# Thymol, alpha tocopherol, and ascorbyl palmitate supplementation as growth enhancers for broiler chickens

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**ABSTRACT** Consumer concern on the quality of products and animal welfare has greatly increased during the past decades. Dietary synthetic antibiotic products used as growth promoters have been restricted or banned in many countries. Edible plants, essential oils, or their main components were suggested as natural feed supplements to improve growth, products' quality, and welfare-related parameters. Thymol (THY), a main component of oregano essential oil, has been proved as an effective antimicrobial and antioxidant compound. Tocopherol (TOC) evidenced antioxidant activity with potential as a growth promoter and a synergic antioxidant activity between TOC and ascorbyl palmitate (AP) has also been reported. Herein, we evaluated whether broiler diet supplementation with THY, and THY with a formulation mix containing TOC and AP (1:0.5:0.5, respectively) have potential as growth enhancers under commercial conditions. Potential protective effects against foot pad dermatitis and hock burns were also evaluated. Newly hatched male broiler chicks with similar body weight (BW) were randomly assigned to 1 of 7 groups (4 replicates each) as follows: Basal (no feed supplements added), Promotor (Basal + 6.26 $\mu$mol flavomycin/kg feed), BHT (Basal + 1.33 mmol of buthylated hidroxytoluene (BHT)/kg feed), Prom-BHT (Basal + 6.26 $\mu$mol flavomycin/kg feed + 1.33 mmol of BHT/kg feed), TOC-AP (Basal + 0.67 mmoles of TOC + 0.67 mmoles of AP/kg feed), THY (Basal + 1.33 mmoles of THY/kg feed), and THY-TOC-AP (Basal + 0.67 mmoles of THY + 0.67 mmoles of a mix 1:1 of TOC-AP). Along 7 wk, BW, feed intake, and feed conversion ratio were evaluated. Skin injuries were assessed at 35 d of age. At the end of the study (42 d), compared to Basal group, similarly enhanced final BW were observed in all groups but TOC-AP. No main differences between groups were detected in feed intake, feed conversion ratio, or skin injuries. Findings suggest that THY itself or in combination with TOC-AP may have value as a natural growth enhancer alternative for broilers.

**Key words:** broiler chicken, diet, tocopherol, thymol, vitamin E

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## INTRODUCTION

Consumer concern on the quality of meat products and animal welfare has greatly increased during the past decades (Min and Ahn, 2005). In fact, "quality" and "healthfulness" were reported to be important factors influencing consumer's choice for foods. Consequently, for example, dietary synthetic antibiotics products used as growth promoters have been restricted or even banned in many countries including the US (AccessScience, 2018). In the same line, a trend to search for natural feed alternatives to synthetic compounds is being observed (Zeng et al., 2015; Ezzat Abd El-Hack et al., 2016; Luna et al., 2017). In this regard, edible plants, herbs spices, and essential oils have been suggested as non-traditional natural feed supplements in broiler diets to improve growth performance, products quality, and even their welfare.

Although several beneficial effects have already been mentioned when supplementing plant extract mixtures, their provision at industry levels appears still difficult to implement. On the other hand, single natural compounds, such as thymol, are nowadays commercially synthesized and distributed, and therefore can be easily incorporated in the birds' diets with potential beneficial effects. Thymol have already shown

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interesting bioactive properties as for example modifying the bacterial cell membrane permeability (Lambert et al., 2001), reacting with lipid and hydroxyl radicals converting them into stable products (Yanishlieva et al., 1999) and reducing birds' fear responses probably through its activity as GABAergic modulator (Garcia et al., 2006; Labaque et al., 2013; Delgado-Marín et al., 2017). Hashemipour et al. (2013) also indicated that the use of thymol as feed additive stimulates digestive enzyme activity, and it is mentioned that it might induce a higher secretion of bile acids. On the other hand, other antioxidants as alpha tocopherol (**TOC**) have also been suggested as potential growth promoters (Giannenas et al., 2005; Yesilbag et al., 2011). Considering that a synergic antioxidant activity was recently reported between TOC and ascorbyl palmitate (**AP**) (Bodoira et al., 2017), it is likely that a dietary supplement containing a combination of these compounds with thymol could have added value for bird's performance.

The objective of the present study was to assess whether feeds supplemented with thymol and a formulation mix with TOC and AP can improve broiler growth performance under commercial conditions. The effects of treatments were also compared with the effects of flavomycin, an antibiotic that was widely used as growth promoter in the poultry industry (Butaye et al., 2003), and also with BHT, still nowadays widely used as antioxidant but also with proved antibiotic properties (Turcotte and Saheb, 1978; Mastelić et al., 2008). Because thymol has shown strong antimicrobial effects, and this property can alter the production of litter ammonia and consequently affect the development of foot pad dermatitis (**FPD**) and hock burn (**HB**) (skin ulcerations affecting the plantar surface of the feet and the hock, respectively) (Kjaer et al., 2006; Haslam et al., 2007; Elson, 2015), this study also explored whether the groups supplemented with thymol can also show a reduced prevalence of FPD and HB.

## MATERIALS AND METHODS

### *Animals and Husbandry*

One thousand one hundred and twenty newly hatched male Cobb-500 broilers weighing 43.35 ± 1 g were obtained from a commercial hatchery (INDACOR S.A.) and reared to 42 d (age at slaughter) at a farm operated by INDACOR S.A. Upon arrival, birds were individually weighed, and randomly housed in one of 28 pens measuring 2.4 × 1.2 m. Birds were identified with leg or wingbands according to their age. All birds within each pen were randomly assigned to 1 of 7 dietary groups (4 replicates each) as follows: (1) Basal (no feed supplements added), (2) Promotor (Basal + 6.26 $\mu$mol flavomycin/kg feed), (3) BHT (Basal + 1.33 mmol of BHT/kg feed), (4) Prom-BHT (Basal + a mix of 6.26 $\mu$mol flavomycin/kg feed and 1.33 mmol of BHT/kg feed), (5) TOC-AP (Basal + 0.67 mmol of TOC + 0.67 mmol of AP/kg feed), (6) THY (Basal + 1.33 mmol of THY/kg feed), and (7) THY-TOC-AP (Basal + 0.67 mmol of THY + 0.67 mmol of a mix 1:1 of TOC-AP). The 6.26 $\mu$mol of flavomycin, 1.33 mmol of BHT, 0.67 mmol of TOC, 0.67 of AP, and 1.33 mmol of THY are respectively equivalent to 10, 293, 288, 278, and 200 mg/kg of feed. The mentioned 0.67 mmol of TOC is equivalent to 430.75 UI of d-$\alpha$-tocopherol. The Basal, Promotor, BHT, Prom-BHT, and TOC-AP groups were used as controls. THY and THY-TOC-AP were considered the experimental groups. Doses were selected based on previous reports (Luna et al., 2010, 2017) and the actual promoter and BHT doses used in the farm. All supplements but flavomycin were chosen to achieve the same final (1.33) molar concentration.

**Table 1.** Feed composition of each basal experimental diet where dietary supplements were added.

| Composition of basal diets (kg %) | | | |
|---|---|---|---|
| Compound | Starter | Grower | Finisher |
| Maize | 47.6 | 51.2 | 61.67 |
| Soybean pellets | 34.3 | 28.6 | 17.55 |
| Soybean | 8.7 | 13.50 | 13.17 |
| Animal meal | 4.75 | 1.50 | 3.25 |
| Crude soy oil | 2.5 | 2.50 | 1.95 |
| DL Methionine 88% (liq.) | 0.55 | 0.47 | 0.37 |
| Salt | 0.32 | 0.32 | 0.3 |
| Emulsifier | 0.25 | 0.25 | 0.03 |
| L-Lysine HCL | 0.22 | 0.24 | 0.22 |
| Bisodium/Bisulfate Butyrate | 0.2 | 0.3 | 0.3 |
| Mineral Premix[1] | 0.15 | 0.15 | 0.1 |
| Vitamin Premix[2] | 0.15 | 0.12 | 0.1 |
| L-Threonine | 0.12 | 0.1 | 0.07 |
| Acidity reg. (sodium lactate) | 0.1 | 0.15 | 0.2 |
| Choline chloride | 0.07 | 0.07 | 0.07 |
| Limestone powder | – | 0.52 | 0.6 |
| Calculated to contain | | | |
| Crude protein, % | 22.2 | 19.9 | 18.2 |
| ME, Kcal/kg | 12.5 | 12.6 | 12.8 |
| Calcium, % | 8.90 | 8.70 | 7.70 |
| Phosphorous (total) | 6.8 | 6.0 | 6.0 |

[1]Vitamin premix provided the following amounts per kilogram of diet: vitamin D3, 200 IU; vitamin A, 1.500 IU; vitamin E, 101 IU; niacin, 35 mg; D-Pantothenic acid, 14 mg; riboflavin, 4.5 mg; pyridoxine, 3.5 mg; menadione, 2 mg; folic acid, 0.55 mg; thiamine, 1.8 mg.

[2]Mineral premix provided the following amounts per kilogram of diet: Mn, 11.0%; Zn, 11.0%; Fe, 6.0 %; I, 2.0 ppm; Mg, 2.68%; Se, 600 ppm.

The feeding regimen consisted of a starter (1–10 d), grower (11–26 d), and finisher (27–42 d) diet. The ingredients and chemical composition of the basal diets are shown in Table 1. Added supplements were dissolved in soybean oil, sprayed to the basal diet, and homogenized prior to extrusion. Prepared feeds were stored between 1 and 3 wk before its consumption to minimize feed deterioration (Luna et al., 2017). Water and feed were supplied ad libitum throughout the experiment. Rice husk was used for bedding material. When birds were 21 d old, stocking density in each pen was randomly reduced to 32 birds per pen.

It is important to mention that animals of the present experiment were raised with research objectives exclusively, and were not commercialized.

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## Variables Measured

Along the experimental period (7 wk), body weight per bird (BW), and feed consumption per pen was registered weekly. Feed intake (**FI**) per bird was estimated from feed consumption per pen as: feed consumption per pen/number of birds per pen. Feed conversion ratio (**FCR**) was calculated for the whole study as: $FI_{0\text{–}42d}/BW_{42d}$.

At 35 d, foot pads and hocks were visually inspected to evaluate FPD and HB respectively following Welfare Quality (Welfare Quality®, 2009) recommendations. The presence of injuries was assessed with regard of severity scale and scored in categories from 0 to 4. A 0 score represented no evidence of FPD or HB, scores 1 and 2 represented minimal evidence of lesions, and scores 3 and 4, clearly evidenced lesions. Both pads and hocks were scored but just the higher value was registered per bird. If the feet were dirty, they were gently hand cleaned before scoring. For FPD only the central plantar was scored.

## Statistical Analysis

Analyses were performed through an "R" (The R Foundation for Statistical Computing) user-friendly interface implemented in InfoStat (Di Rienzo et al., 2016). BW and FI were analyzed with the General Linear Mixed Models (GLMM) procedure of INFOSTAT. The dietary treatment (each diet provided) and the birds' age (0, 7, 14, 21, 28, 35, 42 wk) were included as fixed effects and the pen identity was included as random effects. Data from skin injuries (% of incidence related to severity) and FCR were also analyzed with GLMM, but the model did not included bird's age. Means were compared for significant differences at $P \leq 0.05$, by applying Fisher test with a Bonferroni correction.

# RESULTS

BW, FI, and FCR of male broiler chickens fed with the experimental diets along 7 wk are shown in Table 2. While no main effects of the dietary treatments were detected on the FI and the FCR ($P = 0.75$ and $P = 0.46$, respectively) an interaction effect between dietary treatment and age ($P < 0.001$) was found on BW. In this regard, post hoc analysis showed that no significant differences were observed between groups until 35 d of age. However, at 42 d of age, compared to Basal group, similarly enhanced final BW were observed in all groups but TOC-AP ($P < 0.05$).

Registered means at 35 d of age for FPD and HB within groups ranged from 1.55 and 1.89, and 0.79 and 1.19 respectively, with no dietary treatment effects detected ($P > 0.65$ in both cases).

**Table 2.** Mean ± SE of body weight, feed intake, and feed conversion ratio of male broiler chickens fed with the experimental diets along 7 wk.

| Age (d) | Basal | PROM | BHT | PROM-BHT | TOC-AP | THY | THY-TOC-AP |
|---|---|---|---|---|---|---|---|
| *BW (g)* | | | | | | | |
| 1 | 43.30 ± 0.05 | 43.38 ± 0.05 | 43.32 ± 0.05 | 43.37 ± 0.05 | 43.36 ± 0.05 | 43.32 ± 0.05 | 43.39 ± 0.05 |
| 7 | 142.48 ± 1.58 | 145.6 ± 1.55 | 143.01 ± 1.6 | 147.49 ± 1.54 | 135.22 ± 1.62 | 142.79 ± 1.56 | 148.57 ± 1.57 |
| 14 | 424.02 ± 4.15 | 425.09 ± 4.08 | 411.4 ± 4.2 | 398.81 ± 4.04 | 408.45 ± 4.25 | 404.17 ± 4.1 | 435.51 ± 4.12 |
| 21 | 870.56 ± 8.66 | 854 ± 8.53 | 881.20 ± 8.76 | 875.08 ± 8.46 | 842.29 ± 8.87 | 872.79 ± 8.56 | 901.54 ± 8.63 |
| 28 | 1482.18 ± 12.8 | 1494.62 ± 12.55 | 1575.36 ± 12.96 | 1545.74 ± 12.65 | 1519.33 ± 13.12 | 1517.6 ± 12.65 | 1548.66 ± 12.75 |
| 35 | 2206.68 ± 16.79 | 2262.61 ± 16.59 | 2266.6 ± 16.99 | 2215.20 ± 16.72 | 2200.42 ± 17.13 | 2239.21 ± 16.65 | 2233.83 ± 16.59 |
| 42 | $2937.28^{b}$ ± 24.83 | $3047.85^{a}$ ± 24.64 | $3009.36^{a}$ ± 25.23 | $2999.87^{a}$ ± 25.03 | $2941.89^{b}$ ± 25.44 | $3062.09^{a}$ ± 24.73 | $3009.22^{a}$ ± 25.23 |
| *FI (g)* | | | | | | | |
| 0 to 7 d | 190.09 ± 6.32 | 189.41 ± 6.32 | 195.45 ± 6.32 | 204.42 ± 6.32 | 178.79 ± 6.32 | 196.57 ± 6.32 | 198.32 ± 6.32 |
| 8 to 14 d | 273.85 ± 9.57 | 271.93 ± 9.57 | 272.61 ± 9.57 | 283.48 ± 9.57 | 266.91 ± 9.57 | 284.73 ± 9.57 | 292.52 ± 9.57 |
| 15 to 21 d | 558.10 ± 20.28 | 567.14 ± 20.28 | 610.57 ± 20.28 | 586.14 ± 20.28 | 550.86 ± 20.28 | 567.30 ± 20.28 | 606.76 ± 20.28 |
| 22 to 28 d | 983.41 ± 53.36 | 967.31 ± 53.36 | 958.13 ± 53.36 | 958.78 ± 53.36 | 1088.53 ± 53.36 | 865.65 ± 53.36 | 1001.60 ± 53.36 |
| 29 to 35 d | 1384.57 ± 49.25 | 1359.96 ± 49.25 | 1426.18 ± 49.25 | 1432.56 ± 49.25 | 1462.98 ± 49.25 | 1459.39 ± 49.25 | 1398.03 ± 49.25 |
| 36 to 42 d | 1623.38 ± 73.30 | 1658.71 ± 73.30 | 1720.34 ± 73.30 | 1669.06 ± 73.30 | 1714.77 ± 73.30 | 1692.75 ± 73.30 | 1750.95 ± 73.30 |
| 0 to 42 d | 4988.41 ± 125.09 | 5014.47 ± 125.09 | 5183.27 ± 125.09 | 5134.44 ± 125.09 | 5271.67 ± 125.09 | 5116.39 ± 125.09 | 5273.19 ± 125.09 |
| *FCR (FI/BW)* | | | | | | | |
| 0 to 42 d | 1.70 ± 0.04 | 1.68 ± 0.04 | 1.72 ± 0.04 | 1.71 ± 0.04 | 1.76 ± 0.04 | 1.69 ± 0.04 | 1.75 ± 0.04 |

Basal = no feed supplements added; PROM = Basal + 6.26 μmol flavomycin/kg feed; BHT = Basal + 1.33 mmol of BHT/kg feed; Prom-BHT = Basal + a mix of 6.26 μmol flavomycin/kg feed and 1.33 mmol of BHT/kg feed; TOC-AP = Basal + 0.67 mmoles of TOC + 0.67 mmoles of AP/kg feed; THY = Basal + 1.33 mmoles of THY/kg feed; THY-TOC-AP = Basal + 0.67 mmoles of THY + 0.67 mmoles of a mix 1:1 of TOC-AP.

[a,b]Means within a row with no common superscripts differ significantly at $P < 0.05$.

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## DISCUSSION

This study evaluated whether broiler diet supplementation with THY and THY with a formulation mix including TOC and AP under commercial conditions has potential as growth enhancers for broilers. Whereas no main effects were detected on FI and FCR, compared to Basal control group, final BW increases were evidenced in all feed supplement groups but TOC-AP. Increased BW as consequence of feed supplementation with thymol mixtures, essential oils or plants containing thymol as main component (oregano or thyme between others) were also reported previously. Hashemipour et al. (2016) informed increases in BW supplementing broilers fed on wheat-based diet with a mixture of thymol plus carvacrol. Hafeez et al. (2016) has also reported increased BW for broilers supplemented with a powder mixture of thymol, carvacrol, and limonene, and similar results were also observed by Vazquez et al. (2015) supplementing broilers chickens with Mexican oregano oil. Recently, Ocel'ová et al. (2016) has demonstrated the important absorption of small lipophilic molecules as THY in gastrointestinal track of broiler chickens. It has been also proposed that THY modulate intestinal microbial counts in broilers (Hashemipour et al., 2016), and possibly the cause of the growth promoting effect observed in our study is in that line. However, more research is needed in order to clarify the detailed mechanism of THY, either through its antimicrobial and/or its antioxidant activity. Considering that BHT has a relatively low antimicrobial activity (Mastelić et al., 2008), it is possible that the observed growth enhancing effect is not only related to its antimicrobial properties, but also to the antioxidant activity. On the other hand, the absence of effect of the TOC-AP dietary group, although consistent with the results informed by Yesilbag et al (2011) showing that 200 mg of alpha TOC/kg feed did not affect male broilers BW at 42 d, suggests that just an antioxidant activity would not be enough to induce a growth enhancing effect. Nevertheless, as suggested by Lee et al (2003), potential effects of dietary supplements may be masked when birds are fed with a well-balanced diet and/or are reared in clean environments. In that study, Lee et al. (2003) reported no effect of THY supplementation on female broilers performance measured at 28 d of age. Thus, our results are consistent with their findings and also complement them since we found that the dietary supplementation effects only became statistically evident at the end of the study (42 d of age). The BW increase observed in groups supplemented with flavomycin, a widely known antibiotic with growth promoter activity, was expected. Interestingly, the flavomycin increases also became evident only in the last week of the study. The growth enhancer effects were evident in the last week of the study and may be due to a cumulative effect (inducing a divergent growth) during developing. It is important to highlight that flavomycin, BHT, flavomycin + BHT, and THY-TOC-AP supplemented groups showed the same BW increase magnitude than that observed in the THY supplemented group. Furthermore, since the TOC-AP supplementation did not influence broilers BW, the observed effect in the THY-TOC-AP supplemented group might suggest that only 100 mg of THY/kg feed could be enough to induce a growth enhancer effect at 42 d age. However, possible interactive effects between THY, TOC, and AP should not be discarded.

Like our study, Hafeez et al. (2016) and Yesilbag et al. (2011) reported no differences in FI as consequence of dietary supplementation when respectively supplemented with a mixture containing THY, carvacrol and limonene or rosemary, rosemary volatile oil and alpha TOC to broilers. The absence of changes in FI reported in our study suggests that the supplements used did not affect feeding behavior.

This study also explored whether the THY as a feed supplement may also have protective effects against FPD and HB skin injuries. As it was mentioned above, those lesions are of high relevance regarding welfare and profitability of intensive broiler production systems (Dawkins et al., 2017). Compounds with antimicrobial effects can also alter the production of litter ammonia and consequently affect the development of FPD and HB (Elson, 2015). However, in the present study, although an incidence of minor lesions was observed, those scores were not related to the different dietary treatments applied. Nevertheless, the lesions incidence was observed in a relatively clean environment, and the questions of whether the different dietary treatments can help to reduce skins lesions in more challenging environments or with different bedding material still remain open.

In conclusion, the present results suggest that THY itself or in combination with TOC-AP may be a natural growth enhancer alternative for broiler production. Furthermore, previous findings showed that THY had similar antioxidant effects to synthetic BHT both on feed mash and meat lipid oxidation (Luna et al., 2010, 2017). Thus, considering these results collectively, THY could be considered a useful natural alternative for poultry feed formulations that combines growth enhancer effects while helping to sustain the quality of poultry feed and meat products.

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