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Assessing of Peppermint as a Replacement for Antibiotic and Probiotic Using Technique for Order of Preference by Similarity to the Ideal Solution

Research Article

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ABSTRACT

Antibiotic resistant is a critical issue that can be the first cause of death in developing countries in near future. Poultry nutritionist should determine antibiotic alternatives for applying in the broiler farms to ensure the meat quality. The effects of replacing antibiotic and/or probiotic with peppermint oil were investigated in a completely randomized design with 5 treatments, 5 replicates, and 25 mixed-sex broiler chicks (Arian strain, average body weight=40±0.12) in each replicate. The experimental treatments were: 1) basal diet (control), 2) basal diet + 150 mg/kg antibiotic Avilamycin, 3) basal diet + 100 mg/kg probiotic Protexin, 4) basal diet + 200 mg peppermint essential oil/kg, 5) basal diet + 400 mg peppermint essential oil/kg. After obtaining data in performance, production index, heterophil to lymphocyte ratio, digestion index and ileum microflora of broilers, the multiple attribute decision making (MADM), and the technique for order of preference by similarity to ideal solution (TOPSIS) was applied. Results showed that adding 200 mg peppermint essential oil per kg diet caused higher livability (93% *vs.* 90%), higher production index (224 *vs.* 222), lower ratio of hetrofil/limfosit (0.38 *vs.* 0.40), higher digestion index (7.45 *vs.* 5.20), higher Lactobacillus count (7.37 *vs.* 7.21), lower *E. coli* count (7.05 *vs.* 7.61) compared to the control group. Also, comparing 200 ppm peppermint essential oil versus probiotic treatment revealed higher production index (224 *vs.* 216), lower *E. coli* count (7.05 *vs.* 7.95), so it can be concluded that 200 ppm peppermint essential oil has priority to probiotic treatment. The group which fed with 400 ppm peppermint essential oil had the lowest body weight (1974 g), the highest feed conversion ratio (FCR) (2.03), the highest livability (96%), and the lowest dimension index (4.49). In conclusion, using peppermint at the level of 200 mg/kg of diet has the potential to be considered as a probiotic replacement in broilers' diet.

KEY WORDS antibiotic, broilers, essential oil, peppermint, probiotic, TOPSIS.

INTRODUCTION

Today, poultry meat is the most produced meat around the world and it is the second most produced meat in European Union (EuroStat, 2022). It is clear that producing this vast volume of poultry meat needs intensive farms which practically uses antibiotics to ensure birds health, so antimicrobial resistance happens due to antibiotic residue in poultry meat and causes a global public health concern (Abreu *et al.* 2023). The beneficial biological effects of herbal essential oils are a crucial character that has found an application in broilers' nutrition and kicked back antibiotic growth promoters from the broilers' industry. Simultaneously, people all around the world, are interested in the use of organic products such as herbal essential oils. Therefore, there has been a rigorous attempt to substitute antibiotic growth promoters with probiotics or essential oils, *etc*. in poultry feeds (Khattak *et al*. 2014). Using probiotic needs some considerations such as the count of microorganism which reach to gut, health state of the birds, condition of storage place, *etc*. which directly affected the bird's performance. Essential oils are complex materials obtained from different parts of a variety of plants (leaves, flowers, seeds, fruits, roots, etc.) that contained many chemical components (Jäger *et al*. 2022). Essential oils have some nutritionally beneficial effects on poultry such as their antibacterial, antiviral and antioxidant activities and digestion and immunity stimulation (Atanda and Oluwafemi, 2007; Abd El-Hack *et al*. 2016). Production of broilers meat focuses on enhancing growth performance (the fastest growth with the lowest feed intake) and improving the immunity of the flock; which almost all essential oils can the ability to simultaneously stimulate weight gain and health in broilers (Adaszynska and Szczerbinska, 2016). Peppermint (*Mentha piperita*) is a domestic herb in Europe and the Middle East. Peppermint contains terpenoids and flavonoids such as eriocitrin, hesperidin, and kaempferol and its oil contains small amounts of many compounds including menthol and p-menthone, monoterpenes, menthofuran, limonene, pulegone, caryophyllene, pinene and terpene oxides (Eftekhari *et al*. 2021). It is a strongly antimicrobial substance (Inouye *et al*. 2001; Kędzia *et al*. 2007). It has positive effects on the broiler's performance (Ahmed *et al*. 2016; Asadi *et al*. 2017). Decision support systems (DSS) have been used in different issues to solve difficulties and to assess profits (Josaputri *et al*. 2016). It can be used for decision-making process. One of the methods used to make the decision process is technique for order of preference by similarity to ideal solution (TOPSIS).

Mude (2016) found that TOPSIS is blended into the Multi-Attribute Decision Making model and needs a decision matrix and weight value to perform calculations. During the last decades, increasing concerns over food and feed safety, the development of antibiotic resistance and unpleasant side effects of some antibiotics, have resulted in particular attention to find new antibiotic alternative agents in poultry diets (Helmy *et al*. 2023).

The present study aimed to evaluate the efficacy of peppermint essential oil and probiotics on the performance, digestibility, immunity response and ileal microflora of broiler chickens by TOPSIS technique.

MATERIALS AND METHODS

The protocol of the experiment approved by the Animal Care Committee of the Animal Science Research Institute of Iran, Karaj (Project#12-13-13-9154-91008).

Analysis of peppermint essential oils

The peppermint oil was analyzed using GC (Shinadzu-9A system equipped with F.I.D detector, chromate Pac data-processor and capillary column of DB-5), and GC/MS (Varin-3400 GC system connected to Saturn 2 mass spectrometer with ion trap detector and capillary column of DB-5). The carrier gas was helium at a flow rate of 22.7 cm/s and 50 cm/s in GC and GC/MS, respectively. The ionization energy in the mass spectrometer was 70 electron volts. The oven temperature program in GC was raised up to 100-220 ˚C at a rate of 2 ˚C /min and the injector temperature was 230 ˚C. The compounds of essential oils were identified according to the method described by Yazdanpanah Goharrizi and Tasharofi (2017).

Experimental design and dietary treatments

The effects of using peppermint essential oil were investigated in broilers as growth promotors instead of antibiotic and probiotic. In every trial, five hundred mixed sex day-old broilers (Arian strain) weighing 40 ± 0.12 g were allocated to five experimental treatments in a balanced completely randomized design (n=4) with 20 pens and 25 chicks in every pen (2×1.5×1 cm^3). Treatments were as follows: 1) basal diet (control), 2) basal diet + 150 mg/kg antibiotic Avilamycin, 3) basal diet + 100 mg/kg probiotic Protexin (each g or mL contains: *Lactobacillus acidophilus*, *Lactobacillus* delbrueckii subspecies bulgaricus, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*; *Bifidobacterium bifidum*; *Enterococcus faecium*; *Streptococcus salivarius* subspecies thermophilus), 4) basal diet + 200 mg/kg peppermint mint, 5) basal diet + 400 mg/kg pepper mint. Peppermint essential oil was prepared from peppermint leaf according to Sustrikova and Salamon (2004). Table 1 presents the chemical composition of the diet (based on Arian requirement recommendations and formulated with UF-FDA) and all chicks had free access to feed and water (*ad-libitum*).

Chicks were raised under similar environmental conditions (lighting, temperature, humidity) based on Arian management recommendations for 42 days. Before the beginning of experiments, all animals were vaccinated for bronchitis (at day 1), Newcastle at days 7, 12, 19 and 26 and Gambaro at days 16 and 23.

Performance

Chicks were weighed weekly and the amount of feed consumption during all trial was also measured at the last day (day 42) to calculate body weight gain (BWG) and the feed intake (FI), respectively. By having these two measurements, the feed conversion ratio (FCR) was calculated as feed consumed per unit of weight gain.

Table 1 Ingredients and chemical compositions of diets

Ingredients of diets (g/kg)	**Growth periods**		
	1-14 d	14-28	28- 42 d
Corn	486	457	455.5
Wheat	67.8	150	200
Soybean meal (44%)	365	320	279
Fish powder (60%)	21	14	5
Plant Oil	16	21	20
Sodium bicarbonate	2	1.5	1.5
Oyster shell	12.5	10.5	11
DL-Methionine	2.7	1.7	1.8
L-lysine hydrochloride	0.5	-	0.7
Di calcium phosphate	19	16.8	18
Salt (NaCl)	2.5	2.5	2.5
Vitamin and mineral premix[1]	5	5	5
Chemical compositions (calculated)			
Metabolizable energy (kcal/kg)	2851	2937	2965
Crude protein (%)	22.23	20.39	18.50
Ca (%)	1.06	0.90	0.90
Available P (%)	0.50	0.45	0.45
Lysine (%)	1.28	1.10	1.00
Methionine (%)	0.63	0.49	0.47
Methionine-cysteine (%)	0.99	0.83	0.78
Threonine (%)	0.85	0.77	0.69
Na (%)	0.18	0.16	0.16
Balance of anion cation	258	234	216

[1] Provides the following per kg of diet: vitamin A: 9000 IU; vitamin B_1: 1.8 mg; vitamin B_2: 6.6 mg; vitamin B_6: 3 mg; vitamin B_{12}: 0.015 mg; Calcium pantothenate: 10 mg; Folic acid: 1 mg; Biotin: 0.1 mg; vitamin D_3: 2000 IU; vitamin E: 18 IU; vitamin K_2: mg; Choline chloride: 500 mg; Mn (manganese oxide): 100 mg; Zn (zinc oxide): 100 mg; Fe (ferrous sulphate): 50 mg; Cu (copper sulphate): 10 mg; Se (sodium selenite): 0.2 mg and I (calcium iodate): 1 mg.

During the experiment, mortalities were checked out to calculate broilers' livability (Li). The FCR was corrected to mortality rate. Also, the production index (PI) which is a very valuable tool to compare different flocks, was calculated by the following equation:

PI= (Li (%) × average body weight gain (g) / FCR × trial days) / 10 (Zaghari *et al.* 2020)

Jejunum histomorphology

On day 42, 4 chicks per replicates with similar average weights were sacrificed by cervical dislocation to carry out histomorphological analysis. From the jejunum of chicks, 0.5 cm tissue samples were obtained and fixed in 10% buffered formalin (100 mL of 40% form aldehyde, 4 g phosphate, 6.5 g dibasic sodium phosphate and 900 mL of distilled water) for 24 h and then the 10% buffered formalin solutions were renewed. Tissues were dehydrated by transferring through a series of alcohols with increasing concentrations, placed into xylene and embedded in paraffin. A microtome (semi-automatic microtome model DS9209) was used to make 5 cuts that were 5 µm. The paraffin sections were stained with hematoxylin-eosin (Thompson and Applegate, 2006). The values were measured with a LEICA light microscope [using the LEICA Queen 550 software (Germany)].

Measurements of villus height and crypt depth (digestion index=villus height/crypt depth) were determined at a magnification of 10X. A minimum of 5 measurements per slide were made for each parameter and averaged into one value.

Ileum microflora

Digesta samples was collected from the ileum of the sacrificed birds, and stored in sterile bags for *Lactobacillus* (Lact) and *E. coli* quantification. Digesta samples were homogenized with 1 mL physiological serum. Five µL aliquot was mixed with blood agar and eosin methylene blue (EMB) and incubated at 37 ˚C for 24 h using Isotherm® Natural Convection Lab Incubator (model IFA-170-8).

After incubation, bacteria colonies were counted in selective agar media for the enumeration of target bacterial groups. The microbial counts were determined as colony-forming units (cfu) per gram of wet samples (Boyd and Mulvey, 2013).

Heterophil to lymphocyte ratio (H/L)

On the 35th day of the experiment, blood samples of one chick from every replicate were taken into EDTA tubes to evaluate heterophil to lymphocyte ratio. Then, by Giemsa stain processing, white blood cells (WBC) were counted.

Statistical analysis

The data were statistically analyzed based on a completely randomized design using the GLM procedure of SAS (SAS, 2003). The means were compared by Duncan's multiple comparisons procedure (Duncan, 1955). Statistical models were as follows:

$Y_{ij} = \mu + T_i + e_{ij}$

Where:

μ: overall mean.

T_i: treatment effect.

e_{ij}: experimental error.

Use of technique for order of preference by similarity to ideal solution (TOPSIS)

TOPSIS is a method to choose alternatives that simultaneously had the shortest distance from the positive ideal solution and the furthest distance from the negative ideal solution. A positive ideal solution maximizes the benefit criteria and minimizes the cost criteria, and vice versa for the negative ideal solution (Hosseini *et al.* 2014; Pungky *et al.* 2018). In the present study, TOPSIS is applied to make a multiple attribute decision-making (MADM) based on replacement of antibiotics and/or probiotics with essential oils. There are 5 steps in completing a MADM case with TOPSIS as follows:

Step 1. Make a normalized decision matrix to omit positive and negative quantity indexes.

$$n_{ij} = \frac{a_{ij}}{\sqrt{\sum_{i=1}^{m} a_{ij}^2}}$$

n_{ij}: normalized value of the decision matrix

a_{ij}: original value of the decision matrix

Step 2. Create a normalized weighted decision matrix.

$V = N \times W_{n \times n}$

V: weighted normalized decision matrix.

N: weighting against criterion *i*.

$W_{n \times n}$: normalized value of the decision matrix.

Step 3. Determine the matrix of positive and negative ideal solutions.

$A^+ = (y1^+, y1^+, \ldots, y1^+)$.

$A^- = (y1^-, y1^-, \ldots, y1^-)$.

$y1^+ = Max\ y_{ij}$: if *j* is a benefit attribute, $Min\ y_{ij}$: if *j* is a cost attribute.

$y1^- = Max\ y_{ij}$: if *j* is a cost attribute, $Min\ y_{ij}$: if *j* is a benefit attribute.

A^+= positive ideal solution A^+ matrix.

A^-= negative ideal solution A^- matrix.

$y1^+$= Max y_{ij} if *j* is a benefit attribute (*benefit*).

$Max\ y_{ij}$= if j is a cost attribute (*Cost*).

$y1^-$= Min y_{ij} if *j* is a benefit attribute (*benefit*).

$Miny_{ij}$= if *j* is the cost attribute (*Cost*).

Step 4. Determine the distance between the value of each alternative with the matrix of positive and negative ideal solutions.

$$d_i^+ = \sqrt{\sum_{j=1}^{n} (v_{ij} - v_j^+)^2}$$

$$d_i^- = \sqrt{\sum_{j=1}^{n} (v_{ij} - v_j^-)^2}$$

di^+= distance to a positive ideal solution.

di^-= distance to a negative ideal solution.

Step 5. Determine the preference value (the final value to rank all previously assessed alternatives) in which the highest value is the best one and shows that it has been appropriately selected.

$$CL = \frac{d_i^-}{d_i^- + d_i^+}$$

RESULTS AND DISCUSSION

Based on the percent of compounds in peppermint essential oil (Table 2), carvone (62.24%), limonene (20.47%), Dihydro-carvone (8.69%), and β-Pinene (1.47%) are the main substances of the peppermint essential oil, respectively. In agreement with this result, Giménez-Santamarina *et al.* (2022) reported that Carvone (41.1%) and limonene (14.4%) were the major effective substances in peppermint. Bardaweel *et al.* (2018) reported that carvone (49.5%) and limonene (16.1%) had the highest value among other substances in peppermint. Also, Brahmi *et al.* (2017) found that carvone amount was about 20.8% and limonene amount was about 48.5%; Nikšić *et al.* (2018) reported that the amount of carvone was about 56.4% and limonene was about 16.2%. Table 3 shows the deciding matrix to determine the best experimental treatment.

Table 2 composition of active substances of peppermint essential oil

Constituents	Amount %	Constituents	Amount %
α-Pinene	0.97	Dihydrocarveol acetate	0.43
Camphene	0.05	Trans-Carveyl	0.90
β-Phellandrene	0.46	Copaene	0.01
β-Pinene	1.47	Bourbonene	0.62
Limonene	20.47	β-Humulene	0.02
Limonene oxide	0.03	Caryophyllene	0.50
Artemiseole	0.10	Cuvebene	0.07
Borneol	0.03	Farnesene	0.10
Carvone	62.24	α-Caryophyllene	0.21
Dihydrocarvone	8.69	Aromadendrene	0.02
Cis-Carveol	0.51	Caryophyllene oxide	0.49

Table 3 Decision-making matrix (crude results of the experimental treatments)

Matrix	BW (g)	FI (g)	FCR	Li (%)	PI	H/L	DI	*Lactobacillus* (Log_{10})	*E. coli* (Log_{10})
Control	2092	4171	1.99	90	222	0.40	5.20	7.21	7.61
Antibiotic	2066	4009	1.94	95	241	0.41	7.18	8.13	6.35
Probiotic	2043	4087	2.00	89	216	0.37	7.73	7.82	7.59
Peppermint 200 ppm	2041	4116	2.01	93	224	0.38	7.45	7.37	7.05
Peppermint 400 ppm	1974	4006	2.03	96	222	0.38	4.49	7.42	7.45
Criteria type	Positive	Negative	Negative	Positive	Positive	Negative	Positive	Positive	Negative
Criteria weight	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

BW: body weight; FI: feed intake; FCR: feed conversion ratio; Li: livability; PI: production index; H/L: heterophyl to lymphocyte and DI: digestion index.

In this table, a positive scale was considered for the traits in which higher values were desired, and a negative scale was considered for the traits in which lower values were desired. At this study, using antibiotic caused the best performance of broiler chickens, however using antibiotics are banned in poultry industry in lots of countries around the world. So regards to higher livability (93 % *vs.* 90 %), higher production index (224 *vs.* 222), lower ratio of H/L (0.38 *vs.* 0.40), higher digestion index (7.45 *vs.* 5.20), higher *Lactobacillus* count (7.37 *vs.* 7.21), lower *E. coli* count (7.05 *vs.* 7.61) compared to the control group, using 200 ppm peppermint essential oil is preferred. Also, comparing 200 ppm peppermint essential oil versus probiotic treatment revealed higher production index (224 *vs.* 216), lower E. Coli count (7.05 *vs.* 7.95), so we conclude it has priority to probiotic treatment. The group which fed with 400 ppm peppermint essential oil had the lowest body weight (1974 g), the highest FCR (2.03), the highest livability (96%), and the lowest dimension index (4.49). Table 4 shows the normalization of the data of the decision-making matrix to evaluate the optimum level of adding peppermint essential oil in broiler chickens' diet. Regards to relative importance of the traits, relative weights which are considered at Table 3, was considered for each trait and the obtained data to evaluate the weights of traits by entropy method is shown in Table 5. Regards to Table 5, production index as a valuable index for comparing economic benefits of the flock was highest for antibiotic and then 200 ppm peppermint essential oil among all the groups.

Considering the issue of antibiotic resistance problems, using 200 ppm peppermint essential oil is preferred. Then, regards to decision matrix, positive or negative of positive or negative ideal ways was determined for each trait (Table 6). Determining of the distance between the values and positive and negative ideal solutions for evaluating the optimum level of addition of peppermint essential oil to broiler chickens' diet is shown in Table 7. Table 8 represents the relative closeness of each parameter to the ideal solution. In this table, each item its value is bigger is preferred compared to other items. Accordance to this table the birds which received antibiotic or peppermint at the level of 200 mg/kg had the highest score regards to considered traits among the experimental treatments. In agreement with this result, Hassan (2019) reported that adding peppermint essential oil to broilers' diet is beneficial for the birds' growth performance. Abdel-Wareth *et al.* (2012) reported that the body weight and body weight gain of broilers improved by using peppermint leaves in their diet. Essential oils are oily, volatile or aromatic liquid substances which prepared from different parts of plants such as flowers, seeds, herbs, leaves, fruits, roots and bark (Brenes and Roura, 2010). Previous researchers have reported that essential oils can improve increase body weight gain (Falaki *et al.* 2016; Yang *et al.* 2018), and feed conversion ratio (Yang *et al.* 2018), however we did not see improvement in body weight gain (BWG) or FCR of the birds fed with essential oils, which can be due to differences in dosage of usage or strain of the birds.

Table 4 Normalization of the data of decision-making matrix to evaluate the optimum level of adding peppermint essential oil in broiler chickens' diet

Unscaled matrix	BW (g)	FI (g)	FCR	Li (%)	PI	H/L	DI	*Lactobacillus* (Log$_{10}$)	*E. coli* (Log$_{10}$)
Control	0.4578	0.4574	0.4466	0.4345	0.4402	0.4635	0.3555	0.4245	0.4709
Antibiotic	0.4521	0.4396	0.4345	0.4586	0.4778	0.4692	0.4908	0.4786	0.3931
Probiotic	0.4471	0.4482	0.4479	0.4296	0.4283	0.4259	0.5284	0.4601	0.4696
Peppermint 200 ppm	0.4466	0.4513	0.4513	0.4490	0.4441	0.4339	0.5093	0.4341	0.4363
Peppermint 400 ppm	0.4320	0.4393	0.4555	0.4634	0.4402	0.4419	0.3069	0.4366	0.4613

BW: body weight; FI: feed intake; FCR: feed conversion ratio; Li: livability; PI: production index; H/L: heterophyl to lymphocyte and DI: digestion index.

Table 5 Weighting the data of normalized decision-making matrix to evaluate the optimum level of adding peppermint essential oil in broiler chickens' diet

Weighted matrix	BW (g)	FI (g)	FCR	Li (%)	PI	H/L	DI	*Lactobacillus* (Log$_{10}$)	*E. coli* (Log$_{10}$)
Control	0.0458	0.0457	0.0447	0.0434	0.0440	0.0464	0.0355	0.0424	0.0471
Antibiotic	0.0452	0.0440	0.0434	0.0459	0.0478	0.0469	0.0491	0.0479	0.0393
Probiotic	0.0447	0.0448	0.0448	0.0430	0.0428	0.0426	0.0528	0.0460	0.0470
Peppermint 200 ppm	0.0447	0.0451	0.0451	0.0449	0.0444	0.0434	0.0509	0.0434	0.0436
Peppermint 400 ppm	0.0432	0.0439	0.0456	0.0463	0.0440	0.0442	0.0307	0.0437	0.0461

BW: body weight; FI: feed intake; FCR: feed conversion ratio; Li: livability; PI: production index; H/L: heterophyl to lymphocyte and DI: digestion index.

Table 6 Determination of positive and negative ideal solutions to evaluate the optimum level of adding the peppermint essential oil to broiler chickens' diet

Best solution	BW (g)	FI (g)	FCR	Li (%)	PI	H/L	DI	*Lactobacillus* (Log$_{10}$)	*E. coli* (Log$_{10}$)
Positive ideal	0.0458	0.0439	0.0434	0.0463	0.0478	0.0426	0.0528	0.0479	0.0393
Negative ideal	0.0432	0.0457	0.0456	0.0430	0.0428	0.0469	0.0307	0.0424	0.0471

BW: body weight; FI: feed intake; FCR: feed conversion ratio; Li: livability; PI: production index; H/L: heterophyl to lymphocyte and DI: digestion index.

Table 7 Determination of the distance between the values and positive and negative ideal solutions to evaluate the optimum level of adding the peppermint essential oil to broiler chickens' diet

Distance	Positive	Negative
Control	0.0207	0.0058
Antibiotic	0.0058	0.0217
Probiotic	0.0101	0.0229
Peppermint 200 ppm	0.0079	0.0211
Peppermint 400 ppm	0.0241	0.0051

Table 8 Calculation the closeness coefficient to positive and negative ideal solutions and rank the treatments to evaluate the optimum level of adding the peppermint essential oil to broiler chickens' diet

Result	Closeness coefficient
Control	0.2202
Antibiotic	0.7899
Probiotic	0.6945
Peppermint 200 ppm	0.7284
Peppermint 400 ppm	0.1743

At present study, adding essential oils increased livability and production efficiency of the birds. These positive effects can be related to their characteristics such as antioxidative (Silva *et al.* 2012), antimicrobial (Du *et al.* 2016), or immunological effects (Hosseini *et al.* 2016). At present study using peppermint essential oil decreased ileal *E. coli* count. Parallel to these results, some researchers reported that essential oils improve the ecological situation of the intestine and simulate the activity of the digestive enzymes (Cross *et al.* 2007; Jang *et al.* 2007).

CONCLUSION

Today, probiotics are used extensively in broilers diet. However, the quality of probiotics may be decrease after its production at the probiotic factory. Usually, this problem happens because of mistakes in storage condition. So introducing other additives which may be more accessible in some region is a good way to have higher feed efficiency and production index in broilers farm. In conclusion, regard to the results applying peppermint at the level of 200 mg/kg of diet has the potential to be considered as a probiotic alternative in broiler diets.

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