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The use of peppermint (*Mentha piperita*) leaves meal reduces ammonia excreta, increases egg production, and egg quality of laying hens

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ABSTRACT: A study was carried out to determine the effect of peppermint (Mentha piperita) leaves meal on ammonia production, dry matter excreta, egg production, and the quality of laying hens. A total of 80 laying hens of Lohmann Strain aged 20 weeks old were used as experimental animals. The birds were allocated in individual battery pens. The 20-week-old laying hens were vaccinated for New Castle diseases protection by using Vaksimune®ND B1on day 5 after arrival. The hens were kept for 8 weeks and fed four experimental diets. The diets used were basal diet (BSL), basal + 1% peppermint leaves meal (PLM) (BSL+1PLM), basal + 2% PLM (BSL+2PLM) and basal + 3% PLM (BSL + 3PLM). Feed and drinking water were present at all times. Parameters measured were ammonia concentration, dry matter excreta, hen day production, total egg mass, feed intake, FCR, dry matter digestibility, and quality of 14-days-stored eggs. A completely randomized design with 4 experimental diets and 5 replications was used. Data collected from this study were analyzed by using analysis of variance and tested with the Tukey test. The addition of peppermint leaf meal reduced ammonia production and increases dry matter excreta, total egg mass production, and dry matter digestibility. The Haugh unit, yolk height, and albumen height were improved when the eggs were kept for 14 days at room temperature. The addition of peppermint leaves meal decreased the mass loss of 14 days-stored eggs. In conclusion, supplementation of diets with peppermint leaf meal decreased ammonia concentration and watery excreta and increased the quality of eggs stored for 14 days at room temperature.

Keywords: Ammonia; Dry matter excreta; Egg quality; Peppermint; Poultry

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INTRODUCTION

The world's chicken population has increased rapidly by 90% over the last 2 decades, from 13.64 billion in 1999 to 25.92 billion in 2019 (FAO 2019). This increase in the chicken population causes an increase in manure production which is high in uric acid concentration (Ritz et al 2004). Through the enzymatic process, uric acid is converted into ammonia and carbon dioxide by the involvement of bacteria-producing urease (Bacharach 1957; Carlile 1984). It is accordingly the increase in the chicken population enhances ammonia production and thus has an impact on the decrease in air quality.

When the world's chicken population in 1998 was about 13.1 billion (FAO 2019), ammonia production generated from the chicken excreta was 2.1 million tons, equivalent to 9.0% of the total ammonia production from domestic animals (Asman et al 1998; Ritz et al 2004). Extrapolating from the chicken population in 2019, ammonia production generated by the chicken excreta was predicted to be doubled from the reported production in 1998.

A 500% increase in Indonesia's chicken population from 1999 to 2019 (FAO 2019) has triggered several animal nutritionists in Indonesia to find a technology to minimize ammonia production in the poultry industry (Jenny et al 2012; Puspani et al 2016; Mahardika et al 2019). Understandably, the high ammonia concentration in the poultry farm could negatively affect not only chicken production but also the health of the farmers. Ritz et al (2004) have well-reviewed the impact of ammonia on chicken health and production.

Decreased poultry production, digestive tract diseases, increased activity of New Castle Diseases virus, and Escherichia coli bacteria are to name some of the negative effects of ammonia (Ritz et al 2004). Efforts to reduce ammonia production from chicken excreta have received special attention from poultry nutritionists through the use of enzymes (Praes et al 2016), probiotics (Mahardika et al 2019), and bioactive substances from plants (Jenny et al., 2012). The modus operandi of decreasing ammonia production can occur through increasing the digestibility of protein which is the source of the raw material for ammonia and reducing the production of bacteria that can produce urease and uricase (Ritz et al 2004).

Peppermint is an herb from the family of Lamiaceae that has long been believed to have medicinal properties. Peppermint leaves had essential oils with a lot of bioactive compounds, functioning as anti-anti-bacteria and anti-oxidant (Tsai et al 2013; Zaia et al 2016). Of 0.5 to 4% essential oil in peppermint leaves, more than 50% of the compounds are in the forms of menthol, menthone, iso menthone, 25 to 78%, 14 to 36%, and 2.8 to 10% respectively.

Small quantities of menthyl acetate and tannin were also found in peppermint leaves (Azis et al 2011; Bupesh et al 2007; Grigoleit and Grigoleit 2005). The anti-bacterial substance in peppermint can inhibit the growth of bacteria that can produce ammonia (Ritz et al 2004). The production of watery excreta can also be inhibited because of the presence of the anti-bacterial fraction (Malone 2002). The anti-oxidant content in peppermint can function in maintaining egg quality (Centigul et al 2008).

A study was conducted to determine the effect of using peppermint leaves in reducing the ammonia concentration of excreta, dry matter of excreta, and improving egg production and quality of laying hens.

MATERIALS AND METHODS

Experimental Animals and cages

The study was approved by the Animal Ethics Committee at the Faculty of Animal Science and Fisheries. A total of 80 laying hens of Lohmann Strain aged 20 weeks old were used in this study. The hens

were placed in individual battery pens. On the arrival, the laying hens were offered Vita stress® (vitamin mix) as an anti-stress product. The 20-weeks-old laying hens were vaccinated for New Castle Diseases protection by using Vaksimune®ND B1on day 5 after arrival. The hens were kept for 8 weeks. The feeding trough was placed outside along the pen and one nipple drinker was provided for each pen. The house and surroundings were cleaned whenever necessary.

Feed and feeding

Peppermint leaf meal was purchased from the local traditional market. Corn and rice bran were bought from the local farmers. Commercial concentrate used was the product of Japfa Comfeed company and purchased from the local poultry shop. The basal diet can be seen in Table 1 and experimental diets were shown in Table 2. Drinking water and the diets were supplied *ad-libitum*. The feeding trough was topped up twice a day at 07.00 am and 04.00 pm.

Table 1. Basal diet Ingredients Concentration (%)

Ingredients	Concentration
Commercial concentrate	35
Corn	50
Rice bran	15
Calculated nutrients	
Protein	18.0
Metabolizable energy	2730
Calcium	3.52
Phosphorus	0.60
Lysine	0.81
Methionine	0.41

Table 2. Treatment diets

Treatments	Peppermint level	Replicates	Hens
BSL	100% basal diet	5	4
BSL+1PLM	99% basal diet + 1% peppermint leaves meal	5	4
BSL+2PLM	98% basal diet + 2% peppermint leaves meal	5	4
BSL+3PLM	97% basal diet + 3% peppermint leaves meal	5	4

NH_3 (ammonia) excreta, dry matter excreta, and feed digestibility analysis

Analysis of ammonia excreta was done by using a Conway micro diffusion method as described by Cunnaro and Weiner (1974). Two grams of fresh excreta were diluted with 20 ml of distilled water. The mixture was sieved and centrifuged for 3 minutes at 100 rpm. The supernatant was collected for the analysis of ammonia. One ml supernatant was placed in the left side of the outer chamber of Conway micro diffusion dishes. A total of 1 ml of saturated Na_2CO_3 was placed on the right side of the chamber and 1 ml of 2% H_2BO_3 with Bromocresol green was placed in the center of the chamber. The Conway micro diffusion dishes were sealed. To mix the supernatant with Na_2CO_3, the dishes were tilted gently. After 24 hours of incubation at room temperature, titration was done by using 0.005 N H_2SO_4 until the color change from black to pink. The levels of N-NH_3 were calculated as follows: mM N-NH_3 = Titration volume x N H_2SO_4 x 1000.

Digestibility study procedure

For the digestibility study, a total collection of excreta applied by Sundu et al (2019) was used in this study. To collect 1-day fecal discharge, a plastic tray of the same size as the pen was put underneath each pen. Excreta was collected each day at 08.00 am for 3 consecutive days. The excreta were individually weighed after

discarding feather and feed particles. The excreta from each pen were sampled and oven-dried at 50^{o}C for 3 days to measure the dry matter of excreta. Dry matter digestibility was calculated based on the formula:

$$\text{Dry matter (DM) digestibility (\%)} = \frac{\text{DM feed intake (g)} - \text{DM excreta (g)}}{\text{DM feed intake (g)}} \times 100$$

Egg production and egg quality measurements

Eggs were collected twice a day to minimize the broken eggs and weighed on the day of collection. Two eggs from each pen were sampled and stored for 14 days at room temperature for the measurement of egg quality. Egg mass loss was done by weighing the same eggs on day 1 and day 14. Egg mass loss, yolk index, and the Haugh unit (HU) were calculated by the following formula. Egg mass loss is expressed in percentage.

$$\text{Egg mass loss (\%)} = \frac{\text{Initial egg mass} - \text{egg mass after storage}}{\text{Initial egg mass}} \times 100$$

$$\text{Yolk index} = \frac{\text{Yolk height}}{\text{Yolk diameter}}$$

$$\text{The Haugh unit} = 100 * \text{LOG}\,(H - 1.7W^{0.37} + 7.6)$$

Where; H= albumen height (mm)
W = egg mass (g)

Experimental design and analysis of statistics

The study used a Completely Randomized Design with 4 experimental diets and 5 replicate pens. Data collected from this study were analyzed by using analysis of variance (Steel and Torrie 1980) to detect the significant effect of the treatments at $P<0.05$.

To run this analysis, the statistical program of Minitab 16 (Pesti et al 1986) was used. Any differences identified by analysis of variance were tested with the Tukey test by using Minitab statistical software.

RESULT AND DISCUSSION

Laying hens fed diets supplemented with peppermint leaves meal produced lower ammonia concentration and higher dry matter excreta and digestibility (Table 3). Although hen day production and average egg mass were not affected by peppermint leaves supplementation, the total egg mass that was produced for 8 weeks was heavier than the control egg mass (Table 3).

Feed intake and FCR were found unaffected due to peppermint addition in the diets. The eggs produced by laying hens fed the control diet without peppermint supplementation lost more weight the those of eggs from the peppermint-fed hens when the eggs were kept for 14 days at room temperature (Table 4). Yolk height, albumen height, and the Haugh unit were better in the eggs produced by hens fed the peppermint-containing diets. Yolk height, yolk color, and eggshell thickness were found of the same quality among all experimental eggs. Percentages of yolk, albumen, and eggshell were not affected by the experimental diets (Table 4).

Table 3. Ammonia production, dry matter excreta, egg production, feed intake, FCR and dry matter digestibility of laying hens fed the experimental diets

Parameters	Experimental diets			
	BSL	BSL + 1PLM	BSL + 2PLM	BSL 3PLM
NH_3 (Mol/L)	0.493±0.031[a]	0.328±0.024[b]	0.319±0.019[b]	0.349±0.019[b]
NH_3 (ppm)	8.34±0.533[a]	5.60±0.407[a]	5.39±0.329[a]	5.95±0.323[b]
DM Excreta (%)	27.24±0.44[a]	30.22±0.34[b]	30.41±0.52[b]	29.68±0.22[b]
Hen day (%)	88.5±3.71	89.6±1.09	90.4±0.17	89.6±1.12
Average egg mass (g)	61.4±1.09	62.1±0.80	62.0±1.11	62.5±0.76
Total egg mass (g)	12248±42.6[b]	12425±39.9[ab]	12572±33.0[a]	12533±70.7[a]
Feed intake (g)	28686±1102	28996±235	28665±484	28757±623
FCR	2.34±0.088	2.33±0.026	2.28±0.036	2.29±0.044
DM digestibility (%)	80.1±0.31[a]	81.9±0.23[b]	82.0±0.26[b]	81.2±0.12[b]

BSL: basal; BSL+1PLM: basal + 1% peppermint leaves meal (PLM); BSL+2PLM: basal +2% PLM; BSL+3PLM: basal + 2% PLM

[ab] Values with a different superscript in the same row are significantly different

Table 4: Effect of diets containing peppermint leave meal on egg quality stored for 14 days

Parameters	Experimental diets			
	BSL	BSL+1PLM	BSL+2PLM	BSL+3PLM
Egg mass loss (%)	2.80±0.21[a]	2.48±0.02[b]	2.49±0.03[b]	2.48±0.04[b]
Yolk height (mm)	10.4±0.16[a]	11.2±0.22[b]	11.3±0.16[b]	11.8±0.23[b]
Yolk index	0.35±0.0180	0.38±0.0040	0.39±0.0041	0.38±0.0041
Albumen height (mm)	7.2±0.17	8.5±0.44	8.2±0.31	8.7±0.24
HU	84.8±1.10[a]	90.8±2.30[b]	90.0±1.91[b]	93.6±1.08[b]
Shell thickness (mm)	0.46±0.016	0.49±0.013	0.48±0.021	0.47±0.029
Yolk (%)	28.7±0.86	29.0±0.93	26.6±0.51	29.0±1.07
Albumen (%)	59.2±1.03	58.6±0.98	60.3±0.48	58.4±0.79
Eggshell (%)	12.1±0.25	12.4±0.19	13.1±0.27	12.6±0.39

BSL: basal; BSL+1PLM: basal + 1% peppermint leaves meal (PLM); BSL+2PLM: basal +2% PLM; BSL+3PLM: basal + 2% PLM

**Values with a different superscript in the same row are significantly different*

Ammonia (NH3) excreta production, dry matter excreta, and feed digestibility

Ammonia production has been the main issue in commercial poultry farming due to its negative effect both on human and poultry health (Ritz et al 2004). The production of ammonia occurs when raw materials in the form of nitrogen-containing substances such as uric acid and ammonia-producing bacteria such as *Bacillus pasteurii* were present.

Hence ammonia production can be minimized through two mechanisms. First, nitrogen and uric acid discharge in the excreta should be minimal. Second, the population of the ammonia-producing microorganisms present in the excreta or litters should be in a lower concentration. The two modus operandi can be acted by optimizing digestibility and using anti-bacterial substances.

The presence of anti-bacterial properties in peppermint leaves can function as a digestibility enhancer (Emami et al 2012) through the increase in villus height (Mehri et al 2015) and phytobiotic (Schumacher et al 2003) through the increased population of *Lactobacillus* (Mehri et al 2015). This might be the reason why the addition of peppermint leaves meal in the laying hen diet in the present study decreased ammonia production by about 28.7 and 35.4%. Dilawar et al (2019) did a study on the use of *Mentha arvensis* (the same family as *Mentha piperita* used in this present study) on ammonia concentration.

Their finding on ammonia reduction due to *Mentha arvensis* supplementation supported our current finding.

Since ammonia production in poultry farms is affected by the temperature and moisture content of the litter (Ritz et al 2004), producing drier excreta in the tropical region has become a pivotal aspect in decreasing strong odor in the farm. The tropical climate in Indonesia with high daily temperature and high humidity worsens ammonia emissions in the poultry farm. Decreasing the dry matter content of the excreta can be a way to minimize the ammonia concentration on the farm. Interestingly, the inclusion of peppermint leaves meal in the diet up to 3% increased the dry matter content of the excreta from 27.24% to between 29.68 and 30.41% in the present study. As indigestible dietary fiber could bind more water than other dietary fractions (Sundu et al 2005), the more indigestible fraction in the excreta might indicate the dry matter excreta.

This present study showed a very strong correlation between dry matter digestibility and dry matter excreta with the correlation value of R^2= 0.96 (Figure 1). It can be said here that dry matter excreta was affected by dry matter digestibility.

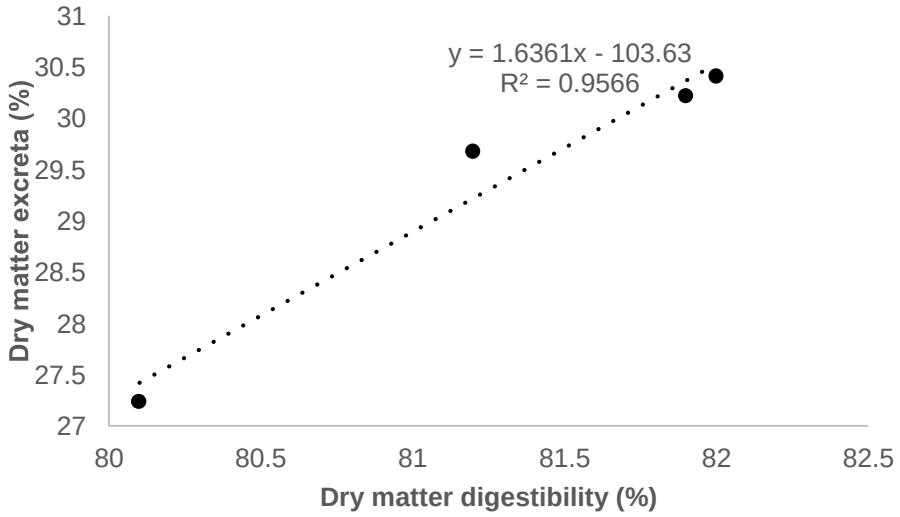

Figure 1: Correlation between dry matter digestibility and dry matter excreta

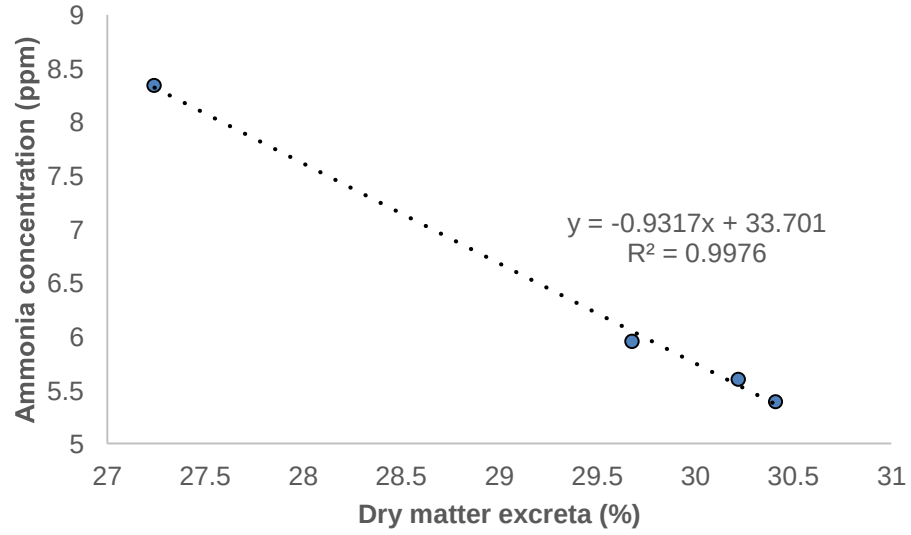

Figure 2: Correlation between dry matter excreta and ammonia concentration

Feed digestibility can be the main indicator of either ammonia production or wet dropping as indigestible and metabolizable protein in the excreta can be converted into ammonia. Accordingly, improving feed digestibility can tackle the problem of ammonia emission and watery excreta. There was a correlation between feed digestibility and ammonia concentration in this present study (Figure 2), being $R^2 = 0.99$.

The current finding indicates that supplementation of the diet with up to 3% peppermint meal increased feed digestibility by about 1.4 to 2.4%. The mechanism of increased digestibility might be similar to the mechanism of antibiotic usage. Anti-microbial substances present in the peppermint leaves protect the digestive tract of birds from deterioration by pathogenic bacteria colonization (Schumacher et al 2003).

Once the healthy gut can be maintained, optimal digestibility and absorption are achieved. Another mechanism might be through the increase in enzyme activity. Sharathchandra et al (1995) stated that increased lipase and amylase activity in the rats was found when the rats were fed with mint. Our findings of the increased feed digestibility were in accordance with the finding of Emami et al (2012), who found that feeding the birds with peppermint essential oil increased feed digestibility from 76.9% to 86.5%.

Effect of peppermint leaves meal on egg Production

Studies on the effect of peppermint on the production of eggs have been reported by several workers (Rahman et al 2021; Abdel-Wareth and Lohakare 2020). Supplementation of the diets with peppermint up to 3% did not increase hen day production and egg mass in the present study.

This finding is contradictory with the previous finding of Abdel-Wareth and Lohakare (2014), who reported that the addition of peppermint leaves up to 2% in the diets linearly increased egg mass and hen day production. However, the total egg mass collected for 8 weeks, in this current study, increased in the birds fed the 2 and 3% peppermint (BSL+2PLM and BSL+3PLM) from 12,248 g to between 12,533 and 12,572 g. The increase in the total egg mass might be due to the increase in feed digestibility. Understandably when the digestibility increases, more nutrients might be released and absorbed for the formation of eggs. This finding shows the possibility of using this herb to increase digestibility, total egg mass and reduce ammonia excreta. However, supplementation of the diets with only 1% peppermint leaves meal was not sufficient to increase total egg mass production.

It has long been believed that feed intake is attributed to the diet, either chemically or physically. Feed intake of the laying hens fed the diets supplemented with peppermint were similar among treatments. A contradictory finding of Abdel-Wareth and Lohakare (2014) reported that the addition of peppermint up to 2% increased the feed intake of laying hens aged 64-76 weeks. The different findings between the current study and the previous study of Abdel-Wareth and Lohakare (2014) might be due to the study location and the age of hens used.

The present study was done in the tropical country of Indonesia with a warm temperature and used 20 weeks-old hens, while the study of Abdel-Wareth and Lohakare (2014) was in Egypt with a cooler temperature and used much older hens. The feed conversion ratio was also not affected by the experimental diets in this present study.

Effects of peppermint leaf meal on egg quality

As a biological product, the egg undergoes deteriorated process due to a biochemical reaction during storage. Free water present in the egg is easily evaporated when the storage temperature is high. Accordingly, egg mass loss between 2.48 and 2.80% found in this present study was much related to the evaporation during 2 weeks of storage at room temperature. The 11.4% decrease in weight loss of eggs produced by hens fed the diets containing

peppermint leaves meal might be related to the thickness of the eggshell, the porosity of the shell, and the size of the egg's surface (Feddern et al 2017). Since eggshell thickness was not affected by treatment diets in the present study, the high loss of egg mass in control eggs (BSL diet) might be due to the accumulation of the three factors mentioned above. The lower egg mass loss from the hens fed the peppermint-supplemented diets could benefit egg producers as the eggs were sold on a weight basis and thus minimizing mass loss of egg means saving the profit.

The effects of peppermint on egg quality have been studied by several workers (Centigul et al 2008; Rahman et al 2021; Abdel-Wareth and Lohakare 2020). Lower yolk height, albumen height, and HU found in the eggs produced by laying hens in the current study indicate that eggs underwent biochemical and physical changes when they were stored at room temperature for 2 weeks. The addition of peppermint leaves meal could slow down the deterioration process. The 7.7 to 13.5%, 13.9 to 20.8% dan 6.1 to 10.4% higher in the respective yolk height, albumen height, and HU might indicate that the antioxidant substances in the peppermint worked well to protect the yolk and albumen from biological, physical, and chemical reactions.

Even though the yolk index did not improve due to peppermint supplementation, the yolk height of eggs produced by birds fed the peppermint-supplemented diets was better than those of control eggs. When compared to the HU, the unimproved yolk index due to peppermint supplementation was possible as a result of the different speeds of biochemical reaction in the yolk and albumen. It can be speculated here that yolk takes a longer time to be deteriorated than in the Albumen. It is therefore 2 weeks of storage might not be enough to detect yolk index change in this present study. Hatta et al (2020) detect a significant change in yolk index when the eggs were stored for 28 days. The slower changes found in the yolk index than the HU could be explained by the fact that yolk is physically more intact or solid than the albumen and thus the biochemical reaction needs longer a time to fully penetrate to the whole part of the yolk.

Parts of the eggs were unchanged due to the peppermint supplementation, where the percentages of yolk, albumen, and eggshell were from 26.6 to 29.0, 58.4 to 60.3, and 12.1 to 13.1% respectively. This finding might show that the portion of the egg was nothing to do with diet but perhaps something to do with genetic potential. As the strain used in this study was the same, the percentage of albumen, yolk, and eggshell were also similar among treatment diets. This finding was supported by Abdel-Wareth and Lohakare (2014). Eggshell was made of calcium and phosphorus and thus the eggshell thickness was much dependent upon the availability of calcium and phosphorus.

The unchanged eggshell thickness due to peppermint supplementation in this present study might indicate that the available minerals of calcium and phosphorus for eggshell formation remain the same.

CONCLUSIONS

Supplementation of the diets with peppermint leaves meal decreased ammonia excreta concentration and dry matter excreta of laying hens. Total egg mass production for 8 weeks and dry matter digestibility increased when the laying hens were fed with peppermint leaves-supplemented diets. The hens were fed the diets containing peppermint leaves meal produced better quality in the forms of egg mass loss, yolk height, and Haugh unit when the eggs were stored for 14 days at room temperature. Feed intake, FCR, eggshell thickness, and percentages of yolk, albumen, and eggshell were not affected by the inclusion of peppermint leaves up to 3% in the diets.

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