

EFFECT OF PHYTOGENES (THYMOL AND TRANS-CINNAMALDHYDE) ON GROWTH PERFORMANCE AND CECAL BACTERIAL POPULATIONS IN BROILERS

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ABSTRACT

A 35-d trial was conducted to study the effect of phytogetic additives on growth performance and cecal bacterial populations of broilers. The feeding program consisted of a starter diet until 21-d and a grower diet until 35-d. There were 4 treatment groups: control, 50g EO (essential oil)/ ton, 100g EO/ton and 150g EO/ton. Slight non significant improvements have been observed for body weight, weight gain, feed intake and feed conversion ratio in the starter stage when birds fed diets contained 50g EO/ton. In both grower stage and through out the entire period of growth study no differences in growth parameters have been observed when birds fed diets contained 100g EO/ton and those fed the control diet. The worst performance has been observed by the birds fed diets contained 150 EO/ton through the whole stages. Yet, in general, there were no significant difference in growth performance between birds fed the control diet and those fed diets supplemented with EO. No mortality occurred during the whole period of the growth study. Using a commercial blend from thymol and trans-cinnamaldehyde revealed a disparate impact on cecal microflora population. Yet, this was not reflected on the bird growth performance. This may be referred to the balanced diet and controlled condition under which the trial was conducted. The present data suggest, further investigation should focus on the mechanism of EO, the ratio between components of the blend and their effect on bird performance under uncontrolled conditions such as (heat stress, unbalanced dietetc.) by increasing levels of EO.

Keywords: Phytogetic additives, Performance, Cecal microflora, Broilers.

INTRODUCTION

Livestock performance and feed efficiency are closely related with the qualitative and quantitative microbial load of the host animal. Poultry possess a limited natural resistance and immunity against colonization or infection by potentially pathogenic microorganisms (Huyghebaert, 2005).

Antimicrobial feed additives have made a tremendous contribution to the profitability in the intensive husbandry, providing people with healthy and nutritious poultry products (Huyghebaert, 2005).

The use of antibiotics in animal nutrition, as anti-microbial growth promoters (AGP), has been without doubt beneficial for the improvement of zootechnical performance parameters and prevention of diseases. However, bio-security threats for human and animal health, arising from the escalating resistance of pathogens to antibiotics and accumulation of antibiotic residues in animal products and the environment, call for a worldwide removal of antibiotics from animal diets. The European Union has banned completely the use of antibiotics as (AGP) since 2005 (Reglamento, (Council Europeo

(2005). As a result, the demand for alternative products to antibiotics that can be used as prophylactic and as growth promoting agents is very high.

Ongoing research highlights the beneficial potential of various microbes and bioactive materials like probiotics, prebiotics, enzymes, organic acids and phytogenic compounds, in improving animal performance and health. Phytogenic compounds refer to the utilized parts of various aromatic herbs and spices as well as to their respective extracts in the form of essential oils (EO) (Kamel, 2000 and Windisch *et al.* 2008).

The EO consists basically of two classes of compounds, the terpenes and phenylpropenes; thymol is the most important terpenes, while trans-cinnamaldehyde is the most important phenylpropenes.

According to Helander *et al.* (1998) and Hammer *et al.* (1999), thymol display antimicrobial activity against intestinal microbes such as *Colstridium perfringers*, *Salmonella typhimurium* and *E.coli*. Lee *et al.* (2004a) suggested that dietary essential oils may act not only on intestinal microflora, but also on nutrient utilization. El-Ghousein and Al-Beitawi (2009) recommended supplementing broiler rations with 1.5% or 2.0% of crushed thyme as natural growth promoter. Chang *et al.* (2001) stated that cinnamaldehyde had antibacterial activity against *E.coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Klebsiella pneumoniae*, *Salmonella sp.*, and *Vibrio parahemolyticus*. According to Michiels *et al.* (2009) Trans-cinnamaldehyde was very effective against coliform at low doses, while it hardly inhibited *lactobacilli*. However, Jang *et al.* (2007) stated that, the effect of EO on gastrointestinal microflora is not consistent, even though EO has been generally recognized as an anti-microbial agent.

It was reported by (Bölükbaşı *et al.* 2006; Al-Kassie, 2009; Calislar *et al.* 2009; EL-Ghousein and Al-Beitawi 2009 and Scheuermann *et al.* 2009) that using EO caused significant improvement in growth performance and nutrient utilization in broiler. While (Lee *et al.* 2003; Hernández *et al.* 2004; Grilli *et al.* 2006; Jang *et al.* 2007; Muhl and Liebert, 2007 and Isabel and Santos 2009) stated that EO didn't cause any improvement in growth performance and nutrient utilization.

Accordingly, the aim of the current study was to make further investigations on the quantification of commercial mixture of phytogenic feed additives (thymol and cinnamaldehyde) and assess their effects on growth performance, and cecal bacterial populations in broilers

MATERIALS AND METHODS

Experimental system and chicks

Four hundred and sixty eight chicks (ROSS 308) one-day-old were brought from a commercial farm (El-Wadee). The trial was carried out at a poultry house in Nubaria, Regional Center for Food and Feed, Alexandria, Egypt. The chicks were randomly divided into four groups, each group composed of nine replicates and each replicate contained 13 chicks per pen. The trial was conducted under controlled lighting period (24hrs.).

Diets formulation and growth study

Four graded levels; (zero control), 50ppm, 100ppm and 150ppm of the commercial EO (Enviva™ EO 101 G) were added to both starter (23% crude protein) and grower (22% crude protein) experimental diets. The diets were prepared according to management recommendation guide data (ROSS). Starter and grower diets were both iso-caloric (3000 and 3100 Kcal. ME/ Kg diet, respectively) (Table 1).

Table (1): Composition of dietary treatments on as-fed basis.

Ingredients (%) and compositions	Starter (1-21 days)				Grower (22-35 days)			
	Control	5g E.O.	10g E.O.	15g E.O.	Control	5g E.O.	10g E.O.	15g E.O.
Ground yellow, corn.	58.035	58.030	58.025	58.020	57.000	56.995	56.990	56.985
Soybean meal (47 % CP).	28.000	28.000	28.000	28.000	28.000	28.000	28.000	28.000
Corn gluten meal (60 % CP).	8.500	8.500	8.500	8.500	8.500	8.500	8.500	8.500
Essential oil ^a	—	0.005	0.010	0.015	—	0.005	0.010	0.015
Vegetable oil.	1.140	1.140	1.140	1.140	2.678	2.678	2.678	2.678
Di-calcium phosphate.	2.180	2.180	2.180	2.180	1.890	1.890	1.890	1.890
Limestone.	0.707	0.707	0.707	0.707	0.620	0.620	0.620	0.620
Vit.&Min. Mixture ^b	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400
Cholinchlorid	0.082	0.082	0.082	0.082	0.075	0.075	0.075	0.075
Salt	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300
L-lysine HCL ^c	0.429	0.429	0.429	0.429	0.317	0.317	0.317	0.317
DL-Methionine ^c	0.227	0.227	0.227	0.227	0.220	0.220	0.220	0.220
Total	100	100	100	100	100	100	100	100
Calculated values%								
Crude protein	23	23	23	23	22	22	22	22
Metabolizable energy (Kcal/Kg)	3070	3070	3070	3070	3160	3160	3160	3160
Lysine	1.400	1.400	1.400	1.400	1.300	1.300	1.300	1.300
Methionine.	0.600	0.600	0.600	0.600	0.570	0.570	0.570	0.570
Methionine +Cystine	1.040	1.040	1.040	1.040	1.000	1.000	1.000	1.000
Calcium.	1.000	1.000	1.000	1.000	0.900	0.900	0.900	0.900
Available Phosphorus.	0.500	0.500	0.500	0.500	0.450	0.450	0.450	0.450

^a Essential oil; Enviva™ EO 101 G, mixture of thymol and cinnamaldehyde min 180g/kg, Maltodextrin (carrier).

^b Vitamin-mineral mixture supplied per kg of diet; Vit. A, 12000 IU Vit. D₃ 2000 IU Vit. E, 10mg Vit. K, 2mg Vit. B₁, 1mg Vit. B₂, 5mg Vit. B₆, 1.5mg Vit. B₁₂, 10ug Biotin, 50ug Choline chloride, 500mg Pantothenic acid, 10mg Niacin, 30mg Folic acid, 1mg Manganese, 60mg Zinc, 50mg Iron, 30mg Copper, 10mg, Iodine, 1mg Selenium, 0.1mg Cobalt, 0.1mg.

^c Lysine and Methionine were added according to management recommendation guide data (ROSS).

Calcium and available phosphorus were adjusted using di-calcium phosphate and limestone. Vitamins and trace minerals were added to cover broiler's requirements.

Birds were fed the respective starter (1-21 days) and grower (22-35 days) diets *ad libitum* and had free access to water for the entire experimental period. Body weight and feed intake were recorded at the end of starter and

grower stages. Feed conversion ratio, weight gain and mortality percentages were calculated at the end of the trial.

Microbial enumeration

At the fifth week of the trial, ten random birds from each treatment were slaughtered to enumerate *Salmonella*, *Campylobacter*, *Bifidobacterium*, *Clostridium*, *E.coli*, *Lactic acid bacteria* and anaerobic bacterial count.

To enumerate the bacterial content of the ceca, immediately after slaughtering ceca were removed following the method of Xiang *et al.* (2002). The samples of the fresh cecal contents were diluted 10-folds by weight in buffered peptone (peptone 1g/l, NaCl 8.5 g/l) then serially diluted in 0.85% sterile saline solution. Enumeration of each bacterium was performed using specific media, incubation temperatures and durations (ISO 1986, 1998, 2002, 2003, 2010 and Andrew *et al.* 2003) for *Clostridium*, *Lactic acid bacteria*, anaerobic bacterial count, *Salmonella*, *Campylobacter* and *Bifidobacterium*, consequently. Four measurements were taken for each bacterial species.

Statistical analysis

The data obtained were subjected to a one way analysis of variance using the linear model (GLM) of SAS (SAS institute, 1991). Means were compared using Duncan's new multiple range test ($P < 0.05$) (Duncan, 1955).

RESULTS

Growth performance and mortality percentage

Effect of supplementing broiler diets with commercial EO on growth performance is shown in (Table 2), where in the starter stage slight non significant improvements in growth parameters have occurred (feed intake, body weight, body weight gain) when birds fed diets contained 50g EO/ton comparing to those fed the control diet. No differences in feed conversion were detected between birds fed diets contained 50g and 100g EO/ton and those fed the control diet. In the grower stage and through out the entire period of growth study, no differences have been observed in body weight and body weight gain between birds fed the control diet and those fed diet contained 100g EO/ton. The worst growth performance was for the group of birds fed diet contained 150g EO/ton in both starter stage and grower stage. No mortality occurred during the whole period of the growth study.

Microflora enumeration

Supplementing broiler diets with commercial EO showed disparate impact on the bacterial cecal populations (Table 3).

Whereas, birds fed diets supplemented with the commercial EO have shown a reduction in the *E.coli* counting nearly by one \log_{10} compared to those fed the control diet. Supplementing diet with 150g EO/ton significantly ($***P < 0.001$) reduced the count of anaerobic bacteria, *Bifidobacter*, *Colestridium*, and *Salmonella*. While, supplementing diet with 100g EO/ton has reduced the count of anaerobic bacteria and *Colestridium*. However, *Bifidobacter* and *Salmonella* were weakly influenced by this dose. *Lactobacilli* counting were not influenced by the dietary supplementation with EO.

Table (2): Effect of supplementing boiler diets with commercial EO on growth performance

Parameters (g)	0-21 days				22-35 days				0-35 days			
	Control	50g EO/ton	100g EO/ton	150g EO/ton	Control	50g EO/ton	100g EO/ton	150g EO/ton	Control	50g EO/ton	100g EO/ton	150g EO/ton
Body weight	748 ±16.33	762 ±23.21	746 ±29.55	715 ±41.63	1680 ±75.11	1663 ±50.71	1681 ±79.77	1625 ±77.42	1680 ±75.11	1663 ±50.71	1681 ±79.77	1625 ±77.42
Body weight gain	703 ±16.33	717 ±23.21	701 ±29.59	670 ±41.63	932 ±78	901 ±37.08	935 ±55.56	910 ±43.93	1635 ±75.11	1618 ±50.70	1636 ±79.77	1580 ±77.42
Feed intake	1145 ±58.53	1152 ±51.01	1135 ±47.78	1126 ±46.62	1853 ±107.43	1830 ±109.72	1868 ±139.20	1822 ±41.30	2998 ±136.46	2976 ±135.13	3009 ±163.62	2949 ±71.53
Feed conversion ratio	1.62 ±0.09	1.61 ±0.10	1.62 ±0.07	1.68 ±0.10	1.99 ±0.13	2.03 ±0.11	1.99 ±0.06	2.00 ±0.08	1.83 ±0.04	1.84 ±0.09	1.84 ±0.04	1.87 ±0.08

There was no significant effect of the treatment on the parameters ($P>0.05$).

Table (3): Effect of boiler diets supplementation with commercial EO on cecal bacterial populations.

Treatment	<i>E.coli</i> (Cfu/g)	Anaerobic Bacteria (Cfu/g)	<i>Lactobacilli</i> (Cfu/g)	<i>Bifidobacter</i> (Cfu/g)	<i>Colestridium</i> (Cfu/g)	<i>Salmonella</i> (Cfu/g)
Control	51x 10 ^{5a} ±5x10 ⁵	59 x 10 ^{6a} ±5x10 ⁶	105	275 x 10 ^{4ab} ±15x10 ⁴	28.75 x 10 ^{3a} ±12.5x10 ³	28.55x10 ^{5a} ±1.6 x10 ⁵
50g EO/ton	80 x 10 ^{4b} ±5x10 ⁴	54 x 10 ^{6a} ±5x10 ⁶	105	297.5 x 10 ^{4a} ±7.5x10 ⁴	23.75 x 10 ^{3ab} ±13.75x10 ³	28.65x10 ^{5a} ±2 x10 ⁵
100g EO/ton	81 x 10 ^{4b} ±5x10 ⁴	38 x 10 ^{6b} ±5x10 ⁶	105	270 x 10 ^{4b} ±30x10 ⁴	12.5 x 10 ^{3b} ±7.0 x 10 ³	21.5x10 ^{5ab} ±8 x10 ⁵
150g EO/ton	19 x 10 ^{4c} ±5x10 ⁴	22 x 10 ^{6c} ±5x10 ⁶	105	163.5 x 10 ^{4c} ±27x10 ⁴	ND	10x10 ^{5b} ±5 x10 ⁵

Results are means ± S.E. (n = 3); there was no significant effect of treatment on *Lactobacilli* (P>0.05).

¹ Cfu, Colony forming unit.

ND, not detected

DISCUSSION

Earlier studies have proved that EO have antimicrobial and antioxidant properties (Ultee *et al.* 2002; Valero and Salmeron, 2002 and Shan *et al.* 2005). These properties have elucidated the idea of using EO as growth promoters. The beneficial effect of growth promoter substances is related to a more efficient use of nutrients, which in turn results in an improved feed conversion ratio (Devriese *et al.* 1993).

In the current study, even though the dietary factor EO supplementation had an effect on the bacterial cecal populations; yet, this effect was not significant on improving growth performance and nutrient utilization by the birds. This agrees with the findings of Lee *et al.* (2003), Hernández *et al.* (2004), Grilli *et al.* (2006), Jang *et al.* (2007), Muhl and Liebert, (2007) and Isabel and Santos (2009) who reported that no improvement in growth performance and feed utilization have occurred when EO was used as dietary additive. Adversely, Bölükbaşı *et al.* (2006), Al-Kassie (2009), Calislar *et al.* (2009), EL-Ghousein and Al-Beitawi (2009) and Scheuermann *et al.* (2009) reported an improvement in growth performance and feed utilization by broilers.

The contradiction here might be referred to the possibility that the effect of EO may be masked by diet composition and/or environment, in that no effect of EO on growth performance was seen when a well-balanced diet was fed and the birds were kept in clean environment, as in the case of the present trial.

This claim agree with Lee *et al.* (2003) who stated that there was no significant effects on feed intake, body weight gain and feed conversion when thymol, cinnamaldehyde and commercial formulation were added at 100mg/kg diet based on corn-soybean meal for female Cobb broilers. When carboxymethyl cellulose (CMC) was added in the corn- soybean meal diet as

a mean to increase intestinal viscosity, the addition of cinnamaldehyde and commercial formulation partly counteracted the negative effect of CMC on broiler body weight gain during the first 21 days of age (Lee *et al.* 2004a). Similarly when basal diets were based on rye instead of corn, the rye induced suppression of weight gain between 1-14 days of age, this was partially overcome by the addition of cinnamaldehyde (Lee *et al.* 2004b).

In the current study, using a commercial blend from thymol and trans-cinnamaldehyde caused a disparate impact on cecal microflora population.

This agrees with Jang *et al.* (2007) who stated that, the effect of EO on gastrointestinal microflora is not consistent.

The disparate impact of thymol and trans-cinnamaldehyde on cecal microflora population might have resulted from the doses added from thymol and trans-cinnamaldehyde in the commercial blend and/or the mechanism of antimicrobial action of thymol and trans-cinnamaldehyde.

This agrees with Helander *et al.* (1998) who investigated how two isomeric phenols (carvacol and thymol) and the trans-cinnamaldehyde, exert their antibacterial effects on *E.coli* and *Salmonella typhimurium*. They found that both carvacol and thymol have attacked the membrane of bacteria, leading to the release of membrane-associated materials from the cells to the external medium. On the other hand, trans-cinnamaldehyde failed to affect the membrane, but exhibited antibacterial activity, indicating that the two molecules have different mechanisms.

Kurita *et al.* (1979) investigated the mechanism of cinnamaldehyde as antifungal. They proposed that the action of cinnamaldehyde was through the reaction with sulfhydryl groups, causing complexes with fungus cell leading to inhibition of cell division and thus interfere with cell metabolism.

Conclusion

Even though the dietary factor EO supplementation had an impact on the bacterial cecal populations; yet, this was not reflected on the bird growth performance, we suggest that this might have resulted from the balanced diet and controlled condition under which the trial was conducted. Further research on the use of EO must focus on the mechanism and the ratio between components of the blend and their effect on bird performance under uncontrolled conditions.

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تأثير الإضافات الفيتوجينية على أداء النمو و المحتوى الأعوري من البكتريا في دجاج التسمين

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في تجربه تصل الي ٣٥ يوماً تم دراسة تأثير إستخدام الإضافات الفيتوجينية علي أداء النمو و المحتوى الأعوري من البكتريا في دجاج التسمين. إشتمل برنامج التغذية على عليقة بادئة لفترة ٢١ يوماً إتبعته بفترة تغذية على عليقة ناميه حتى عمر ٣٥ يوماً. كان عدد المعاملات اربع معاملات: عليقة قياسية، عليقة إحتوت على ٥٠ جرام زيوت ضرورية / طن علف، ١٠٠ جرام زيوت ضرورية/ طن علف و ١٥٠ جرام زيوت ضرورية/ طن علف. لوحظ تحسن طفيف في نمو الطيور من حيث وزن الجسم، مقدار الزيادة الوزنية المكتسبة ، مقدار العلف المأكول و معامل التحويل الغذائي في مرحلة الباديء عند تغذية الطيور على عليقة إحتوت على ٥٠ جم زيوت ضرورية/طن علف. في كل من مرحلة النامي وعلى مستوى التجربة ككل لوحظ عدم تباين مقياس النمو بين الطيور التي تغذت على عليقة إحتوت على ١٠٠ جم زيوت ضرورية/طن علف وتلك التي تغذت على العليقة القياسية. كان أقل مقاييس النمو بالنسبة للطيور التي تغذت على عليقة إحتوت على ١٥٠ جم زيوت ضرورية/طن علف وذلك على مستوى مراحل النمو المختلفة، الا انه بوجه عام لم يكن هناك فارق معنوي بالنسبة لمقاييس النمو بين الطيور التي تغذت على العليقة القياسية وتلك المغذاة على العلائق المدعمة بالزيوت الضرورية. لم تحدث حالات نفوق على مدار التجربة. اظهر إستخدام خليط من الثيمول والترنزنمالدهيد تأثيراً متبايناً على المحتوى البكتيري في الأعور. إلا ان هذا التأثير لم ينعكس على نمو الطيور. وقد يعود ذلك لإستخدام علائق متزنة بالإضافة إلى إجراء التجربة تحت ظروف متحكم بها.

وعليه ومن خلال نتائج تلك الدراسة، يجب إجراء مزيد من الدراسات تركز على مكانية عمل الزيوت الضرورية و نسب خلط بعضها ببعض وتأثير ذلك على أداء الطيور لعلائق تحت الظروف غير المحكومة مثل (الإجهاد الحراري، العلائق غير المتزنة..... إلخ).

قام بتحكيم البحث

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