

## **EFFECT OF PHYTOGENIC ADDITIVES ON GROWTH PERFORMANCE AND INTESTINAL MORPHOLOGY OF BROILERS.**

**T.M. El-Afifi; M.F. Emar; M.A. El-Sherbiny; H.F.A. Motawe; G.M. El-Moghazy; A.H.M. Gomaa and N.A. Hashem**

*Regional Center for Food and Feed, Agriculture Research Center, Giza, Egypt*

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### **SUMMARY**

**A** 35-d trial was conducted to study the effect of phytogetic additives on growth performance and intestinal morphology of broilers. The feeding program consisted of a starter diet until 21-d and a grower diet until 35-d. There was 4 treatment groups: control, 50g EO (essential oil)/ ton, 100g EO/ton and 150g EO/ton. Slight non significant improvements of growth parameters have been observed as the incorporation level of the commercial EO have increased. No mortality has occurred during the whole period of the growth study. Non-significant increase in hemoglobin levels with continuous increase EO incorporation level in the diet. There was no effect for addition of EO to basal ration on kidneys functions, liver enzymes, cholesterol and triglycerides. There was a slight morphological change in the intestine of broilers fed different experimental diets at the first 21-d, in the second stage (from 22-35d) an increase in EO incorporation level in the diet is paralleled by an improvement of intestinal morphology. Yet, this was not reflected on the bird growth performance. This may be resulting from the balanced diet and controlled condition under which the trial was conducted. The present data suggest, further investigation should be focused on studying the effect of supplementation with EO under uncontrolled conditions such as (heat stress, unbalanced diet .....etc.) by increasing levels of EO.

***Keywords: phytogetic additives, growth performance, morphology, broilers.***

### **INTRODUCTION**

Dietary antibiotics, at low levels as growth promoter to improve animal performance have been a usual practice for more than half a century (Kamphues and Hebel, 1999). However, antibiotics have been banned by the EU since 2005 to prevent the development of antibiotics-resistant human pathogenic bacteria (Reglamento (CE) no. 183/2005).

This ban has led to investigate alternative feed additives that could be used as growth promoters. One of the alternatives is phytogetic feed additives. Phytogetic feed additives are defined as plant-derived compounds (from herbs, spices, thyme and cinnamon) incorporated into diets to improve the productivity of livestock through amelioration of feed properties (Windisch *et al.*, 2008).

Phytogenic feed additives are mainly essential oils (EO). EO are complex compounds, their chemical composition basically consist of two classes of compounds, terpenes and phenylpropenes which have a lipophilic property (Cornner, 1993), this property can make them penetrate the membranes of bacteria and reach the cellular content (Lee *et al.*, 2004). Due to this property, EO has demonstrated biological characteristics such as antimicrobial, antioxidant and antiseptic activities (Hertrampf, 2001). Ipek, *et al.*, (2005) found that the amplitude of EO effects depend on their concentration.

Thyme oil containing thymol and cinnamon oil containing cinnamaldehyde are essential oils. According to Helander, *et al.*, 1998; Hammer *et al.*, 1999, thymol displayed antimicrobial activity against intestinal microbes such as *Colistridium perfringers*, *Salmonella typhimurium* and *E.coli*. 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 anti-bacterial 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.

Previous studies on the effect of EO on liver functions have shown contradiction in results, where Bölükbaşı *et al.*, (2006) reported increase in triglycerides, LDL and HDL levels in broilers. Lee *et al.*, (2003); Calislar *et al.*, (2009) reported that no effect on triglycerides, LDL and HDL had occurred, while EL-Ghousein and Al-Beitawi (2009), Al-Kassie (2009) reported a decrease in serum levels of triglyceride, LDL and HDL.

Accordingly, the aim of the current study was to make further investigations on the quantification of phytogenic feed additives (thymol and cinnamaldehyde) and assess their effects on growth performance, liver functions and intestinal morphology of broilers.

## **Materials and Methods**

### ***Experimental system and chicks***

468 chicks (ROSS 308) one day old were brought from commercial farm (El-Wadee). The trial was carried out in 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 (%)	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.
Corn, yellow, ground.	58.04	58.03	58.03	58.02	58.50	58.50	58.49	58.49
Soybean meal (48 %).	28.00	28.00	28.00	28.00	28.00	28.00	28.00	28.00
Corn gluten meal.	8.50	8.50	8.50	8.50	8.500	8.50	8.50	8.50
Essential oil.	---	0.005	0.01	0.015	---	0.005	0.01	0.015
Vegetable oil.	1.14	1.14	1.14	1.14	2.68	2.68	2.68	2.68
Di-calcium phosphate.	2.18	2.18	2.18	2.18	1.89	1.89	1.89	1.89
Limestone.	0.71	0.71	0.71	0.71	0.62	0.62	0.62	0.62
Vit.&Min. <sup>1</sup>	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Cholinechlorid	0.082	0.082	0.082	0.082	0.075	0.075	0.075	0.075
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
L-lysine <sup>2</sup> .	0.43	0.43	0.43	0.43	0.32	0.32	0.32	0.32
DL-Methionine <sup>2</sup>	0.23	0.23	0.23	0.23	0.22	0.22	0.22	0.22
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	1.40	1.40	1.40	1.40	1.30	1.30	1.30	1.30
Methionine.	0.60	0.60	0.60	0.60	0.57	0.57	0.57	0.57
Methionine + cystine	1.04	1.04	1.04	1.04	1.00	1.00	1.00	1.00
Calcium.	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Available Phosphorus.	0.50	0.50	0.50	0.50	0.45	0.45	0.45	0.45

<sup>1</sup> Vitamin-mineral mixture supplied per kg of diet: Vit. A, 12000 IU Vit. D<sub>3</sub> 2000 IU Vit. E, 10mg Vit. K, 2mg Vit. B<sub>1</sub>, 1mg Vit. B<sub>2</sub>, 5mg Vit. B<sub>6</sub>, 1.5mg Vit. B<sub>12</sub>, 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.

<sup>2</sup> 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. At the third and fifth weeks of the trial, ten random birds from each treatment were slaughtered, immediately after slaughtering intestines were collected and stored in 10% formalin solution for morphological measurements (villi length, thickness and surface area for the jejunum). Blood samples were collected at the end of the growth trial to study the effect of treatments on total serum cholesterol, HDL and triglycerides, kidney functions and liver enzymes.

#### *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 and DISCUSSION**

### *Growth performance and mortality percentage*

Compared with birds fed the control diet (Table 2), slight non significant improvements of growth parameters (feed intake, body weight and body weight gain) have been observed as the incorporation level of the commercial EO was increased. No differences in feed conversion have occurred between birds fed diets contained 50g and 100g EO and those fed the control diet. Birds fed diet contained 150g has the worst feed conversion (starter stage), yet this difference has vanished in both (grower stage) and throughout the entire period of the growth study. No mortality has occurred during the whole period of the growth study.

Earlier studies have proved that EO have antimicrobial and antioxidant properties (Ultee *et al.*, 2002; Valero and Salmeron, 2002; Shan *et al.*, 2005), these properties have enhanced 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, the dietary factor EO supplementation didn't cause significant improvement in growth performance and nutrient utilization, this agrees with the finding of (Lee *et al.*, 2003; Hernández *et al.*, 2004; Grilli *et al.*, 2006; Muhl and Liebert, 2007; Jang *et al.*, 2007; Isabel and Santos 2009). Other authors have shown improvement in growth performance and feed utilization when they used 200 ppm or more from EO ( Bölükbaşı *et al.*, 2006; Al-Kassie, 2009; Calislar *et al.*, 2009; EL-Ghousein and Al-Beitawi 2009; Scheuermann *et al.*, 2009).

In the current study the used dosages were 50, 100 and 150 ppm, these dosages might not be efficient enough to yield a measurable response in respect of growth performance

**Table (2). Effect of supplementing broiler diets with commercial EO on growth performance**

Items	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 (BW) gm	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 (BWG) gm	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 (FI) gm	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 (FCR)	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).*

and feed utilization. This agrees with Michiels *et al.*, (2009) who reported that minimum concentration of thymol and trans-cinnamaldehyde to reduce the number of anaerobic bacteria was 258 and 56 ppm respectively. Another possibility is 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 this trial.

***Blood constituents***

Table (3) shows non-significant increase in hemoglobin levels with continuous increase in the supplemented amount from commercial EO to the basal ration. There was no effect for addition of EO to basal ration on kidneys functions (uric acid mg/dl and creatinine mg/dl), liver enzymes (AST U/L and ALT U/L), cholesterol and triglycerides (mg/dl).

**Table (3). Effect of supplementing broiler diets with commercial EO on Hemoglobin, Kidneys function, Liver function, Serum cholesterol and triglycerides.**

Items	Control	50g EO/ton	100g EO/ton	150g EO/ton
Hemoglobin (g/dl)	12.3	12.8	13.3	13.8
Uric acids (mg/dl)	3.5	3.5	3.6	3.7
Creatinine (mg/dl)	0.34	0.32	0.34	0.33
AST (U/L)	250	246	235	246
ALT (U/L)	36	36	36	41
Cholesterol (mg/dl)	141	141	143	141
Triglycerides (mg/dl)	73	77	83	82

According to El-Ghousein and Al-Beitawi (2009) triglyceride and serum cholesterol had decreased significantly as the inclusion level of crushed thyme increased from 1% to 2 %. Yet, in the current study, supplementation with EO had no effect on triglyceride and serum cholesterol. This agrees with finding of (Lee *et al.*, 2003; Calislar *et al.*, 2009).

Slight non-significant increase in hemoglobin was recognized, this agrees with the finding of (Kassie, 2009) who observed increase in hemoglobin level when broiler fed diet containing 200 ppm EO derived from thyme and cinnamon. The improvement in hemoglobin level means improvement in the bioavailability and assimilation of iron; as a result bird with good health is produced. There was no effect for EO on liver and kidneys functions.

***Histopathology***

At the first 21 days of the growth trial, Fig. (1and 2) show normal appearance of intestinal villi length and thickness for the group of birds fed basal diet or EO 100g/ton. While, there was a slight infiltration in villi *lamina propria* Fig. (3) for the group fed EO 50g/ton and increase in mucous secretion of intestinal epithelium Fig. (4) for the group fed EO 150g/ton.

Starting from day 22 till 35, Fig. (5) shows normal appearance of intestinal villi for birds fed the control diet. Fig (6 and 7) show hyperplasia, marked folding and an increase in length of the intestinal villi for birds fed diets contained 50g/ton and 100g/ton EO with a

form of finger-like project in the lumen for those fed 100g/ton EO. Group fed diets containing 150g/ton Fig. (8) shows thickness in intestinal wall and an increase in villi length.

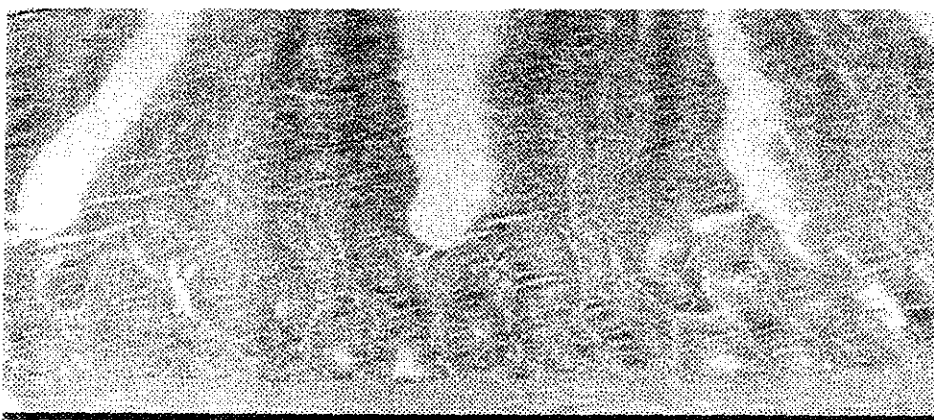
Morphologically, significant increase in villi folding has occurred in this trial as the level of supplementation of EO increased in bird diets. Annabell and Frank (2007), observed an increase in mucus secretion and villi-related protective, others have reported unchanged and reduced villi length and crypt depth in the jejunum and colon for birds and pigs fed diets containing the phytogetic feed additives (Demir *et al.*, 2005; Oetting *et al.*, 2006). In the present study the morphological changes may be occurred due to improvement in bird health resulting from the decrease of microbial lode in the digestive tract.

The suggestion here agree with Kroismayer *et al.*, (2007) who compared a blend of essential oils from oregano, anise and citrus peels with an antibiotic growth promotant and reported a decrease in microbial activity in the terminal illeum, cecum, and colon for both feed additives.

Windisch *et al.*, (2008) suggested that morphological improvement in gastrointestinal tissues caused by EO may be an indication for beneficial effect of EO on the digestive tract.

## CONCLUSION

Even though there was a morphological change in the intestinal villi, this was not reflected on the bird growth performance, this may be resulting from the balanced diet and controlled condition under which the trial was conducted. To reveal the effect of EO on bird performance further investigation should be focused on studying the effect of supplementation with EO under uncontrolled conditions such as (heat stress, unbalanced diet .....etc.)



**Fig. (1). Normal appearance of intestinal villi length and thickness for chick fed the control diet after 21 days from the trial start (H and E x 100).**



**Fig. (2).** Normal appearance of intestinal villi length and thickness for chick fed diets supplemented with 100g EO/ton after 21 days from the trial start (H and E x 100).



**Fig. (3).** Slight infiltration in villi lamina propria for chick fed diets supplemented with 50g EO/ton after 21 days from the trial start (H and E x 200).

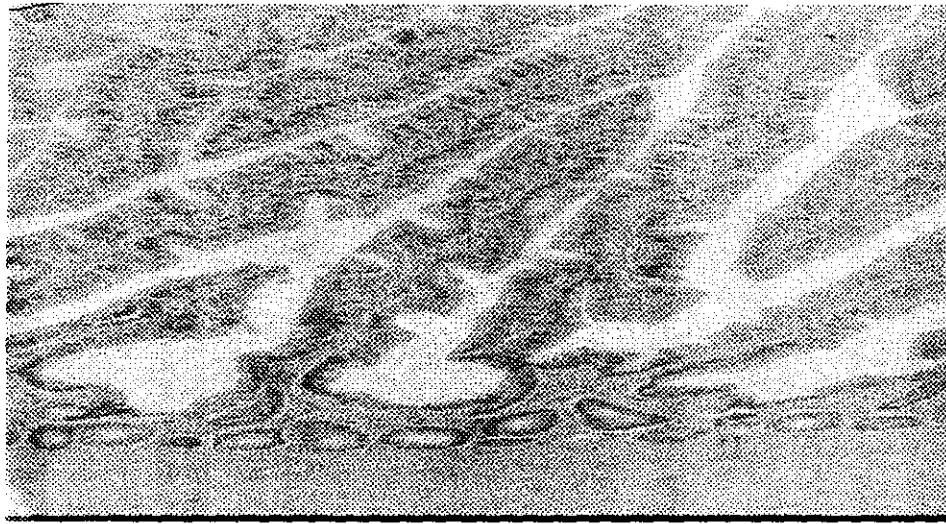




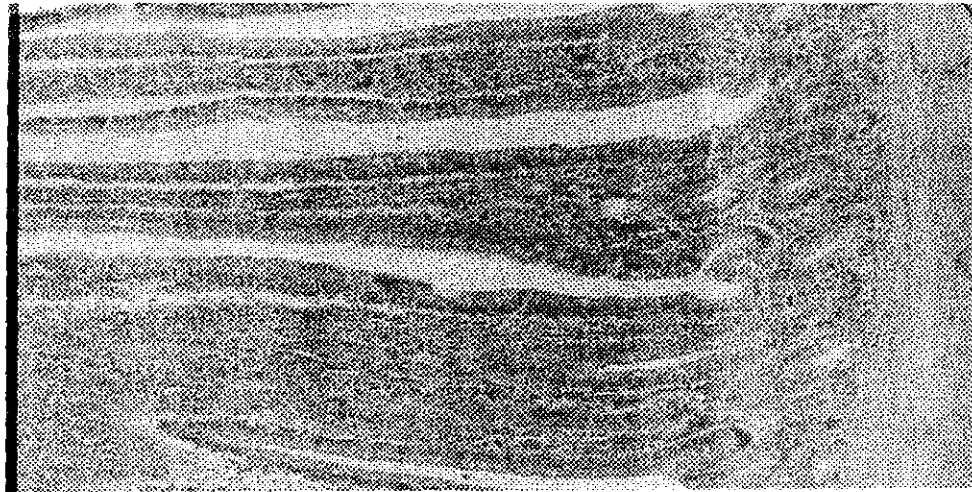
**Fig. (4).** Increase in mucous secretion of intestinal epithelium for chick fed diets supplemented with 150g EO/ton after 21 days from the trial start (H and E x 100).



**Fig. (5).** Normal appearance of intestinal villi length and thickness for chick fed the control diet after 35 days (H and E x 100).



**Fig. (6).** Hyperplasia, marked folding and increase of the intestinal villi length for birds fed diets containing 50g/ton after 35 days (H and E x 100).



**Fig. (7).** Hyperplasia and elongation in intestinal villi length for birds fed diets containing 100g/ton after 35 days (H and E x 100).



Fig. (8). Thickness in intestinal wall and increase in villi length for birds fed diets containing 150g/ton after 35 days (H and E x 100).

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## تأثير الإضافات الفيتوجينية على اداء النمو و مورفولوجية الإمعاء في دجاج التسمين

طارق محمد العفيفي، محمد فهمي عماره، محمد أحمد الشرييني، هادي فتحي مطاوع، جيهان محمد المغازي، اشرف هاشم محمد جمعه، نصر احمد هاشم  
المركز الاقليمي للاغذية والاعلاف-مركز البحوث الزراعية-جيزه-مصر

في تجريره تصل الي ٣٥ يوم تم دراسة تأثير استخدام الإضافات الفيتوجينية على اداء النمو ومورفولوجية الأمعاء في دجاج التسمين.

اشتمل برنامج التغذية على عليقه باندن لفترة ٢١ يوم إتبعه بفترة تغذيه على عليقه ناميه حتى عمر ٣٥ يوم. كان عدد للمعاملات اربع معاملات: عليقه قياسييه، عليقه احتوت على ٥٠ جرام زيوت ضروريه / طن علف، ١٠٠ اجرام زيوت ضروريه/طن علف و ١٥٠ جرام زيوت ضروريه/طن علف. لوحظ تحسن طفيف في نمو الطيور مع زيادة مقدار إضافه الزيوت الضروريه التجاريه الي العليقه. لم تحدث حالات نفوق على مدار التجربه. صاحب زيادة مقدار الزيوت الضروريه المضافه الي العليقه تحسن غير معنوي في مستويات الهيموجلوبين. لم تؤثر إضافه الزيوت الضروريه على كل من وظائف الكلتيان، انزيمات وظيف الكبد، الكوليستيرول و الجلوسريدات الثلاثيه. عند تغذيه الطيور على العلائق الباندنه لقره ٢١ يوم والمحتويه على مستويات مختلفه من الزيوت الضروريه حدث تغير طفيف في التركيب المورفولوجي للأمعاء. خلال الفتره الثقيه (من ٢٢-٣٥ يوم) صاحب زيادة الكمييه المضافه من الزيوت الضروريه الي العلائق تحسن في مورفولوجية الأمعاء غير ان هذا للتحسن لم ينعكس على نمو الطيور. وقد يعود ذلك لإستخدام علائق متزنه بالإضافه الي إجراء التجربه تحت ظروف متحكم بها.

وعليه ومن خلال نتائج تلك الدرسمه، يجب إجراء مزيد من الدراسات تركّز على تأثير إضافه الزيوت الضروريه لعلائق الطيور تحت الظروف غير المحكومه مثل ( الإجهاد الحراري، العلائق غير المتزنه..... إلخ) وذلك ببستخدام مستويات متزايدة من الزيوت الضروريه.