

Article

Effect of Essential Oil of Thyme (*Thymus vulgaris* L.) or Increasing Levels of a Commercial Prebiotic (TechnoMOS®) on Growth Performance and Carcass Characteristics of Male Broilers

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Simple Summary: Since the ban of in-feed antimicrobial with auxin purposes, a wide range of herbal plants have been tested as growth promoters. In particular, thyme and prebiotic compounds, such as mannan-oligosaccharides, have been tested in animal production due to their antibacterial, antifungal, and antioxidant properties and antimicrobial, anticoccidial, and anti-inflammatory actions. Thyme essential oils and TechoMOS® positively affected the growth performance of Ross male broiler during the grower phase, reducing the feed intake and improving feed conversion ratio. However, increasing dose of TechoMOS® only affected the broiler performance on a week-to-week basis and no difference has been registered between same dosage of TechoMOS® and TEO. Dietary treatments, both TechoMOS® and TEO, did not affect the carcass characteristics at 42 days.

Abstract: To investigate the effect of thyme (*Thymus vulgaris* L.) essential oil (TEO) or increasing inclusion of a prebiotic (TechnoMOS®) on growth performance and carcass characteristics of Ross 308 broilers, 400 one-day-old male broilers (43.5 g, as mean of body weight) were placed in 20 pens (2.0 × 1.0 m, with a floor area of 0.10 m² per bird) in groups of 20, and each pen cage was assigned to a specific dietary treatment (four replicates per each one). The dietary treatments included basic diet (no additive; CTR), basic diet including 0.025%, 0.075%, or 0.125% of TechnoMOS® (MOS025, MOS075, and MOS125, respectively), or basic diet including 0.075% thyme extract (TEO075). All dietary treatments were offered from the beginning of the study until the end of the trial. There were no effects of MOS or TEO on carcass characteristics. No significant effects of treatment on weight gain were obtained on a week-by-week basis; however, CTR birds gained less weight during the grower phase and overall compared with MOS birds. The same contrast for feed intake revealed that CTR birds had greater feed intake than MOS birds during both the grower phase and overall (492.18 g and 486.35 g, respectively). In conclusion, treated groups showed an improved feed conversion ratio.

Keywords: *Thymus vulgaris*; prebiotics; feed conversion ratio; growth; broiler

1. Introduction

The poultry sector is considered as the fastest growing and most flexible of all the livestock sectors [1] and is frequently subjected to various kind of stresses due to intensive production pressure at farming level [2]. According to the increase of consumer's demand of poultry products, the agricultural stage is faced with the need to improve the animal welfare conditions [3] and to reduce the environmental impact of the production cycle. Not least, in recent decades particular attention has been focused on reducing the use

of antibiotics [4], through the reduction of disease incidence, better feed efficiency, and feed conversion ratio. Optimizing broiler health, and in particular gastrointestinal health, is a critical aspect necessary to achieve optimum performance in the relatively short production cycle.

In-feed antimicrobials have been used for decades, generating further challenges such as the antibiotic-resistance in the human and domestic livestock and poultry microbiota, reflecting what Aminov [5] referred to as the “interminable adaptive capabilities of bacteria”.

The trend towards decreased use of historical antibiotic compounds in animal production, for prophylactic and growth-promotion purposes, was codified at European level in 2006 [6] leading to the adoption of a wide range of herbal plants, their extracts, essential oils, and purified components as replacements [7]. Moreover, a broad range of substances, including organic acids [8], mannan- and fructo-oligosaccharides, prebiotics [9], bacterial probiotics [10], enzymes [11–13], and synbiotics [14–16] have been tested under experimental and commercial conditions.

Thyme (*Thymus vulgaris* L.), an aromatic member of the *Lamiaceae* family, is a recognized source of essential oil with several food, feed, and medicinal applications. Indeed, in vitro experiments showed pharmacological activities [17]. Different plant species could be different in content and type of components [18]; however, thyme generally contains essential oil, carvacrol, flavonoids, and phenol compounds [19,20]. Regarding the thyme essential oil, this mainly contains oxygenated monoterpenes, monoterpene hydrocarbons, sesquiterpene hydrocarbons and oxygenated sesquiterpenes like thymol (10–64%) [11,21], as well as carvacrol (2–11%), γ -terpinene (2–31%) and p-cymene (10–56%) [11,22]. Thyme is also a source of several vitamins such as B-complex, folic acid, beta-carotene, vitamin A, K, E, and C, and minerals like potassium, calcium, iron, manganese, magnesium, and selenium [23].

Taken together, the composition of thyme essential oil leads to antiseptic, antibacterial, antifungal, and antioxidant properties and antimicrobial, anticoccidial, and anti-inflammatory actions [23,24]. Thyme essential oil has been shown to increase the production of digestive enzymes, which in turn improve the digestion of nutrients [25].

Another important feed additive is represented by prebiotic compounds. These substances are non-digestible small carbohydrate fragments, called oligosaccharides, and are composed of galactose, fructose, or mannose monosaccharides [26,27]. Prebiotics have gained increased attention as beneficial feed additives and have been reported to have a positive effect at gastrointestinal level mainly stimulating the growth of positive bacteria and reducing pathogenic bacteria [26,28–32]. Among prebiotic substances, mannan-oligosaccharides (MOS) have been extensively studied as an alternative to antibiotic growth promoters in broiler diets. In this regard, MOS are perceived as more ‘natural’, feasible, and affordable alternatives to traditional antibiotic growth promoters which do not incur the disadvantages of microbial resistance or contaminating residues [33]. MOS are typically derived from yeast outer cell walls which consist of proteins and carbohydrates including glucose, mannose, and N-acetylglucosamine. Briefly, the way of action of MOS involves provision of alternative attachment sites on MOS for pathogenic bacteria which prevents or reduces bacterial adhesion to intestinal enterocytes and the adhesion-induced pathogenesis [34–38]. Other mechanisms may include effects on villus height (uniformity and integrity) and immune system stimulation [39–44]. Additionally, MOS have been shown to effect gene expression in the jejunum which could contribute to their observed beneficial effects [45–47].

The objective of the present study was to investigate the effects of thyme extract and of three levels of a commercial prebiotic product (TechnoMOS[®]) on growth performance and carcass characteristics of male broiler chicks.

2. Materials and Methods

2.1. Animals, Housing, and Experimental Design

Four hundred one-day-old Ross 308 male broiler chickens (Aviagen, Newbridge, Scotland, UK) purchased from a commercial hatchery were involved in a 42-days study.

The birds were randomly allocated into 5 treatment groups, composed by 2 birds, with 4 replicates per each treatment. The broiler chickens were placed in 20 cages (2.0 × 1.0 m) with earth flooring (floor area of 0.10 m² per bird covered with paper roll litter) in a thermostatically controlled curtain side-wall poultry barn for the entire duration of the experiment.

The farming conditions were set according to the standard brooding practice for the rearing stages of the birds, as reported in *Ross Broiler Management Manual* [48].

Relative humidity was maintained between 55–65%. The initial temperature was kept at 32 °C, was decreased periodically to 24 °C at 3 weeks of age, and maintained until the end of the study. Constant light (23 W fluorescent tubes in ceiling fixtures) was provided on day 1, and then it was established at 23 h per day until the end of the study. Air circulation within the poultry barn was provided by tunnel ventilation.

2.2. Experimental Diets

A two-phase feeding program, according to Ross 308 catalogue recommendations (Aviagen, 2014), was used and consisted of provision of starter feed from the 1 to the 21 days of age, and of grower feed from the 22 to the 42 days of age (Tables 1 and 2) [49].

Table 1. Ingredient composition of diets during the starter (1–21 days of age) and grower (22–42 days of age) periods.

| Ingredient (%, Fresh Weight Basis) | Starter Period (1–21 Days of Age) | Finisher Period (22–42 Days of Age) |
|---|--------------------------------------|--|
| Corn | 54.32 | 58.69 |
| Soybean Meal | 39.43 | 31.87 |
| Corn oil | 2.16 | 5.83 |
| Mineral oysters | 0.90 | 0.79 |
| Dicalcium phosphate dihydrate | 2.05 | 1.68 |
| NaCl | 0.37 | 0.37 |
| DL-Methionine | 0.20 | 0.22 |
| Lysine-HCl | 0.07 | 0.05 |
| Vitamin and mineral premix [#] | 0.50 | 0.50 |
| Total | 100 | 100 |

[#] Supplied per kilogram of feed: vitamin A, 12,500 I.U.; vitamin D3, 1250IU; vitamin E 18IU; vitamin K3 3.7 mg; thiamine 1.8 mg; riboflavin 6.6 mg; calcium pantothenate 10 mg; niacin, 37.5 mg; pyridoxine, 32.5 mg; vitamin B12 2.5 mg; manganese 50 mg; zinc, 37.5 mg; iron, 25 mg, copper 7.5 mg.

Table 2. Proximate composition and specific nutrient analysis of diets during the starter (1–21 days of age), and grower (22–42 days of age) periods.

| Nutrient Analysis [#] | Starter Period (1–21 Days of Age) | Finisher Period (22–42 Days of Age) |
|------------------------------------|-----------------------------------|-------------------------------------|
| Metabolizable Energy (ME, kcal/kg) | 2900 | 3200 |
| Metabolizable Energy (ME, MJ/kg) | 12.13 | 13.38 |
| Crude protein (%) | 22.16 | 19.20 |
| Calcium (%) | 1.00 | 0.85 |
| Available Phosphorus (%) | 0.50 | 0.42 |
| DCAB (mEq/kg) [§] | 236 | 202 |
| Lysine (%) | 1.15 | 0.96 |
| Methionine (%) | 0.50 | 0.48 |
| Methionine + Cysteine (%) | 0.83 | 0.78 |
| Threonine (%) | 0.79 | 0.71 |

[#] Calculated nutrients (according to the National Research Council, 1994). [§] DCAB: dietary cation-anion balance, expressed as milliequivalents per kg.

The dietary treatments used were as follows: (1) basic diet (no additive; CTR); (2) basic diet including 0.025% (0.25 g/kg) TechnoMOS[®] (MOS025); (3) basic diet including 0.075% (0.75 g/kg) TechnoMOS[®] (MOS075); (4) basic diet including 0.125% (1.25 g/kg) TechnoMOS[®] (MOS125); (5) basic diet including 0.075% (0.75 g/kg) thyme extract (TEO075). All dietary treatments were offered from the beginning until the end of the trial.

The additives used in the current experiment were a commercial prebiotic product, TechnoMOS[®] (Biochem, Kuestermeyerstraße 16, D-49393, Lohne, Germany), and thyme essential oil extract product (Zarband Co., Tehran, Iran).

2.3. Growth Performance, Feed Intake and Carcass Characteristics

Body weight (BW) and feed intake was weekly recorded throughout the study for each replicate within each treatment. Average of feed intake, average weight gain, and feed conversion ratio (FCR) was also calculated.

At slaughter (42 d of age), 4 birds per replicate (16 birds per treatment) were randomly selected, weighed, and killed by cervical dislocation by qualified personnel. After slaughter and picking operations, the head and legs were removed. Broilers were eviscerated before determining the carcass components. Weights of defeathered body weight, breast, thighs, wings, abdominal fat, vertebrae, gastrointestinal (GIT), liver, heart, pancreas, spleen, and gizzard were also assessed. Furthermore, the eviscerated carcass weight was measured.

2.4. Statistical Analysis

Data were analyzed by SAS (version 9.2; Institute Inc., Cary, NC, USA) using the generalized linear model (GLM) procedure (completely randomized design with five treatments and four replicates per treatment). The post-hoc analysis was performed by Duncan test. Linear (L) and quadratic (Q) effects of increasing MOS inclusion levels were estimated. Contrasts were performed between CTR and MOS, CTR and TEO075, and between TEO075 and MOS075 to assess differences between CTR and MOS, between CTR and thyme, and between MOS and thyme inclusion at 0.75 g/kg, respectively. The significance was declared at $p \leq 0.05$.

3. Results

3.1. Growth Performance

Overall and weekly weight gains are reported in Table 3.

Table 3. Mean weekly weight gain (g) and mean weight gains (g) during the starter (1 to 21 days), grower (22 to 42 days), and overall (1 to 42 days) production periods for Ross 308 broilers offered increasing levels of a commercial prebiotic (TechnoMOS).

| Days | Dietary Treatments [#] | | | | | SD | p Value | Effects | | Contrasts | | |
|---------|---------------------------------|-----------------------|-----------------------|----------------------|-----------------------|--------|---------|---------|-------|------------|---------------|------------------|
| | CTR | MOS025 | MOS075 | MOS125 | TEO075 | | | L | Q | CTR v. MOS | CTR v. TEO075 | MOS075 v. TEO075 |
| 1–7 | 113.73 ^b | 108.97 ^{ab} | 108.25 ^{ab} | 109.88 ^{ab} | 107.60 ^a | 1.90 | 0.043 | 0.112 | 0.030 | 0.0086 | 0.0057 | 0.7373 |
| 8–14 | 212.12 | 208.19 | 222.06 | 210.21 | 219.57 | 8.42 | 0.421 | 0.769 | 0.311 | 0.8446 | 0.3900 | 0.7710 |
| 15–21 | 381.30 | 361.92 | 374.91 | 348.80 | 380.76 | 21.24 | 0.507 | 0.291 | 0.754 | 0.2803 | 0.9800 | 0.7863 |
| 22–28 | 539.35 | 548.93 | 518.78 | 523.87 | 500.07 | 45.60 | 0.843 | 0.610 | 0.908 | 0.8156 | 0.4023 | 0.6873 |
| 29–35 | 668.00 | 803.25 | 832.50 | 793.75 | 824.75 | 84.80 | 0.334 | 0.230 | 0.165 | 0.0585 | 0.0845 | 0.9284 |
| 36–42 | 543.50 ^a | 785.00 ^{ab} | 706.50 ^{ab} | 869.75 ^b | 747.00 ^{ab} | 94.20 | 0.040 | 0.014 | 0.684 | 0.0064 | 0.0473 | 0.6733 |
| Starter | 707.15 | 679.08 | 705.23 | 668.88 | 707.93 | 20.79 | 0.236 | 0.262 | 0.599 | 0.2000 | 0.9704 | 0.8982 |
| Grower | 1750.85 ^a | 2137.18 ^{ab} | 2057.78 ^{ab} | 2187.37 ^b | 2071.82 ^{ab} | 136.60 | 0.047 | 0.027 | 0.265 | 0.0042 | 0.0329 | 0.9195 |
| Overall | 2458.00 | 2816.25 | 2763.00 | 2856.25 | 2779.75 | 143.10 | 0.090 | 0.045 | 0.252 | 0.0085 | 0.0400 | 0.9084 |

[#] CTR: basic diet (control, no additive); MOS025: basic diet including 0.025% (0.25 g/kg) TechnoMOS[®]; MOS075: basic diet including 0.075% (0.75 g/kg) TechnoMOS[®]; MOS125: basic diet including 0.125% (1.25 g/kg) TechnoMOS[®]; TEO075: basic diet including 0.075% (0.75 g/kg) thyme extract (Zarband Co., Tehran, Iran). SD: Standard Deviation. ^{a,b} Different letters represent significant differences among challenge treatments ($p < 0.05$).

Diet treatments affect weight gain ($p = 0.043$) during the first (1 to 7 days) and the last (36 to 42 days) week of the trial. Specifically, dietary treatments showed a significant effect during the grower phase (22 to 42 days) of broilers. During the first week, weekly weight gains of CTR broilers resulted the greatest (113.73 ± 2.13 g), whereas for TEO075 group the lowest (107.6 ± 2.82 g; $p = 0.043$). In the last week, CTR group had the lowest value

of weight gain (543.50 ± 121.83 g) and MOS125 the greatest (869.75 ± 59.42 g; $p = 0.040$). Moreover, no significant differences have been found between MOS groups, MOS025 or MOS075, and TEO075 group. During the middle weeks of the trial, overall MOS or TEO treatments did not show effects on weight gains. Treatments affected weekly weight gain only during the grower phase ($p = 0.047$). CTR group showed the lowest weight gain (1750.85 ± 101.46 g) compared with the others treatment groups. Specifically, the treatment MOS125 led to the greatest weight gain (2187.37 ± 67.77 g).

The level of treatment showed a linear effect during the grower phase (22 to 42 days), during the week 36 to 42 ($p = 0.014$), and the overall period (1 to 42 days) ($p = 0.027$ and $p = 0.045$, respectively). A quadratic effect was registered only during the first week of trial ($p = 0.03$). Hence, the weight gain values of MOS and TEO075 groups resulted overall lower than CTR group in the period 1 to 7 days ($p = 0.0086$ and $p = 0.0057$, respectively) and 36 to 42 days ($p = 0.0064$ and $p = 0.0473$ respectively); likewise, a similar response was reached during the grower phase and the overall cycle period.

In addition, no differences were found between MOS075 and TEO075 for weekly weight gain or mean weight gains during the three phases of production.

3.2. Weekly Feed Intake

Feed intake data are reported in Table 4.

Table 4. Mean weekly feed intake (g) and mean feed intakes (g) during the starter (1 to 21 days), grower (22 to 42 days), and overall (1 to 42 days) production periods for Ross 308 broilers offered increasing levels of a commercial prebiotic (TechnoMOS[®]) or thyme essential oil.

| Days | Dietary Treatments [#] | | | | | SD | p Value | Effects | | Contrasts | | |
|---------|---------------------------------|----------------------|-----------------------|-----------------------|-----------------------|--------|---------|---------|--------|---------------|------------------|---------------------|
| | CTR | MOS025 | MOS075 | MOS125 | TEO075 | | | L | Q | CTR v. MOS | CTR v. TEO075 | MOS075 v. TEO075 |
| 1–7 | 141.50 ^b | 133.00 ^a | 132.50 ^a | 130.75 ^a | 129.75 ^a | 2.28 | <0.001 | 0.002 | 0.058 | 0.0001 | 0.0001 | 0.2458 |
| 8–14 | 300.75 ^a | 349.25 ^b | 355.25 ^b | 356.25 ^b | 352.00 ^b | 7.47 | <0.001 | <0.001 | <0.001 | <0.0001 | <0.0001 | 0.6696 |
| 15–21 | 630.00 | 585.75 | 582.44 | 609.04 | 572.44 | 22.97 | 0.138 | 0.411 | 0.011 | 0.0634 | 0.0248 | 0.6695 |
| 22–28 | 922.50 ^{bc} | 787.35 ^a | 819.80 ^{ab} | 937.36 ^c | 797.40 ^a | 51.50 | 0.024 | 0.329 | 0.006 | 0.0975 | 0.0282 | 0.6698 |
| 29–35 | 1221.25 ^b | 953.99 ^a | 1160.78 ^{ab} | 1168.31 ^{ab} | 1064.41 ^{ab} | 86.10 | 0.052 | 0.513 | 0.124 | 0.0910 | 0.0884 | 0.2804 |
| 36–42 | 1506.25 ^b | 1130.75 ^a | 1233.30 ^a | 1281.84 ^{ab} | 1201.16 ^a | 80.30 | 0.003 | 0.097 | 0.003 | 0.0005 | 0.0017 | 0.6945 |
| Starter | 1072.25 | 1068.00 | 1070.19 | 1096.04 | 1054.19 | 28.12 | 0.683 | 0.277 | 0.407 | 0.8032 | 0.5304 | 0.5778 |
| Grower | 3650.00 ^b | 2872.09 ^a | 3213.88 ^{ab} | 3387.51 ^{ab} | 3062.98 ^{ab} | 193.40 | 0.011 | 0.873 | 0.007 | 0.0071 | 0.0083 | 0.4473 |
| Overall | 4722.25 ^b | 3940.09 ^a | 4284.06 ^{ab} | 4483.55 ^{ab} | 4117.16 ^{ab} | 198.70 | 0.011 | 0.987 | 0.005 | 0.0090 | 0.0082 | 0.4141 |

[#] CTR: basic diet (control, no additive); MOS025: basic diet including 0.025% (0.25 g/kg) TechnoMOS[®]; MOS075: basic diet including 0.075% (0.75 g/kg) TechnoMOS[®]; MOS125: basic diet including 0.125% (1.25 g/kg) TechnoMOS[®]; TEO075: basic diet including 0.075% (0.75 g/kg) thyme extract (Zarband Co., Tehran, Iran). SD: Standard Deviation. ^{a–c} Different letters represent significant differences among challenge treatments ($p < 0.05$).

Average feed intake was significantly greater ($p < 0.001$) for CTR (141.50 ± 2.38 g) than for all other treatments from day 1 to 7 days.

By the second week (8 to 14 days), CTR birds had the lowest feed intake (300.75 ± 6.99 g; $p < 0.001$) compared to other treatment groups.

There were weekly differences in feed intake between treatments in the fourth week ($p = 0.024$), fifth week ($p = 0.052$), and sixth week ($p = 0.003$). In the fourth week, feed intake for MOS125 group was significantly ($p = 0.052$) greater (937.36 ± 38.33 g) than MOS025 and MOS075 and TEO075, whereas feed intake of MOS025 and TEO075 was significantly ($p = 0.052$) lower (787.35 ± 86.45 g and 797.40 ± 89.79 g) compared with CTR and MOS125 (922.50 ± 89.95 g and 937.36 ± 38.33 g, respectively). MOS treatment, both at 0.75 g/kg and 0.125 g/kg dose, was different from MOS025 and TEO075.

During the fifth and sixth weeks, weekly feed intake of CTR group was the greatest (1221.25 ± 78.78 g and 1506.25 ± 67.75 g, respectively), whereas for MOS025 group was the lowest (953.99 ± 176.06 g and 1130.75 ± 147.51 g, respectively) ($p = 0.052$). The greater feed intake of CTR broilers was achieved in the grower phase ($p = 0.011$), where it was greater ($p = 0.011$) than MOS025 but not different to MOS075, MOS125, or TEO075. Similarly, overall feed intake for CTR broilers was significantly ($p = 0.011$) greater than MOS025, but was not significantly different to MOS075, MOS125, or TEO075.

There were linear effects of increasing MOS inclusion in the first ($p = 0.002$) and second ($p < 0.001$) week. A quadratic effect of increasing MOS inclusion was observed in the third week ($p = 0.011$), fourth week ($p = 0.006$), and sixth week ($p = 0.003$). Increasing MOS inclusion had a quadratic effect on feed intake in the grower phase ($p = 0.007$) and overall ($p = 0.005$).

When contrasts were performed, it was observed that feed intake for CTR broilers was significantly greater than MOS broilers in the first ($p = 0.0001$) and last ($p = 0.0005$) week of the production cycle. Conversely, feed intake of CTR was lower ($p < 0.001$) than MOS in the second week. Feed intake of CTR was greater ($p = 0.011$) than MOS in the grower phase and overall ($p = 0.007$ and $p = 0.009$, respectively).

3.3. Weekly Feed Conversion

Feed conversion ratio (FCR) data are reported in Table 5.

Table 5. Feed conversion ratio (FCR, g/g), and mean FCR (g/g) during weeks 1 to 6 and the starter (1 to 21 days), grower (22 to 42 days), and overall (1 to 42 days) production periods for Ross 308 broilers offered increasing levels of a commercial prebiotic (TechnoMOS®) or thyme essential oil.

| Days | Dietary Treatments # | | | | | SD | p Value | Effects | | Contrasts | | |
|---------|----------------------|-------------------|--------------------|--------------------|--------------------|------|---------|---------|-------|------------|---------------|------------------|
| | CTR | MOS025 | MOS075 | MOS125 | TEO075 | | | L | Q | CTR v. MOS | CTR v. TEO075 | MOS075 v. TEO075 |
| 1–7 | 0.90 | 0.87 | 0.87 | 0.852 | 0.86 | 0.02 | 0.135 | 0.028 | 0.769 | 0.0355 | 0.0372 | 0.4418 |
| 8–14 | 1.20 ^a | 1.34 ^b | 1.31 ^{ab} | 1.34 ^b | 1.30 ^{ab} | 0.04 | 0.033 | 0.026 | 0.148 | 0.0026 | 0.0339 | 0.9326 |
| 15–21 | 1.43 | 1.48 | 1.43 | 1.54 | 1.41 | 0.05 | 0.152 | 0.080 | 0.295 | 0.2366 | 0.6655 | 0.6486 |
| 22–28 | 1.55 | 1.46 | 1.49 | 1.65 | 1.48 | 0.07 | 0.096 | 0.036 | 0.010 | 0.7492 | 0.3348 | 0.9071 |
| 29–35 | 1.64 ^b | 1.36 ^a | 1.46 ^{ab} | 1.58 ^{ab} | 1.41 ^{ab} | 0.09 | 0.029 | 0.827 | 0.003 | 0.0243 | 0.0184 | 0.6090 |
| 36–42 | 1.89 ^b | 1.38 ^a | 1.54 ^a | 1.55 ^{ab} | 1.47 ^a | 0.11 | 0.005 | 0.043 | 0.006 | 0.0006 | 0.0020 | 0.5221 |
| Starter | 1.43 | 1.48 | 1.43 | 1.54 | 1.41 | 0.05 | 0.152 | 0.080 | 0.295 | 0.2366 | 0.6655 | 0.6486 |
| Grower | 2.09 ^b | 1.35 ^a | 1.59 ^a | 1.55 ^a | 1.50 ^a | 0.15 | 0.002 | 0.017 | 0.006 | 0.0002 | 0.0010 | 0.5275 |
| Overall | 1.89 ^b | 1.38 ^a | 1.54 ^a | 1.55 ^a | 1.47 ^a | 0.11 | 0.005 | 0.043 | 0.006 | 0.0006 | 0.0020 | 0.5221 |

CTR: basic diet (Control, no additive); MOS025: basic diet including 0.025% (0.25 g/kg) TechnoMOS®; MOS075: basic diet including 0.075% (0.75 g/kg) TechnoMOS®; MOS125: basic diet including 0.125% (1.25 g/kg) TechnoMOS®; TEO075: basic diet including 0.075% (0.75 g/kg) thyme extract (Zarband Co., Tehran, Iran). SD: Standard Deviation. ^{a,b} Different letters represent significant differences among challenge treatments ($p < 0.05$).

There were differences in FCR between treatments in the second ($p = 0.033$), fifth ($p = 0.029$), and sixth week ($p = 0.005$). In the second week, CTR value was significantly lower than MOS025 and MOS125 (1.20 ± 0.06) ($p = 0.033$). In the fifth week, CTR group was significantly higher than MOS025, which in turn was found to be the lowest (1.64 ± 0.07 g and 1.36 ± 0.12 g). During the sixth week, the CTR group (1.89 ± 0.04 g) was found to be significantly higher than MOS025, MOS075, and TEO075 (1.38 ± 0.14 g, 1.54 ± 0.21 g and 1.47 ± 0.24 g, respectively). Furthermore, the CTR group showed greater FCR during both the grower phase (2.09 ± 0.09 , $p = 0.002$) and overall period (1.89 ± 0.04 , $p = 0.005$) compared with MOS and TEO treatments.

There were linear effects of dietary treatments inclusion during the first, second, fourth, and sixth week ($p = 0.028$, 0.026 , 0.036 , and 0.043) as well as during the grower phase and overall ($p = 0.017$ and 0.043). Quadratic effect of treatments inclusion was observed in the fourth, fifth and sixth week ($p = 0.010$, 0.003 , and 0.006) and during the grower phase and overall ($p = 0.006$).

Contrast analysis revealed a treatment effect both for the MOS and TEO075 during the first ($p = 0.0355$ and $p = 0.0372$, respectively), second ($p = 0.0026$ and $p = 0.0339$, respectively), fifth ($p = 0.0243$ and $p = 0.0184$, respectively), and sixth week ($p = 0.0006$ and $p = 0.0010$, respectively).

3.4. Carcass Characteristics

Carcass characteristics are presented in Table 6.

Table 6. Carcass characteristics on the 42nd day of age of Ross 308 broilers offered increasing levels (0.25, 0.75, or 1.25 g/kg of diet) of a commercial prebiotic (TechnoMOS®) or thyme essential oil (0.75 g/kg diet).

| Variable | Dietary Treatments # | | | | | | p Value | Effects | |
|-----------------------------|----------------------|---------|---------|---------|---------|-------|---------|---------|-------|
| | CTR | MOS025 | MOS075 | MOS125 | TEO075 | SD | | L | Q |
| Live weight (g) | 2662.50 | 2545.00 | 2552.50 | 2573.75 | 2722.50 | 104.2 | 0.385 | 0.541 | 0.389 |
| Defeathered body weight (g) | 2097.50 | 1965.5 | 2003.75 | 2031.25 | 2136.25 | 87.3 | 0.327 | 0.729 | 0.297 |
| Eviscerated carcass (%) | 61.82 | 60.09 | 60.56 | 61.47 | 60.44 | 1.143 | 0.534 | 0.939 | 0.185 |
| Breast weight (g) | 582.75 | 526.25 | 542.00 | 546.50 | 550.25 | 37.9 | 0.671 | 0.589 | 0.358 |
| Thighs weight (g) | 488.50 | 464.50 | 438.25 | 494.50 | 478.25 | 23.98 | 0.192 | 0.891 | 0.021 |
| Wings weight (g) | 153.50 | 150.50 | 156.25 | 150.00 | 150.00 | 8.30 | 0.922 | 0.875 | 0.675 |
| Abdominal fat (g) | 45.75 | 52.75 | 45.00 | 42.00 | 61.2 | 12.16 | 0.539 | 0.491 | 0.665 |
| Vertebrae (g) | 375.25 | 356.75 | 358.75 | 353.00 | 388.75 | 16.28 | 0.199 | 0.301 | 0.626 |
| GIT weight (g) | 380.75 | 338.75 | 345.75 | 353.50 | 369.25 | 24.12 | 0.430 | 0.511 | 0.245 |
| Liver weight (g) | 75.50 | 69.75 | 68.25 | 74.75 | 76.75 | 7.55 | 0.740 | 0.988 | 0.250 |
| Heart weight (g) | 16.50 | 15.50 | 15.25 | 16.00 | 16.75 | 1.565 | 0.852 | 0.803 | 0.425 |
| Pancreas weight (g) | 5.75 | 6.25 | 6.50 | 5.25 | 6.75 | 0.856 | 0.443 | 0.454 | 0.094 |
| Spleen weight (g) | 3.25 | 2.75 | 3.50 | 3.25 | 3.25 | 0.447 | 0.573 | 0.534 | 0.931 |
| Gizzard weight (g) | 69.25 | 68.75 | 73.00 | 74.50 | 74.00 | 5.91 | 0.792 | 0.273 | 0.995 |

CTR: basic diet (Control, no additive); MOS025: basic diet including 0.025% (0.25g/kg) TechnoMOS®; MOS075: basic diet including 0.075% (0.75g/kg) TechnoMOS®; MOS125: basic diet including 0.125% (1.25g/kg) TechnoMOS®; TEO075: basic diet including 0.075% (0.75g/kg) thyme extract (Zarband Co., Tehran, Iran). SD: Standard Deviation.

There was no significant effect of treatment on any carcass characteristics. There was only a significant quadratic effect ($p = 0.021$) of MOS inclusion on thigh weight.

4. Discussion

Several studies investigated and reported thyme supplementation effects on blood parameters, growth performance, gut integrity and content, and carcass characteristics of broilers [49–60]. Specifically TechnoMOS® was also tested, either by using it alone or symbiotically with probiotics, and confirmed a positive effect on performance (i.e., weight gain, FCR) [61–63]. Abudabos et al. and Murshed et al. [61,62], unlike our study, used different doses according to the cycle phase and obtained better performance testing MOS alone compared to its combination with probiotics. It is known that animal requirements and digestive capacity, according to digestive enzyme production and intestinal mucosa development, varies according to the animal age [64,65]. These could influence the effective required dose. Moreover, farm management, feeding strategies, and pen hygiene could also affect the animal performance. These factors may modify the impact of MOS dietary inclusion.

From the present study, it could be pointed out that feed additive inclusion may change slightly during the production cycle. However, the latter can only be assessed when parameters are measured weekly. During the first week of treatment, no differences were detected for FCR values, even though weekly feed intake and weight gain were affected. This could be explained by the small quantity of feed consumed during this period. In this study the overall effect of treatments mainly occurred during the grower phase and overall period, and this was in line with previous studies.

Sojoudi et al. [66] used an increasing level of MOS supplementation (TechnoMOS®) in the diet of Ross 308 broilers. Authors reported that total feed intake, total consumed energy (kcal), and total consumed protein were significantly higher than control group when MOS was included in the basic diet at the lowest inclusion of 0.1% or at 0.15%. Thus, since inclusion of MOS likely represents a significant component of the cost of feed, if no additional benefits or efficiencies can be gained by including levels above 0.1%, it does not make economic sense to constrain inclusion to that level.

In a meta-analysis of broiler chicken pen trials evaluating MOS over a decade (1993–2003), Hooze [39] reported that MOS has a positive effect, improving average body weight and reducing FCR and mortality incidence; moreover, authors suggested that the optimal recommended MOS inclusion level in broiler diets is 0.2% between 0 and 7 days, 0.1% between 7 and 21 days, and 0.05% between 21 and 42 days, implying that scope exists to calibrate inclusion levels, a rationale based on a number of factors. In the present study, the inclusion levels used were similar, even though lower (for 0.025% and 0.075%) than

those used by Sojoudi et al. [67], and not different from those recommended by Hooge [39]. The highest inclusion level, 0.125%, was slightly higher than the level used by Sojoudi et al. [67].

Our findings regarding TEO effect showed more effectiveness than MOS treatment at the same dosage. However, MOS also showed a significant effect on weekly feed intake during the second and third weeks. Previous studies confirmed that TEO had positive effect on immune functions [54,68], FCR, and growth [56,69]. In our study TEO was fed only at 0.75 g/kg dose, but Attia et al. and Wade et al. [56,69] suggested that the optimal dose should be set at 1 g/kg; Zhu et al. [68] in contrast, found the optimal dose range to be 0.1 to 0.25 g/kg.

In the present study, significant effects of treatment on weight gain were largely absent on a week-by-week basis, but a contrast between CTR and MOS for weight gain revealed that CTR broilers gained less weight than MOS during the grower phase and overall. The same contrast for feed intake revealed that CTR broilers had greater feed intake than MOS broilers during both the grower phase and overall. Thus, it can be speculated that MOS supplementation led to a better feed efficiency, indicating a beneficial effect of MOS compared with CTR. A similar trend was also observed for TEO, also implying a positive effect of this feed supplement for feed efficiency. Even though a cost-benefit analysis is beyond the aim of the present study, clearly an economic assessment of MOS and TEO use can be faced and taken into account with local availability and the price of prebiotic or bioactive feed additives.

It is widely known that physiological effects of MOS and TEO are mediated by their effects on the chicken intestinal microbiota. The interaction of the gut microbiota with host physiology, both locally in the gut and in more systemic ways, is now emerging as a core concern in livestock production [70,71]. It is also noteworthy that the chicken gut microbiota changes during the production cycle [72]; indeed, the microbiota during early production reflect the maternal influence [73,74] and subsequent changes reflect diet and environment [75]. Thus, if the purpose of supplementing with prebiotic feed additives is to modify performance and health parameters, the use of these compounds should take into account the microbial community dynamics with the aim of being able to have a greater effect. For instance, the fact that MOS broilers gained 376.58 g more weight than CTR broilers during the grower phase (between days 22 and 42) and only 353.83 g more weight than the CTR broilers over the entire production cycle suggests that beneficial effects of MOS supplementation are time dependent. Temporal variation has been observed in the chicken intestinal microbiome [76] and, thus, mediation of such time-dependent effects of prebiotic feed additives on the physiology of the host broilers could be speculated from the present study.

Takebaveh et al. [77] showed that prebiotics and probiotics promoted the Lactobacilli population in the intestinal broiler microbiota, and also efficacy on challenged broiler with *Salmonella* spp. or *Clostridium perfringens*, promoting the surface area of intestinal villi and immune parameters [78,79].

Although there were positive effects on production performance in the present study, there were no effects on carcass characteristics measured, which was in line with data reported by Salehimanesh et al. and De Souza et al. [80,81]. On the contrary, Tayeri et al. [82] showed effects on carcass composition with symbiotic use of probiotics and prebiotics and Ahmadian et al. [83] using thyme essential oil.

5. Conclusions

In conclusion, taking together all the results, the tested prebiotic at different dosage and thyme essential oil affected the grower and overall growth performance of male Ross broiler during a 42-day trial. Considering that the dosage was not modified during the entire trial, the weekly response was different and with a marked action during the grower phase. The latter suggests that the effectiveness could be linked with the feed and feed additive intake. The main effect, of both MOS and TEO, revealed better weight gain, feed

intake, and feed conversion ratio but no improvements on carcass traits compared with control group.

Even though average weight gain and feed intake have been influenced by treatments, no differences in feed conversion ratio had been found between treatments. Moreover, at similar dose level, MOS and TEO treatments did not show differences. Probably a different dosage should be used according mainly to the week of life, in order to reach a greater effect from a performance but also economic standpoint.

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