Acta Scientiarum
http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v47i1.73251



# Comparison of oregano essential oil as antibiotic or probiotic alterative in broilers' diet: Use of technique for order of reference by similarity to ideal solution

**Seyed Abdollah Hosseini, Hosna Hajati*, Amir Hossein Alizadeh-Ghamsari and Houshang Lotfollahian**

Department of Animal Science, Animal Science Research Institute of Iran, Agricultural Research Education and Extension Organization, Karaj, Iran. *Author for correspondence. E-mails: h.hajati2010@gmail.com; h.hajati@areeo.ac.ir

**ABSTRACT.** Antibiotic resistance is a major problem for public health worldwide, and nutritionists are searching for antibiotic alternatives. Oregano essential oil is one of the natural products that has antioxidant and antibacterial substances. In the present study the technique of ordering the preference by similarity to the ideal solution was applied to compare different feed additives regarding the biological parameters of broilers fed diets containing antibiotic, probiotic or different levels of oregano essential oil. A total of five hundred Arian broiler chicks were used in a completely randomized design with 5 treatments and 5 replicates. The experimental treatments were: 1) basal diet (control), 2) basal diet+150 ppm antibiotic Avilamycin, 3) basal diet +100 ppm probiotic Protexin, 4) basal diet+200 ppm oregano essential oil, 5) basal diet+400 ppm oregano essential oil. After obtaining data on performance, production index, heterophil to lymphocyte ratio, 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 indicated that oregano essential oil had the potential to be considered as a natural probiotic or antibiotic replacement in broiler chickens' diet. Oregano essential oil improved FCR, production index, intestinal villus height/crypt depth of the broilers. Also, it decreased the bacterial count of *E.Coli* in the ileum content of the broilers. It conclusion using oregano essential oil at the level of 200 or 400 ppm of diet can be considered as probiotic or antibiotic natural alternative in broilers diet.

**Keywords:** TOPSIS; MADM; Oregano; Broiler Chicken.

Received on August 4, 2024.
Accepted on November 29, 2024.

## Introduction

Applying antibiotics as a growth promoter has been banned in the poultry industry due to health problems (Simitzis, 2017). Thus, poultry nutritionists are searching to find the desired antibiotic alternatives. It was stated that herbal additives, such as plant essential oils and herbal extracts can be considered as antibiotic alternatives to improve the birds' productive performance (Simitzis & Deligeorgis, 2011; Lotfollahian et al., 2023). Essential oils consist of low molecular weight aliphatic hydrocarbons such as phenols, aromatic aldehydes (Dorman & Deans, 2000). Essential oils are volatile secondary metabolites which has been broadly applied in the cosmetic and food industry. These compounds have been also known to have antioxidant properties that could optimize the antioxidant levels in the feed (Simitzis, 2017). Oregano (*Origanum vulgare*) is native to temperate Western and Southwestern Eurasia and Mediterranean region. It has the potential to be used as a highly antimicrobial substance (Avila Ramos et al., 2017). Its essential oil contains carvacrol and thymol, p-cymene, γ-terpinene, caryophyllene, spathulenol, germacrene, beta fenchyl alcohol, and gamma terpineol. Oregano oil is considered Generally Recognized as Safe by the United States Food and Drug Administration (Vázquez et al., 2015). It was shown that oregano has different activities such as antimicrobial, antioxidant, anti-inflammatory, antiparasitic, stomachic, antispasmodic, diuretic, and immunomodulator activity (Alagawany et al., 2018). It was reported that a mixture of essential oils of oregano, thyme, and garlic leads to higher body weight gain, relative growth rate, and lower feed conversion ratio and oocyte count in broiler chicks (Abou-Elkhair et al., 2014). Eler et al. (2019) found that using oregano essential oil at the level of 300 ppm improved growth performance, hemogram, and leukogram of the broilers (Eler et al., 2019). Peng et al. (2016) have found that oregano essential oil at the level of 300 or 600 ppm improved carcass traits and jejunal morphology of broilers, and they concluded that oregano essential oil can be considered as an alternative for antibiotic growth promotors (AGPs). TOPSIS is a method to choose alternatives that simultaneously have 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 (Javaheri Barfourooshi et al., 2023; Pungky et al., 2018). The aim of the present study was using TOPSIS method to compare the effects of Avilamycin, protexin, or oregano essential oil on the performance, gut morphology, immunity and ileum microflora of broiler chickens.

## Material and methods

### GC and GC/MS analysis of essential oil

Oregano essential oil was purchased from Ayat-esans company (Iran). The essential oil was prepared from the leaves of oregano plant by water and steam distillation. The oil was analyzed by GC (Shinadzu-9A system equipped with F.I.D detector, chromatopac data-processor and DB-5 capillary column), and GC/MS (Varin-3400 GC system connected to Saturn II mass spectrometer with ion trap detector and DB-5 capillary column). The carrier gas was helium at flow rate 22.7 and 50 cm $s^{-1}$ in GC and GC/MS, respectively. Ionization energy in 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 $min.^{-1}$ and injector temperature was 230°C. The temperature program in GC/MS was 60-240°C at a rate of 3°C $min.^{-1}$ and injector temperatures was 250°C. The compounds of essential oil were identified by comparison of their retention indices (RI), mass spectra fragmentation with those on the stored Wiley 7 n.1 mass computer library, and NIST (National Institute of Standards and Technology) library (Yazdanpanah Goharrizi & Tasharofi, 2017).

Animals, diets and experimental design. A total of five hundred mixed-sex day-old broilers (Arian strain) with initial body weight of 40 g were divided to five experimental treatments in a completely randomized design with 20 pens and 25 chicks in every pen. Treatments were as follows: 1) basal diet (control), 2) basal diet+150 ppm Avilamycin, 3) basal diet +100 ppm probiotic Protexin, 4) basal diet+200 ppm essential oil, and 5) basal diet+400 ppm Oregano essential oil. Table 1 presents the chemical composition of the basal diet (based on Arian requirement recommendations and formulated with UFFDA) and all chicks had free access to feed and water (*ad-libitum*). Chicks were raised under similar environmental conditions based on Arian management recommendations for 42 days (Yousefi et al., 2024). Before the beginning of the experiment, all birds were vaccinated for bronchitis and routine vaccinations (i.e., Newcastle {at days 7, 12, 19, and 26} and Gumboro {at days 16 and 23}) were done during the growing period.

**Table 1.** Ingredients and chemical composition of diets.

| Ingredients of diets (g $kg^{-1}$) | | | |
|---|---|---|---|
| | 1-14 | 14-28 | 28-42 |
| Corn | 486 | 457 | 455.5 |
| Wheat | 67.8 | 150 | 200 |
| Soybean meal (44%,CP) | 365 | 320 | 279 |
| Fish powder | 21 | 14 | 5 |
| 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 | 2.5 | 2.5 | 2.5 |
| Vitamin and mineral Premix* | 5 | 5 | 5 |
| Chemical compositions of diets | | | |
| ME (Kcal $kg^{-1}$) | 2851 | 2937 | 2965 |
| CP (%) | 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 |

* 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 pantotenate, 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, I (calcium iodate) 1 mg.

### Performance

Body weight (BW), feed intake (FI), feed conversion ratio (FCR), and mortality were recorded. During the trial, livability (Li) was calculated. Production efficiency factor was calculated by the following equation:

PI = (Li (%) × average BW (g) / FCR × trial days) / 10

### Jejunum morphology

At day 42 of the experiment, 4 male broilers from each experimental unit, with similar average weight were sacrificed by cervical dislocation to measure histopathological 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 24h and then the 10% buffered formalin solutions were renewed. Tissues were dehydrated by alcohol and then put into xylene and in paraffin. Then 5 cuts (5 µm diameter) were prepared by microtome. The paraffin sections were stained with hematoxylin-eosin (Thompson & Applegate, 2006). The values were measured by a light microscope (LEICA Queen 550 software). Measurements of villus height and 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 were obtained from the ileum of chicks mentioned above, and collected in sterile bags to count *Lactobacillus* (Lact) and *E. coli*. Digesta samples were homogenized with 1 mL serum physiologic. Five µL aliquot was mixed with blood agar and eosin methylene blue (EMB) and incubated at 37°C for 24h. After incubation, bacteria colonies were counted in selective agar media for enumeration of target bacterial groups. The microbial counts were determined as colony-forming units (CFU) per gram of wet samples (Hidayat et al., 2018).

### Heterophil to lymphocyte ratio (H/L)

On the 35$^{th}$ day of the experiment, blood samples of one male broilers from every replicate were taken in EDTA-contained syringes to evaluate heterophil-to lymphocyte ratio.

### Statistical analysis

The data were statistically analyzed based on a completely randomized design using the GLM procedure of SAS (SAS, 2003), and the means were compared using Duncen's multiple range test. Statistical model was 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)

In the present study, TOPSIS was applied to make a multiple attribute decision making (MADM) based on replacement of antibiotic and/or probiotic with essential oil. There are 5 steps in completing a MADM case with TOPSIS as follows:

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

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

$n_{ij}$ = the normalized value of the decision matrix
$a_{ij}$ = the original value of the decision matrix

**Step 2.** Create a normalized weighted decision matrix.

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

*V* = a weighted normalized decision matrix
*N* = weighting against criterion *i*
$W_{nxn}$= the 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)*

*Min* $y_{ij}$ = 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.

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

$di^{+}$ = 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

Regards to the percent of compounds in oregano essential oil (Table 2), o-cymene (29.15%), carvacrol (17.74%), and thymol (15.39%) are the main substances of the oregano essential oil, respectively. Chromatogram of the oregano essential oil is shown in Figure 1. Table 3 shows the deciding matrix to determine the best experimental treatment. In this table, a positive scale was considered for the traits in which higher figures were desired, and a negative scale was considered for the traits in which lower figures were desired. Using antibiotic or 200 ppm oregano essential oil caused the best FCR (1.67, 1.72 *vs.* 1.77). Also, adding antibiotic or 200 ppm oregano essential oil improved production index of the broilers (224, 208 *vs.* 188). The ratio of villus height/crypt depth was higher in the group consumed 200 ppm oregano essential oil *vs*. control group (7.59 *vs*. 6.44). The bacterial count of *E.Coli* was lower in the group consumed 200 0r 400 ppm oregano essential oil *vs*. control group (6.56, 6.15 *vs*. 7.57). Thus, using oregano essential oil had positive effect on the broiler's growth performance. Table 4 shows the normalization of the data of decision-making matrix to evaluate the optimum level of adding oregano essential oil in broiler chickens' diet. Regards to relative importance of the traits, relative weights which considered at Table 3, was taken into account for each trait and the obtained data to evaluate the weights of traits by entropy method is shown in Table 5. Then, regards to decision matrix, being positive or negative of positive or negative ideal ways were determined for each trait (Table 6). Determining the distance between the values and positive and negative ideal solutions to evaluate optimum level of adding the oregano 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 that its value is bigger is preferred compared to other items. Accordance to this table the birds that received antibiotic or oregano at the level of 400 ppm had the highest score regards to considered traits among the experimental treatments.

**Table 2.** Composition of active substances of oregano essential oil.

| Constituents | % | Constituents | % |
|---|---|---|---|
| α-Pinene | 1.55 | Carvacrol | 17.74 |
| Camphene | 2.12 | δ-Terpinene | 4.66 |
| β-Pinene | 0.49 | Linalool | 1.91 |
| β-Myrcene | 1.10 | Thymol | 15.39 |
| α-Terpinene | 1.27 | Caryophyllene | 1.27 |
| o-cymene | 29.15 | | |

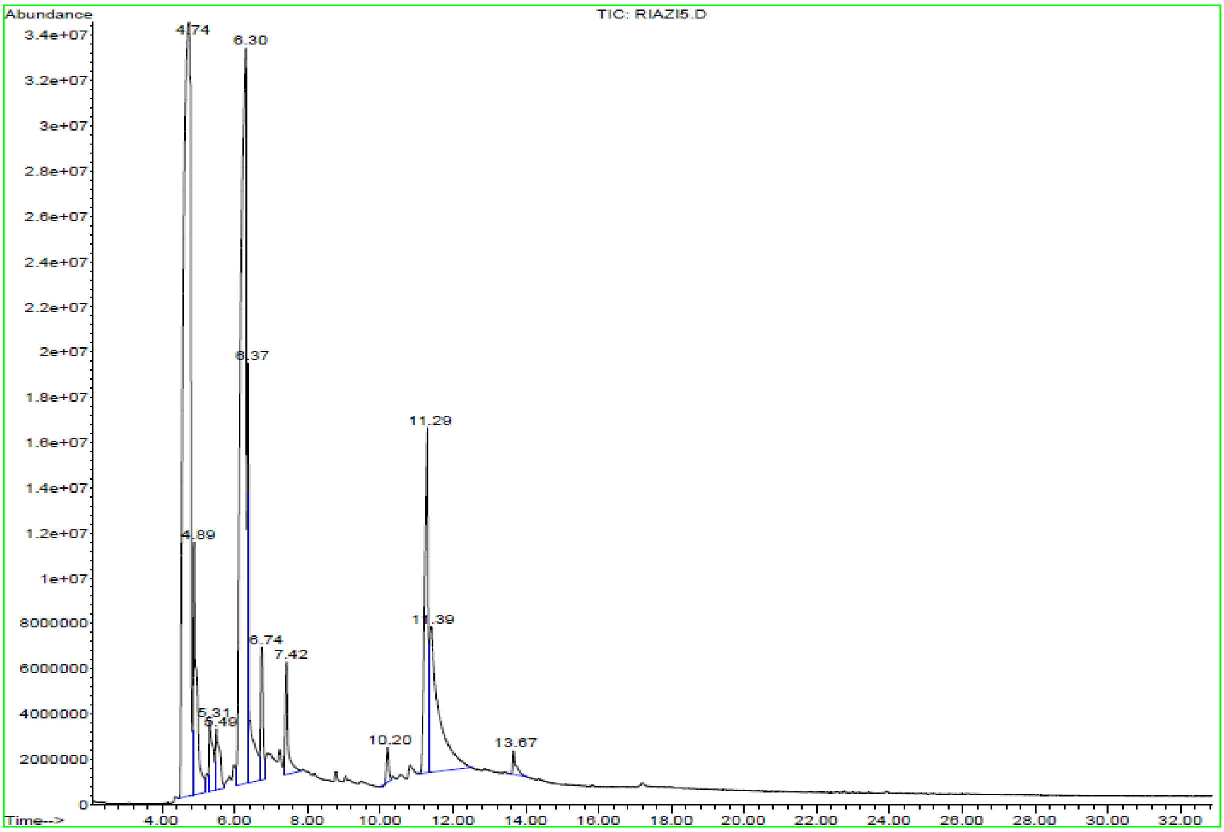


**Figure 1.** Chromatogram of the oregano essential oil.

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

| matrix | BW (g) | FI (g) | FCR | Li (%) | PI | H/L | V/C | Lact ($Log^{10}$) | Ecoli ($Log^{10}$) |
|---|---|---|---|---|---|---|---|---|---|
| control | 1882 | 3343 | 1.77 | 74.8 | 188 | 0.38 | 6.44 | 6.58 | 7.57 |
| antibiotic | 1849 | 3089 | 1.67 | 85.3 | 224 | 0.40 | 7.64 | 6.63 | 7.33 |
| probiotic | 1787 | 3329 | 1.87 | 72 | 169 | 0.40 | 7.11 | 6.54 | 6.46 |
| oregano 200 | 1835 | 3156 | 1.72 | 82 | 208 | 0.45 | 7.59 | 6.52 | 6.56 |
| oregano 400 | 1793 | 3367 | 1.79 | 85 | 196 | 0.38 | 6.99 | 6.50 | 6.15 |
| 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: heterophil to lymphocyte, V/C: Villus height to crypt depth; Lact: *Lactobacillus* bacteria; Ecoli: Ecoli bacteria.

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

| unscaled matrix | BW (g) | FI (g) | FCR | Li (%) | PI | H/L | V/C | Lact ($Log^{10}$) | Ecoli ($Log^{10}$) |
|---|---|---|---|---|---|---|---|---|---|
| control | 0.4600 | 0.4588 | 0.4492 | 0.4177 | 0.4253 | 0.4214 | 0.4018 | 0.4486 | 0.4941 |
| antibiotic | 0.4520 | 0.4239 | 0.4237 | 0.4769 | 0.5062 | 0.4402 | 0.4767 | 0.4525 | 0.4783 |
| probiotic | 0.4368 | 0.4569 | 0.4733 | 0.4026 | 0.3819 | 0.4480 | 0.4436 | 0.4462 | 0.4212 |
| oregano 200 | 0.4485 | 0.4331 | 0.4353 | 0.4585 | 0.4700 | 0.4966 | 0.4736 | 0.4450 | 0.4342 |
| oregano 400 | 0.4383 | 0.4621 | 0.4530 | 0.4752 | 0.4429 | 0.4258 | 0.4361 | 0.4437 | 0.4014 |

BW: body weight; FI: feed intake; FCR: feed conversion ratio; Li: livability; PI: production index; H/L: heterophil to lymphocyte, V/C: Villus height to crypt depth; Lact: *Lactobacillus* bacteria; Ecoli: Ecoli bacteria.

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

| weighted matrix | BW (g) | FI (g) | FCR | Li (%) | PI | H/L | V/C | Lact ($Log^{10}$) | Ecoli ($Log^{10}$) |
|---|---|---|---|---|---|---|---|---|---|
| control | 0.0460 | 0.0459 | 0.0449 | 0.0418 | 0.0425 | 0.0421 | 0.0402 | 0.0449 | 0.0494 |
| antibiotic | 0.0452 | 0.0424 | 0.0424 | 0.0477 | 0.0506 | 0.0440 | 0.0477 | 0.0452 | 0.0478 |
| probiotic | 0.0437 | 0.0457 | 0.0473 | 0.0403 | 0.0382 | 0.0448 | 0.0444 | 0.0446 | 0.0421 |
| oregano 200 | 0.0449 | 0.0433 | 0.0435 | 0.0458 | 0.0470 | 0.0497 | 0.0474 | 0.0445 | 0.0434 |
| oregano 400 | 0.0438 | 0.0462 | 0.0453 | 0.0475 | 0.0443 | 0.0426 | 0.0436 | 0.0444 | 0.0401 |

BW: body weight; FI: feed intake; FCR: feed conversion ratio; Li: livability; PI: production index; H/L: heterophil to lymphocyte, V/C: Villus height to crypt depth; DI: digestion index; Lact: *Lactobacillus* bacteria; Ecoli: Ecoli bacteria.

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

| best solution | BW (g) | FI (g) | FCR | Li (%) | PI | H/L | V/C | Lact (Log[10]) | Ecoli (Log[10]) |
|---|---|---|---|---|---|---|---|---|---|
| positive ideal | 0.0460 | 0.0424 | 0.0424 | 0.0477 | 0.0506 | 0.0421 | 0.0477 | 0.0452 | 0.0401 |
| negative ideal | 0.0437 | 0.0462 | 0.0473 | 0.0403 | 0.0382 | 0.0497 | 0.0402 | 0.0444 | 0.0494 |

BW: body weight; FI: feed intake; FCR: feed conversion ratio; Li: livability; PI: production index; H/L: heterophil to lymphocyte, V/C: Villus height to crypt depth, Lact: *Lactobacillus* bacteria; Ecoli: Ecoli bacteria.

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

| distance | positive | negative |
|---|---|---|
| control | 0.0162 | 0.0094 |
| antibiotic | 0.0080 | 0.0185 |
| probiotic | 0.0165 | 0.0097 |
| oregano 200 | 0.0094 | 0.0148 |
| oregano 400 | 0.0092 | 0.0156 |

**Table 8.** Calculation the closeness coefficient to positive ideal solutions and ranking the treatments to evaluate optimum level of oregano essential oil addition in broiler chickens.

| result | closeness coefficient |
|---|---|
| control | 0.3687 |
| antibiotic | 0.6991 |
| probiotic | 0.3707 |
| oregano 200 | 0.6128 |
| oregano 400 | 0.6274 |

## Discussion

In agreement with this result, Nilkanth et al. (2020) reported that applying oregano oil as phytobiotic growth promoter in broilers diet had a positive impact on intestinal microbiota, dry matter digestibility and nitrogen retention in broilers. It has been shown that both using oregano powder (Ri et al., 2017) and oregano essential oil (Alp et al., 2012) lead to better performance in broilers. Broilers fed with oregano oil at the level of 200 ppm had the lowest FCR. Unlike these results, Botsoglou et al. (2002) reported that adding oregano essential oil up to 200 ppm diet had no significant effect on broilers' performance. This discrepancy in results may be due to differences in the strain of the birds, dosage, and duration of using the supplement, concentration of effective substances, feed formulation, etc. Oregano oil at the level of 400 ppm caused higher livability compared to the control group. The digestion index of all groups improved compared to the control group, and the highest amount of DI was related to the group consumed antibiotic and oregano oil at the level of 200 ppm. Gul et al. (2019) reported that adding 600 ppm Oregano essential oil to basal diet increased villus height in laying hens. Also, it was found that supplementation of oregano extract increased the ileal villus height in quails (Behnamifar et al., 2018). Previous researchers have found that adding dietary oregano essential oil increased production of digestive enzymes and liver functions, which leads to higher digestibility of feed ingredient (Jamroz et al., 2005). Supplementation of oregano essential oil increased the ratio of gasterointestinal tract to whole body weight, empty gizzard and proventriculus weights that a higher capacity for digestion (Johnson et al., 2022). At present study, all dietary treatments decreased the *E. Coli* count, and the oregano at the level of 400 ppm caused the lowest count of *E. Coli* in the ileum. Parallel to these results, Giannenas et al. (2016) reported that adding 25 ppm oregano essential oil had positive effect on growth performance, intestinal microflora, and gut integrity of chickens. It was reported that oregano, thymol and carvacrol had positive effect on the intestinal barrier function, gut bacterial population and morphology (Cheng et al., 2018; Abdel-Latif et al., 2020). It was confirmed that carvacrol and thymol isolated from oregano affected many kinds of bacteria such as *E.Coli*, *Staphylococcus aureus* and *Streptococcus mutans* in-vitro (Liolios et al., 2009). Using oregano oil at the levels of 200 or 400 ppm increased production index of the birds compared to the control group. Lohakare and Abdel-Wareth (2022) reported that adding oregano bioactive lipid compounds at the level of 150 ppm improved livability and production efficiency factor of broiler chickens. Based on the results, oregano oil was appropriate as alternatives of probiotic in broilers diet. In decreasing amounts order, the main ingredients of oregano were o-cymene, carvacrol and thymol. Carvacrol is a monoterpenoid phenol (Abu-Lafi et al., 2008; Naghdi Badi et al., 2017). Limonene is a colorless liquid classified as a cyclic monoterpene (Hafeez et al., 2016). All of these materials are found naturally in many essential oils and used as medicines and food additives in humans and animals. Obviously, a mixture of these components is more effective than their sole usage (Adaszynska & Szczerbinska, 2016). Several studies have demonstrated the positive influence of phytobiotics on intestinal helath and microflora that directly can promote the birds´ performance (Amad et al., 2011; Hashemipour et al., 2013; Khaksar et al., 2012; Zhang et al., 2021).

## Conclusion

In conclusion, using the multidisciplinary decision management method has shown the effectiveness of the addition of oregano essential oil to broilers diet. Applying oregano essential oil at the level of 200 or 400 ppm of diet has the potential to be considered as probiotic or antibiotic alternative in broilers diet.

## Data availability

The datasets generated and/or analyzed during the current study on poultry nutrition are not publicly available as they have not been deposited in any public repository. However, the data are available from the corresponding author upon reasonable request to ensure transparency and reproducibility.

## Acknowledgements

The authors thank the Animal Science Research Institute of Iran (ASRI) staff for their support.

## Refrences

Abdel-Latif, H. M., Abdel-Tawwab, M., Khafaga, A. F., & Dawood, M. A. (2020). Dietary oregano essential oil improved the growth performance via enhancing the intestinal morphometry and hepato-renal functions of common carp (Cyprinus carpio L.) fingerlings. *Aquaculture*, *526*, 735432. https://doi.org/10.1016/j.aquaculture.2020.735432

Abou-Elkhair, R., Gaafar, K. M., Elbahy, N. M., Helal, M. A., Mahboub, H. D., & Sameh, G. (2014). Bioactive effect of dietary supplementation with essential oils blend of oregano, thyme and garlic oils on performance of broilers infected with Eimeria species. *Global Veterinaria*, *13*(6), 977-985.

Abu-Lafi, S., Odeh, I., Dewik, H., Qabajah, M., Hanus, L., & Dembitsky, V. (2008). Thymol and carvacrol production from leaves of wild Palestinian Majorana syriaca. Bioresource Technology, *99*(9), 3914-3918. https://doi.org/10.1016/j.biortech.2007.07.042

Adaszynska, M., & Szczerbinska, D. (2016). Use of essential oils in broiler chicken production – a review. *Annals of Animal Science*, *17*(2). https://doi.org/10.1515/aoas-2016-0046

Alagawany, M., Abd El-Hack, M. E., Farag, M. R., Shaheen, H. M., Abdel-Latif, M. A., Noreldin, A. E., & Patra, A. K. (2018). The usefulness of oregano and its derivatives in poultry nutrition. *World's Poultry Science Journal*, *74*(3), 463-474. https://doi.org/10.1017/S0043933918000454

Alp, M., Midilli, M., Kocabağlı, N., Yılmaz, H., Turan, N., Gargılı, A., & Acar, N. (2012). The effects of dietary oregano essential oil on live performance, carcass yield, serum immunoglobulin G level, and oocyst count in broilers. *The Journal of Applied Poultry Research*, *21*(3), 630-636. https://doi.org/10.3382/japr.2012-00551

Amad, A. A., Männer, K., Wendler, K. R., Neumann, K., & Zentek, J. (2011). Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. *Poultry science*, *90*(12), 2811-2816. https://doi.org/10.3382/ps.2011-01515

Avila Ramos, F., Pro Martinez, A., Sosa Montes, E., Narciso Gaytan, C., Hernandez Cazarez, A. S., Cibrian Tovar, J., Gallegos Sanchez, J., & Rodriguez Castillo, J. C. (2017). Oregano oil use in broiler diet increases accumulation of carvacrol and thymol in breast meat. *Acta Universitaria*, *27*(1), 34-39. https://doi.org/10.15174/au.2017.1074

Barfourooshi, H. J., Sadeghipanah, H., Asadzadeh, N., Hosseini, S. A., & Alizadeh-Ghamsari, A. H. (2023). Multi-attribute decision-making: Use of scoring methods to choose the best form of dietary fat supplement in pregnant Saanen goats. *Veterinary Medicine and Science*, *9*(6), 2912-2919. https://doi.org/10.1002/vms3.1298

Behnamifar, A., Rahimi, S., Karimi Torshizi, M. A., & Mohammad Zade, Z. (2018). Effect of chamomile, wild mint and oregano herbal extracts on quality and quantity of eggs, hatchability, and some other parameters in laying Japanese quails. *Journal of Medicinal Plants and By-products*, *7*(2), 173-180.

Botsoglou, N. A., Florou-Paneri, P., Christaki, E., Fletouris, D. J., & Spais, A. B. (2002). Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. *British poultry science*, *43*(2), 223-230. https://doi.org/10.1080/00071660120121436

Cheng, C., Xia, M., Zhang, X., Wang, C., Jiang, S., & Peng, J. (2018). Supplementing oregano essential oil in a reduced-protein diet improves growth performance and nutrient digestibility by modulating intestinal bacteria, intestinal morphology, and antioxidative capacity of growing-finishing pigs. *Animals*, *8*(9), 159. https://doi.org/10.3390/ani8090159

Dorman, H. D., & Deans, S. G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of applied microbiology*, *88*(2), 308-316. https://doi.org/10.1046/j.1365-2672.2000.00969.x

Eler, G., Gomes, A. V. C., Trindade, B. S., Almeida, L. S. L., Dilelis, F., Cardoso, V. S., & Lima, C. A. R. (2019). Oregano essential oil in the diet of broilers: performance, carcass characteristics, and blood parameters. *South African Journal of Animal Science*, *49*(4), 753-762. https://doi.org/10.4314/sajas.v49i4.17

Giannenas, I., Tzora, A., Sarakatsianos, I., Karamoutsios, A., Skoufos, S., Papaioannou, N., Anastasiou, I., & Skoufos, I. (2016). The effectiveness of the use of oregano and laurel essential oils in chicken feeding. *Annals of Animal Science*, *16*(3), 779-796. https://doi.org/10.1515/aoas-2015-0099

Gul, M., Yilmaz, E., Yildirim, B. A., Sezmis, G. Ü. R. K. A. N., Kaya, A., Timurkaan, S., Onel, S. E., & Tekce, E. (2019). Effects of oregano essential oil (Origanum syriacum L.) on performance, egg quality, intestinal morphology and oxidative stress in laying hens. *European Poultry Science/Archiv für Geflügelkunde*, *83*(290), 1-15. https://doi.org/10.1399/eps.2019.290

Hafeez, A., Manner, K., Schieder, C., & Zentek, J. (2016). Effect of supplementation of phytogenic feed additives (powdered vs. encapsulated) on performance and nutrient digestibility in broiler chickens. *Poultry Science*, *95*(3), 622-629. https://doi.org/10.3382/ps/pev368

Hashemipour, H., Kermanshahi, H., Golian, A., & Veldkamp, T. (2013). Effect of thymol and carvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities, and immune response in broiler chickens. *Poultry science*, *92*(8), 2059-2069. https://doi.org/10.3382/ps.2012-02685

Hidayat, M. N., Malaka, R., Agustina, L., & Pakiding, W. (2018). Effect of Lactobacillus sp. probiotics on intestinal histology, Escherichia coli in excreta and broiler performance. *Journal of the Indonesian Tropical Animal Agriculture*, *43*(4), 445–452. https://doi.org/10.14710/jitaa.43.4.445-452

Jamroz, D., Wiliczkiewicz, A., Wertelecki, T., Orda, J., & Skorupińska, J. (2005). Use of active substances of plant origin in chicken diets based on maize and locally grown cereals. *British poultry science*, *46*(4), 485-493. https://doi.org/10.1080/00071660500191056

Javaheri Barfourooshi, H., Sadeghipanah, H., Asadzadeh, N., Hosseini, S. A., & Alizadeh-Ghamsari, A. H. (2023). Multi-attribute decision-making: Use of scoring methods to choose the best form of dietary fat supplement in pregnant Saanen goats. *Veterinary Medicine and Science*, *9*(6), 2912-2919. https://doi.org/10.1002/vms3.1298

Johnson, A. M., Anderson, G., Arguelles-Ramos, M., & Ali, A. A. (2022). Effect of dietary essential oil of oregano on performance parameters, gastrointestinal traits, blood lipid profile, and antioxidant capacity of laying hens during the pullet phase. *Frontiers in Animal Science*, *3*, 1072712. https://doi.org/10.3389/fanim.2022.1072712

Khaksar, V., Van Krimpen, M., Hashemipour, H., & Pilevar, M. (2012). Effects of thyme essential oil on performance, some blood parameters and ileal microflora of Japanese quail. *The Journal of Poultry Science*, *49*(2), 106-110. https://doi.org/10.2141/jpsa.011089

Liolios, C. C., Gortzi, O., Lalas, S., Tsaknis, J., & Chinou, I. (2009). Liposomal incorporation of carvacrol and thymol isolated from the essential oil of Origanum dictamnus L. and in vitro antimicrobial activity. *Food chemistry*, *112*(1), 77-83. https://doi.org/10.1016/j.foodchem.2008.05.060

Lohakare, J., & Abdel-Wareth, A. A. (2022). Effects of Dietary Supplementation of Oregano Bioactive Lipid Compounds and Silver Nanoparticles on Broiler Production. *Sustainability*, *14*(21), 13715. https://doi.org/10.3390/su142113715

Lotfollahian, H., Tasharofi, S., Hossieni, S. A., Alizadeh-Ghamsari, A. H., & Tasharofi, S. (2023). Assessing of Peppermint as a Replacement for Antibiotic and Probiotic Using Technique for Order of Preference by Similarity to the Ideal Solution. *Iranian Journal of Applied Animal Science*, *13*(3), 527-534.

Naghdi Badi, H. A., Abdollahi, M., Mehrafarin, A., Ghorbanpour, M., Tolyat, M., Qaderi, A., & Yekta, M. (2017). An Overview on Two Valuable Natural and Bioactive Compounds, Thymol and Carvacrol, in Medicinal Plants. *Journal of Medicinal Plants*, *16*(63), 1-32.

Nilkanth, R. H., Vipin, K. K., & Kumar, P. (2020). Effect of feeding of oregano oil with probiotic on gut microbiota and nutrients digestibility of broiler chicken. *Journal of Pharmaceutical Innovation*, *9*(3), 660-664. https://doi.org/10.22271/tpi.2020.v9.i3l.4657

Peng, Q. Y., Li, J. D., Li, Z., Duan, Z. Y., & Wu, Y. P. (2016). Effects of dietary supplementation with oregano essential oil on growth performance, carcass traits and jejunal morphology in broiler chickens. *Animal Feed Science and Technology*, *214*, 148-153.

Pungky, T. K. A., Endang, S., & Alamsyah, A. (2018). Comparison between SAW and TOPSIS methods in selection of broiler chicken meat quality. *Scientific Journal of Informatics*, *5*(1), 90. https://doi.org/10.15294/sji.v5i1.14416

Ri, C. S., Jiang, X. R., Kim, M. H., Wang, J., Zhang, H. J., Wu, S. G., Bontempo, V., & Qi, G. H. (2017). Effects of dietary oregano powder supplementation on the growth performance, antioxidant status and meat quality of broiler chicks. *Italian Journal of Animal Science*, *16*(2), 246-252. https://doi.org/10.1080/1828051X.2016.1274243

Shroha A (2016). Effect of supplementation of ajwain (Trachyspermum ammi) On the performance of broiler chicken. http://krishikosh.egranth.ac.in/handle /1/5810022625.

Simitzis, P. E. (2017). Enrichment of animal diets with essential oils—a great perspective on improving animal performance and quality characteristics of the derived products. *Medicines*, *4*(2), 35.

Simitzis, P. E., & Deligeorgis, S. G. (2011). *The effects of natural antioxidants dietary supplementation on the properties of farm animal products*. Animal Feed: Types, Nutrition, Safety. Nova Science Publishers, Inc.

Thompson, K. L., & Applegate, T. J. (2006). Feed withdrawal alters small-intestinal morphology and mucus of broilers. *Poultry Science*, *85*(9), 1535-1540.

Vázquez, R. S., Meléndez, L. A. D., Estrada, E. S., Rodríguez Muela, C., Villalobos, G. V., Zamora, G. M., & Hume, M. E. (2015). Performance of broiler chickens supplemented with Mexican oregano oil (Lippia berlandieri Schauer). *Revista Brasileira de Zootecnia*, *44*(8), 283-289. https://doi.org/10.1590/S1806-92902015000800003

Yazdanpanah Goharrizi, L, & Tasharofi, S. (2017). Inhibiting Aspergillus flavus Growth and Aflatoxin Decrement in Aquatic Feed with Satureja hortensis Essential Oil. *Research Journal for Veterinary Practitioners*, *4*(4), 71-75. http://dx.doi.org/10.14737/journal.rjvp/2016/4.4.71.75

Yousefi, K., Allymehr, M., Talebi, A., & Tukmechi, A. (2024). A comparative study on the expression of myogenic genes, and their effects on performance and meat quality in broiler chicken strains. *Veterinary Research Forum*, *15*(5), 243-250. https://doi.org/10.30466/vrf.2024.2014542.4046

Zhang, L. Y., Peng, Q. Y., Liu, Y. R., Ma, Q. G., Zhang, J. Y., Guo, Y. P., Xue, Z., & Zhao, L. H. (2021). Effects of oregano essential oil as an antibiotic growth promoter alternative on growth performance, antioxidant status, and intestinal health of broilers. *Poultry science*, *100*(7), 101163. http://dx.doi.org/10.1016/j.psj.2021.101163