat (Avcılar et al. 2019; adapted from Honikel 1998). Left side samples of breast meat were stored at –18 °C until making analyses. Left breast meat samples were subjected to proximate analysis (AOAC 2000). Catalase (CAT) enzyme activities (Goth 1991), levels of total antioxidant (TAS, mmol Trolox equivalent/kg), and total oxidant (TOS, µmol H2O2 equivalent/kg) in the left breast meat were measured with commercial kits (Rel Assay

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**Table 1** Ingredients and chemical composition of diets (as-fed basis)

| Ingredients (%) | Broiler starter (0–3 weeks) | Broiler grower (4–6 weeks) |
|-----------------|----------------------------|---------------------------|
|                 | Normal energy-normal protein | Low energy-low protein | Normal energy-normal protein | Low energy-low protein |
| Sepiolite       | -                           | +                         | -                           | +                         |
| Corn            | 49.23                       | 46.83                     | 57.78                       | 55.00                     |
| Soybean meal    | 20.90                       | 19.00                     | 27.00                       | 24.00                     |
| Full fat soya   | 22.60                       | 25.70                     | 9.00                        | 13.78                     |
| Poultry rendering meal | 2.00   | 2.00                     | 2.00                        | 2.00                     |
| Sepiolite       | 0                           | 1.00                      | 0                           | 1.00                      |
| Soy oil         | 1.20                        | 1.40                      | 0                           | 0                         |
| Limestone       | 1.30                        | 1.30                      | 1.30                        | 1.30                      |
| Dicalcium phosphate | 1.90      | 1.90                     | 1.90                        | 1.90                      |
| Salt            | 0.25                        | 0.25                      | 0.25                        | 0.25                      |
| Sodium bicarbonate | 0.10        | 0.10                     | 0.10                        | 0.10                      |
| DL-methionine   | 0.17                        | 0.17                      | 0.19                        | 0.19                      |
| Lysine          | 0.15                        | 0.15                      | 0.28                        | 0.28                      |
| Vitamin mineral premix | 0.15  | 0.15                     | 0.15                        | 0.15                      |
| Anticoccidial   | 0.05                        | 0.05                      | 0.05                        | 0.05                      |
| Chemical composition (analyzed) | | | | |
| ME (kcal/kg)    | 3026                        | 3020                      | 2887                        | 2890                      |
| Crude protein (g/kg) | 220.3         | 220.2                     | 209.7                       | 209.8                     |
| ME/CP (kcal ME/g CP) | 13.74      | 13.72                     | 13.77                       | 13.78                     |
| Calcium (g/kg)  | 10.58                       | 10.60                     | 10.44                       | 10.47                     |
| Total phosphorus (g/kg) | 7.30    | 7.29                      | 7.16                        | 7.17                      |

1 Supplied the following 1.5 kg of diet: 9.000.000 IU vitamin A, 4.000.000 IU vitamin D₃, 50.000 mg vitamin E, 2.000 mg vitamin K₃, 2.000 mg vitamin B₁, 5.000 mg vitamin B₂, 40.000 mg niacin, 15.000 mg calcium D pantothenate, 2.000 mg vitamin B₆, 10 mg vitamin B₁₂, 1.500 mg folic acid, 100 mg D-biotin, 120.000 mg Mn, 40.000 mg Fe, 100.000 mg Zn, 16.000 mg Cu, 1.250 mg I, 200 mg Co, 300 mg Se, 125.000 mg antioxidant (Etoksiquin, BHA)

2 Salinomycin

3 Calculated (Carpenter and Clegg 1956)
Diagnostics, Gaziantep, Turkey) by colorimetric methods (Erel 2004, 2005). Oxidative stress index (OSI) values were determined with the following formula:

$$\text{OSI} = (\text{TOS, } \mu\text{mol} / \text{TAS, } \mu\text{mol}) \times 100 \quad \text{(Ramay and Yalçın 2020)}.$$ 

On the last day of the experiment, blood samples were taken from 2 broilers from each subgroup and centrifuged at $3220 \times g$ for 8 min. Total protein, albumin, uric acid, triglyceride, and cholesterol in the blood serum were measured by an autoanalyzer (Product code 680–2153, Vitros 350; Johnson-Johnson Company, New York, USA) with commercial kits (Vitros Chemistry Products, Ortho-Clinical Diagnostics; Johnson-Johnson Company). Ca, P, Mg, Fe, Cu, Zn, Na, and K in serum were determined using ICP-OES (Varian Vista MPX CCD Simultaneous ICP-OES).

**Statistical analysis**

Statistical analyses were done using the ANOVA method (SPSS INC., Chicago, IL, USA). Effects of energy-protein level and sepiolite were determined using two-way ANOVA. The significant differences among groups were analyzed by the Tukey test. The level of significance was taken as $P < 0.05$ (Dawson and Trapp 2001).

**Results**

Inclusion of 1% sepiolite in low-protein diets having a constant energy-protein ratio of broilers had no effect on feed intake, body weights, weight gains, and feed conversion ratios of groups during grower and whole of the 6-week trial (Table 2). Feed intake of LPLE groups was increased, and feed intake of sepiolite supplemented groups was reduced during the starter period. However, the FCR of groups was not affected by the changes in feed intake during the starter period. No interaction was seen between the dietary sepiolite and the dietary level of protein and energy in the present study.

Dietary sepiolite supplementation improved nutrient digestibility (Table 3). The 5% reduction in energy and protein levels in diets significantly reduced the digestibility of nutrients. Sepiolite supplementation had higher nutrient digestibility in the NPNE diets and also LPLE diets; however, significant increments were only seen in the digestibility of crude protein. Reducing protein and energy levels in the diets having 1% sepiolite reduced digestibility of crude protein ($P < 0.05$). The 5% reduction in energy and protein levels of diets increased ileal viscosity ($P = 0.05$). However, sepiolite supplementation decreased ileal viscosity significantly ($P < 0.001$) in both of the diets. Reducing protein and energy levels in the diets having 1% sepiolite did not affect ileum viscosity.

### Table 2

| EP level | Sepiolite (%) | IBW (g) | FBW (g) | BWG 1–3 w (g) | BWG 4–6 w (g) | BWG 1–6 w (g) | FI 1–3 w (g) | FI 4–6 w (g) | FI 1–6 w (g) | FCR 1–3 w (g/g) | FCR 4–6 w (g/g) | FCR 1–6 w (g/g) |
|----------|---------------|--------|--------|----------------|----------------|----------------|--------------|--------------|--------------|----------------|----------------|----------------|
| Normal   |               | 44.5   | 2.951  | 781            | 2.126          | 1.097          | 4.007        | 1.41         | 1.65         | 1.50           | 1.45           | 1.60           |
| Low      |               | 44.3   | 2.935  | 794            | 2.089          | 1.133          | 4.084        | 1.43         | 1.70         | 1.62           | 1.70           | 1.70           |
| Low 0    |               | 44.4   | 2.927  | 791            | 2.136          | 1.218          | 4.083        | 1.43         | 1.70         | 1.62           | 1.70           | 1.70           |
| Low 1    |               | 44.7   | 2.915  | 784            | 2.153          | 1.102          | 4.009        | 1.41         | 1.68         | 1.61           | 1.68           | 1.68           |
| Normal   |               | 44.0   | 2.922  | 784            | 2.150          | 1.128          | 4.092        | 1.44         | 1.67         | 1.65           | 1.67           | 1.67           |
| Low      |               | 44.0   | 2.922  | 784            | 2.150          | 1.128          | 4.092        | 1.44         | 1.67         | 1.65           | 1.67           | 1.67           |
| Low 0    |               | 44.4   | 2.911  | 779            | 2.108          | 1.118          | 3.699        | 1.46         | 1.70         | 1.63           | 1.70           | 1.70           |
| Low 1    |               | 44.3   | 2.927  | 791            | 2.153          | 1.128          | 4.092        | 1.44         | 1.67         | 1.65           | 1.67           | 1.67           |
| Normal   |               | 44.7   | 2.915  | 784            | 2.153          | 1.128          | 4.092        | 1.44         | 1.67         | 1.65           | 1.67           | 1.67           |
| Low      |               | 44.0   | 2.922  | 784            | 2.150          | 1.128          | 4.092        | 1.44         | 1.67         | 1.65           | 1.67           | 1.67           |
| Low 0    |               | 44.4   | 2.911  | 779            | 2.108          | 1.118          | 3.699        | 1.46         | 1.70         | 1.63           | 1.70           | 1.70           |
| Low 1    |               | 44.3   | 2.927  | 791            | 2.153          | 1.128          | 4.092        | 1.44         | 1.67         | 1.65           | 1.67           | 1.67           |
| SEM      |               | 0.16   | 14.87  | 4.53           | 14.91          | 0.03           | 0.04         | 1.45         | 0.01         | 0.01           | 0.01           | 0.01           |

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$\text{EP} = \text{energy-protein, } \text{SEM} = \text{standard error of the mean, } \text{IBW} = \text{initial body weight, } \text{FBW} = \text{final body weight, } \text{BWG} = \text{body weight gain, } \text{FI} = \text{feed intake, } \text{FCR} = \text{feed conversion ratio}$
Discussion

Sepiolite supplementation to normal- or low-protein diets having a constant energy-protein ratio in broilers did not affect performance values after the 6-week trial. Similar to the present study results, some researchers reported that 1% (Ouhida et al. 2000), 1.5% (Uzunoğlu and Yalçın 2019), and 2% (Ouhida et al. 2000; Yalçın et al. 2017) addition of sepiolite had no effects on these performance indices in broilers. However, the addition of sepiolite at the levels of 0.5% (Eser et al. 2012), 1% (Ayed et al. 2011; Eser et al. 2012; Yalçın et al. 2017), and 2% (Ayed et al. 2011) increased body weights and weight gains of broilers. Feed efficiency of broilers was improved with dietary usage of 1% (Ayed et al. 2011; Yalçın et al. 2017) and 2% (Ayed et al. 2011) sepiolite. Different results among the studies may be due to the numerous factors such as quality and doses of sepiolite, diet composition, water quality, and management conditions (Yalçın et al. 2017). Reducing...
### Table 4: Effect of addition of sepiolite on low-energy-low-protein diets on intestinal histomorphology of broilers

| EP level | Sepiolite (%) | Duodenum | | | | Jejunum | | | | Ileum | | |
|----------|---------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
|          |               | VH (µm)  | VW (µm)  | CD (µm)  | VH/CD    | VSA (mm²) | VH (µm)  | VW (µm)  | CD (µm)  | VH/CD    | VSA (mm²) | VH (µm)  | VW (µm)  | CD (µm)  | VH/CD    | VSA (mm²) |
| Normal   |               | 1272.4   | 183.2    | 205.1    | 6.55     | 0.73      | 836.8    | 171.3    | 186.7    | 4.79     | 0.45      | 586.8    | 184.4    | 141.9    | 4.27     | 0.34      |
| Low      |               | 1028.4   | 183.9    | 157.4    | 6.98     | 0.60      | 736.9    | 163.1    | 120.3    | 6.44     | 0.38      | 569.1    | 180.6    | 119.7    | 4.83     | 0.33      |
|          | 0             | 1108.1   | 178.9    | 188.3    | 6.22     | 0.63      | 754.5    | 165.9    | 157.0    | 5.17     | 0.40      | 554.9    | 189.2    | 131.6    | 4.31     | 0.33      |
|          | 1             | 1192.7   | 188.2    | 174.3    | 7.31     | 0.70      | 819.3    | 168.5    | 150.0    | 6.06     | 0.44      | 601.1    | 175.8    | 130.1    | 4.79     | 0.33      |
| Normal   |               | 1272.7a  | 189.5b   | 200.8ab  | 6.61a    | 0.76a     | 823.9    | 174.5    | 188.3    | 4.64c    | 0.45      | 583.3    | 190.2    | 140.8    | 4.24b    | 0.35      |
| Normal   | 0             | 1272.1a  | 177.0b   | 209.4a   | 6.49b    | 0.71a     | 849.7    | 168.1    | 185.1    | 4.95bc   | 0.45      | 590.3    | 178.6    | 143.1    | 4.30b    | 0.33      |
| Low      |               | 943.5c   | 168.3b   | 175.7b   | 5.82b    | 0.50b     | 685.0    | 157.3    | 125.6    | 5.70b    | 0.34      | 526.4    | 188.2    | 122.3    | 4.38b    | 0.32      |
| Low      | 1             | 1113.3b  | 199.4a   | 139.2c   | 8.14a    | 0.70a     | 788.8    | 168.8    | 115.0    | 7.18a    | 0.42      | 611.9    | 173.0    | 117.1    | 5.27a    | 0.34      |
| SEM      |               | 13.9     | 3.8      | 4.6      | 0.16     | 0.02      | 11.8     | 4.1      | 4.5      | 0.14     | 0.01      | 8.0      | 3.8      | 2.4      | 0.09     | 0.01      |

$P$ value

|          | EP level      | <0.001   | <0.001   | <0.001   | <0.001   | <0.001   | <0.001   | <0.001   | <0.001   | <0.001   | <0.001   | 0.271    | 0.621    | <0.001   | 0.001    | 0.466     |
|          | Sepiolite     | 0.003    | 0.229    | 0.131    | 0.001    | 0.038    | 0.007    | 0.755    | 0.438    | 0.002    | 0.146    | 0.005    | 0.083    | 0.758    | 0.006    | 0.971     |
|          | EP level*Sepiolite | 0.003    | 0.005    | 0.016    | <0.001   | <0.001   | 0.101    | 0.273    | 0.677    | 0.046    | 0.132    | 0.015    | 0.817    | 0.434    | 0.017    | 0.284     |

$n$: 9

*EP* energy-protein, *SEM* standard error of the mean, *VH* villus height, *VW* villus width, *CD* crypt depth, *VSA* villus surface area

*a,b,c*: values within a column with different superscripts differ significantly at $P < 0.05$
energy and protein levels (constant energy-protein ratio) by 5% had no negative effects on performance indices during the present study. Kamran et al. (2008) stated that body weight gain was decreased and feed intake and FCR values were increased with the reduction in protein and energy levels having a constant energy-protein ratio in the diets during grower, finisher periods, and overall experimental periods, but these parameters were not affected during the starter period. Contrary to the present study, some researchers (Hidalgo et al. 2004; Kamran et al. 2008) showed that feeding broiler chickens with low-protein diets with a constant energy-protein ratio negatively affected the growth performance.

### Table 5
Effect of addition of sepiolite on low-energy-low-protein diets on carcass yield and some relative organ weights in broilers

| EP level | Sepiolite (%) | Carcass yield (%) | Liver (%) | Heart (%) | Gizzard (%) | Spleen (%) | Bursa Fabricius (%) | Abdominal fat (%) |
|----------|---------------|--------------------|-----------|-----------|-------------|------------|---------------------|-------------------|
| Normal   |               | 74.37              | 1.74      | 0.53      | 1.72        | 0.12       | 0.19                | 1.00              |
| Low      |               | 74.43              | 1.72      | 0.52      | 1.64        | 0.13       | 0.18                | 1.23              |
|          | 0             | 74.41              | 1.76      | 0.54      | 1.66        | 0.13       | 0.18                | 1.20              |
|          | 1             | 74.39              | 1.70      | 0.51      | 1.70        | 0.12       | 0.18                | 1.03              |
| Normal   | 0             | 74.30              | 1.72<sup>b</sup> | 0.54 | 1.69        | 0.13       | 0.19                | 1.14              |
| Normal   | 1             | 74.45              | 1.76<sup>b</sup> | 0.52 | 1.76        | 0.12       | 0.19                | 0.86              |
| Low      | 0             | 74.53              | 1.80<sup>a</sup> | 0.54 | 1.64        | 0.13       | 0.18                | 1.26              |
| Low      | 1             | 74.32              | 1.65<sup>b</sup> | 0.51 | 1.65        | 0.13       | 0.18                | 1.20              |
| SEM      |               | 0.12               | 0.02      | 0.01      | 0.02        | 0.00       | 0.01                | 0.05              |

*EP energy-protein, SEM standard error of the mean
a,b: values within a column with different superscripts differ significantly at *P* < 0.05

### Table 6
Effect of addition of sepiolite on low-energy-low-protein diets on breast meat characteristics of broilers

| EP level | Sepiolite (%) | WHC (%) | CL (%) | TAS (mmol/kg) | TOS (µmol/kg) | OSI (%) | CAT (U/l) | DM (%) | Ash (%) | CP (%) | EE (%) |
|----------|---------------|---------|--------|---------------|---------------|---------|------------|--------|---------|--------|--------|
| Normal   |               | 24.83   | 20.98  | 0.62          | 3.04          | 0.51    | 213.06     | 26.68  | 1.14    | 24.32  | 1.22   |
| Low      |               | 24.01   | 21.36  | 0.48          | 3.57          | 0.77    | 178.56     | 26.83  | 1.16    | 24.32  | 1.35   |
|          | 0             | 24.15   | 21.43  | 0.52          | 3.35          | 0.68    | 187.72     | 26.73  | 1.15    | 24.29  | 1.29   |
|          | 1             | 24.69   | 20.91  | 0.58          | 3.26          | 0.61    | 203.89     | 26.78  | 1.15    | 24.36  | 1.27   |
| Normal   | 0             | 24.70   | 21.28  | 0.59          | 3.15          | 0.54    | 204.00     | 26.67  | 1.13    | 24.32  | 1.22   |
| Normal   | 1             | 24.96   | 20.69  | 0.64          | 2.93          | 0.49    | 222.11     | 26.69  | 1.15    | 24.33  | 1.21   |
| Low      | 0             | 23.61   | 21.59  | 0.45          | 3.54          | 0.81    | 171.44     | 26.78  | 1.16    | 24.26  | 1.36   |
| Low      | 1             | 24.41   | 21.14  | 0.51          | 3.59          | 0.72    | 185.67     | 26.87  | 1.15    | 24.38  | 1.33   |
| SEM      |               | 0.10    | 0.09   | 0.02          | 0.10          | 0.03    | 11.62      | 0.09   | 0.01    | 0.08   | 0.03   |

*EP energy-protein, SEM standard error of the mean, WHC water holding capacity, CL cooking loss, TAS total antioxidant status (mmol Trolox equivalent/kg), TOS total oxidant status (µmol H2O2 equivalent/kg), OSI oxidative stress index, CAT catalase, DM dry matter, CP crude protein, EE ether extract

n: 18
Sepiolite supplementation decreased ileal viscosity and increased digestibility in NPNE and also LPLE diets; however, significant increments were seen only in the digestibility of crude protein. This increase in digestibility did not contribute to the improvement in performance indices. This may be due to the short duration of the fattening period in broilers. Reducing protein levels in the diets having 1% sepiolite did not affect ileal viscosity but caused a reduction in the digestibility of crude protein ($P < 0.05$). However, a reduction in digestibility did not cause to decrease in body weight and weight gain significantly. Namroud et al. (2008) reported a decrease in fecal nitrogen from 50.3 to 36.3 mg/g DM and uric acid from 113.5 to 101.1 mg/g DM of excreta digesta by reducing the dietary crude protein from 23 to 17%.

Similar to this present study, Yalçın et al. (2017) reported that 1% sepiolite improved the digestibility of dry matter, organic matter, and crude protein in the ileum. Sepiolite may decrease the passage of nutrients and thus retain digesta longer, increasing the transit time of nutrients. Therefore, the digestibility, and absorption of nutrients could be increased.

| Table 7 | Effect of addition of sepiolite on low-energy-low-protein diets on some blood serum biochemical indices in broilers |
|---------|---------------------------------------------------------------------------------------------------------------|
| EP level | Sepiolite (%) | TP (g/l) | ALB (g/l) | UA (mg/dl) | CHOL (mg/dl) | TG (mg/dl) | ALT (U/l) | AST (U/l) | ALP (U/l) |
| Normal  | 25.90 | 11.55 | 3.83 | 69.32 | 65.42 | 46.00 | 158.44 | 759.22 |
| Low     | 22.82 | 10.84 | 3.66 | 55.81 | 58.87 | 40.39 | 137.89 | 733.67 |
| 0       | 23.13 | 10.89 | 3.78 | 66.54 | 66.04 | 43.31 | 147.89 | 741.00 |
| 1       | 25.59 | 11.51 | 3.71 | 58.59 | 58.25 | 43.08 | 148.44 | 751.89 |
| Normal  | 24.28 | 10.88 | 3.87 | 75.63 | 69.27 | 46.37 | 159.00 | 746.00 |
| Normal  | 27.52 | 12.22 | 3.80 | 63.01 | 61.58 | 45.63 | 157.89 | 772.44 |
| Low     | 21.98 | 10.89 | 3.70 | 57.46 | 62.82 | 40.26 | 136.78 | 736.00 |
| Low     | 23.66 | 10.79 | 3.62 | 54.17 | 54.91 | 40.52 | 139.00 | 731.33 |
| SEM     | 0.32  | 0.10  | 0.08 | 0.88  | 1.06  | 0.66  | 2.45   | 12.75  |

$P$ value
| EP level | <0.001 | 0.001 | 0.252 | <0.001 | 0.004 | <0.001 | <0.001 | 0.324 |
| Seepolite | 0.001 | 0.003 | 0.624 | <0.001 | 0.001 | 0.861 | 0.910 | 0.672 |
| EP level*Seepolite | 0.235 | 0.001 | 0.985 | 0.013 | 0.959 | 0.709 | 0.736 | 0.546 |

$n$: 18

EP energy-protein, SEM standard error of the mean, TP total protein, ALB albumin, UA uric acid, CHOL cholesterol, TG triglyceride, ALT alanine transaminase, AST aspartate transaminase, ALP alkaline phosphatase

| Table 8 | Effect of addition of sepiolite on low-energy-low-protein diets on some blood minerals in broilers |
|---------|------------------------------------------------------------------------------------------------|
| EP level | Seepolite (%) | Ca (mg/dl) | P (mg/dl) | Na (mmol/l) | K (mmol/l) | Mg (mg/dl) | Zn (mg/l) | Cu (mg/l) | Fe (mg/l) |
| Normal  | 7.73 | 5.22 | 126.44 | 3.06 | 2.04 | 2.17 | 0.64 | 3.51 |
| Low     | 7.29 | 5.04 | 125.78 | 3.00 | 1.93 | 2.05 | 0.64 | 3.56 |
| 0       | 7.33 | 5.01 | 126.56 | 3.05 | 2.02 | 2.07 | 0.67 | 3.54 |
| 1       | 7.69 | 5.24 | 125.67 | 3.00 | 1.95 | 2.16 | 0.62 | 3.54 |
| Normal  | 7.55 | 5.08 | 127.00 | 3.09 | 2.04 | 2.15 | 0.68 | 3.48 |
| Normal  | 7.92 | 5.35 | 125.89 | 3.02 | 2.03 | 2.19 | 0.60 | 3.55 |
| Low     | 7.10 | 4.94 | 126.11 | 3.01 | 1.99 | 1.98 | 0.66 | 3.60 |
| Low     | 7.47 | 5.14 | 125.44 | 2.98 | 1.87 | 2.13 | 0.63 | 3.52 |
| SEM     | 0.10 | 0.06 | 1.76 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 |

$P$ value
| EP level | 0.026 | 0.150 | 0.851 | 0.398 | 0.169 | 0.111 | 0.989 | 0.576 |
| Seepolite | 0.064 | 0.063 | 0.802 | 0.452 | 0.390 | 0.210 | 0.485 | 0.985 |
| EP level*Seepolite | 0.991 | 0.784 | 0.950 | 0.801 | 0.456 | 0.439 | 0.753 | 0.404 |

$n$: 18

EP energy-protein, SEM standard error of the mean
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et al. (2015) reported that the improvement in digestion and poor performance of the birds (Qaisrani 2014). Dietary sepiolite in the feed could also reduce the “metabolic cost” for nitrogen catabolism, generating energetic savings which might correspond to a higher net energy availability for body growth (Parisini et al. 1999). High levels of ammonia in the blood may be reduced by enhancing the conversion of ammonia into uric acid which requires 1 mol of glycine to convert each molecule of uric acid in birds (Namroud et al. 2008), and this may lead to amino acid losses resulting in poor performance of the birds (Qaisrani 2014). Dietary sepiolite supplementation at 2% improved digestibility of energy and protein in weaned pigs by 5.3 and 6.1% (Parisini et al. 1999). Castaing and Noblet (1997) also indicated a reduction of nitrogen excretion from sepiolite-fed pigs in balance trials. Qaisrani (2014) reported that improving foregut protein digestion, thereby increasing protein digestion, will reduce the inflow of indigested amino acids in the colon and reduce the potential for protein fermentation. Qaisrani (2014) also concluded that some strategies can be done to increase the digestion and absorption of proteins in the foregut. One of the strategies for improving the digestion in broilers may be the dietary usage of sepiolite.

In the study of Ouhida et al. (2000) on broiler chickens, sepiolite was added to diets with low, medium, and high viscosity values at 1 and 2% levels, the digestibility of organic matter increased due to the reduction in jejunal viscosity, but the ileum viscosity was not affected by dietary sepiolite supplementation. Alzueta et al. (2002) stated that sepiolite (2%) supplementation to the diets of broilers from 22 to 28 days of age did not affect either the digestibility of protein and fat and viscosity of digesta. Zhou et al. (2014) reported that clays increase the intestinal viscosity due to the formation of gel, which slows down the digesta passage rate. Therefore, an increased transit time of digesta may improve the endogenous enzyme activity and the digestibilities of nutrients (Ouhida et al. 2000).

Properties of intestinal morphology such as VH, VW, CD, VH/CD, and VSA are important health indicators and reflect the digestive and absorptive capacity in the gut (Buddle and Bolton 1992). Low-protein diets significantly reduced VH, CD, and VSA and increased VH/CD in duodenum and jejunum (P < 0.001) and reduced CD (P < 0.001) and increased VH/CD (P = 0.001) in the ileum. Khosravinia et al. (2015) reported that the improvement in digestion and absorption was correlated with the increase in the ratio of VH/CD. According to Allameh and Toghyani (2019), the ratio of VH/CD was a marker for the digestive capacity of the small intestine. Abd-Elsamee et al. (2020) indicated that the reduction in dietary protein content without change in energy level in the diet caused a significant reduction in VH, VW, VH/CD, and absorption area. Sepiolite supplementation to low-protein diets increased VH, VW, VH/CD, and VSA and reduced CD in the duodenum, increased VH/CD in the jejunum, and increased VH and VH/CD in the ileum (P < 0.05). Awad et al. (2009) found that longer villi associated with better performance. An improvement in villus height increases the surface area for nutrient absorption thereby increasing nutrient digestibility (Subramaniam and Kim 2015). In the present study, longer villi contributes to higher nutrient digestibility but did not improve performance indices. It can be thought that performance improvement may be seen in longer feeding periods as in turkey fattening or laying hen or in addition to other types or different doses of clay minerals. Yalçın et al. (2017) showed that 1% sepiolite increased VH in the duodenum but did not affect jejunal and ileal histomorphology characteristics.

The main objectives of the poultry industry are to increase the carcass yield with the reduction of abdominal fat. Carcass yield and the relative weight percentages of internal organs were not influenced by sepiolite supplementation. The relative weight of abdominal fat percentage of the group fed diet having low protein with a constant energy-protein ratio was significantly increased (P = 0.024). Excessive fat accumulation is an unfavorable trait for producers and consumers as it is considered as wasted energy in diets, reduces carcass yield, adversely affects consumer acceptance, and is a waste product with low economic value (Emmerson 1997; Luca et al. 2004; Fouad and Senousey 2014; Yalçın et al. 2017). Similarly reducing dietary protein levels caused an increase in the amount of abdominal fat (Kassim and Suwanpradit 1996; Collin et al. 2003; Suartika et al. 2014). Kamran et al. (2008) stated that carcass yield and relative organ weights were not affected by the reduction in the protein of diet having a constant energy-protein ratio in the diets. Some researchers (Eser et al. 2012; Yalçın et al. 2017; Uzunoğlu and Yalçın 2019) also stated that sepiolite supplementation did not affect carcass yield, the relative weight percentages of heart, spleen, liver, and bursa of Fabricius. Similarly, the relative weight of abdominal fat percentage was not affected by 0.5% (Eser et al. 2012) and 1.5% (Uzunoğlu and Yalçın 2019) sepiolite. However, 1% (Eser et al. 2012; Yalçın et al. 2017) and 2% (Yalçın et al. 2017) sepiolite reduced abdominal fat percentages significantly. The reduction in abdominal and subcutaneous fat is a key factor for the success of poultry meat production (Zerehdaran et al. 2004). In this study, dietary 1% sepiolite reduced abdominal fat percentage by 14.17%, but this reduction was not statistically significant.
Broiler meat is one of the main sources of filling animal protein gaps and can play an important role in maintaining a balanced diet. Cooking loss is a parameter relating to meat tenderness. Water loss decreases the nutritional value of meat because nutrients can be removed along with the exudate, resulting in a reduction of tenderness in meat (Yalçın et al. 2017). Poultry meat is susceptible to oxidation. Antioxidant enzymes are widely found in animal tissues and play an important role in removing reactive oxygen species and thus may have important effects in reducing oxidative damage during reoxygenation (Moyo et al. 2012). The increase in total antioxidant activity protects against free radicals and peroxides (Ghore et al. 2021). Catalase is the important antioxidant enzyme and reduces oxidative stress by destroying the mechanism of cellular hydrogen peroxide to produce water and oxygen (Kopec et al. 2016). Catalase enzyme activity was increased in the jejunal mucosa by the supplementation of 2% clay product (zeolite-attapulgite) (Zhou et al. 2014) and in the liver by the supplementation of 2% clinoptilolite (Wu et al. 2013) in the broiler. However, in the present study, sepiolite addition did not affect catalase enzyme activity in breast meat.

Reducing protein with constant energy and protein led to decrease WHC (P < 0.001), increase CL (P = 0.052), decrease TAS (P = 0.002), and increase TOS (P = 0.017) and OSI (P < 0.001) levels in the present study.

The 5% reduction in dietary energy and protein levels significantly increased ether extract percentages (P = 0.047) in breast meat of broilers. However, Castaing and Noblet (1997) 2% sepiolite supplementation reduced carcass fatness and an increase of muscle percentage in the carcass. Quachem and Kaboul (2012) stated that sepiolite may increase the use of proteins in muscle production and, thus, reduce abdominal fat. Similarly, Yalçın et al. (2017) suggested that meat proximate composition was not affected significantly by 1 and 2% sepiolite supplementation. CL in the group fed diet supplemented with 1% sepiolite was decreased by 2% in the study of Yalçın et al. (2017).

Low-protein diets having a constant energy-protein ratio led to reducing albumin, total protein, triglyceride, total cholesterol, ALT, AST, and Ca in blood serum levels. Sepiolite supplementation to the LPLE diets decreased serum albumin and cholesterol concentration. Abd-Elsamee et al. (2020) stated that low-protein diets without any change in energy level in the diet did not affect serum AST and uric acid while a reduction in albumin and total protein levels. Yalçın et al. (2017) concluded that 1 and 2% sepiolite supplementation did not influence the serum levels of calcium, phosphorus, magnesium, potassium, sodium, zinc, iron, protein, albumin, and uric acid but triglyceride and cholesterol levels were decreased. Mizrak et al. (2014) also found that 1.5 and 3% sepiolite in the diets of laying hens did not influence the serum levels of cholesterol, albumin, protein, phosphorus, and calcium. Fernandez et al. (1994) stated that 1.25 and 1.75% sepiolite had no effect on plasma levels of P, Ca, K, and Na but 2.25% sepiolite increased plasma levels of Ca, P, and K, and reduced Mg but did not affect Na and Cl. On the contrary to the present study, Tortuero and Rioperez (1989) reported that sepiolite supplementation to the diets of growing pigs increased serum levels of zinc.

As a result, supplementation of sepiolite to low-protein (5% reduction) diets with a constant energy-protein ratio increased the nutrient digestibility, improved breast meat quality, intestinal histomorphology, and reduced viscosity without any negative effects on performance and carcass traits in broilers. Due to the absorbing capability of ammonia produced from deamination of proteins or urea hydrolysis along the digestive tract of broilers, dietary sepiolite usage will be a powerful strategy in terms of the health of humans, broilers, and the environment. Further study is proposed to clarify the mechanisms involved in the use of sepiolite to improve the nutritive value of diets having different protein and energy levels.

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Data availability Data will be made available on reasonable request from the corresponding author.

Declarations

Ethics approval This study was approved by the Animal Ethics Committee of Ankara University (2013–5-39).

Conflict of interest The authors declare no competing interests.

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