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Research Article
Effect of Different Sources of Growth Promoter Supplementation in Diet on Performance, Carcass Traits and Blood Constituents of Broiler Chicks

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Abstract

One hundred and fifty days old of unsexed broiler chicks were reared on floor pens and distributed into 5 dietary treatments (starter = 0-21 days old and grower = 22-35 days old) including 4 sources of growth promoters (GP's) represented two sources of essential oils, synbiotic preparation (Amio-Flash®) and phytobiotic preparation (Bio-Strong®). The experimental diets were Con. = unsupplemented basal diet, T*gro* = Con.+Geranium oil, T*tno* = Con. +tangerine oil, T*amf* = Con. +Amio-Flash® and T*bis* = Con. +Bio-Strong®. Experimental results showed that Body Weight Gain (BWG) was significantly improved and Feed Intake (FI) increased by dietary GP's inclusion only at starter period but not significantly affected during growing and overall period for both traits. Geranium oil supplementation caused a significant higher FI during grower and overall periods. Although, GP's supplementation didn't significantly affect Feed Conversion Ratio (FCR) but both Con. and T*amf* recorded the best FCR at all experimental periods. The amio-flash (as synbiotic) was the most effective growth promoters between tested ones for improving FCR and BWG. Neither supplementation of diets with GP's nor GP's source significantly affect carcass trait percents or most plasma components except globulin and A/G ratio, which were affect by GP's supplementation. Tangerine oil and Bio-Strong favorably increased total protein and decrease A/G ratio of broiler plasma. Besides, the higher value of plasma ALT for Bio-Strong and AST for both geranium oil and Bio-Strong suggests that both GP's may have a negative effect on liver function of broiler chicks.

Key words: Growth promoters, broilers, performance, carcass, plasma

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Research workers in poultry nutrition field have been interested to investigate the effect of many Growth Promoter (GP's) compounds representing antibiotic preparations, many natural derivatives, microorganisms and synthetic compounds in poultry nutrition as feed additives to enhance overall health, performance, final product and reproductive performance of poultry flocks. Among those tested GP's, synbiotics, phytobiotic preparations and natural essential oils.

Synbiotic preparation is a combination of one or more of prebiotic and probiotic. The mode of action of this mixture comes from that the improvement of the survival of probiotic microorganisms due to its specific substrate is available for fermentation, causing enhancement of the bacterial hostage through the availability of the live microorganisms and prebiotics (Batavani, 2010) and this mechanism improves the beneficial effect of both prebiotics and probiotics in their mode of action on intestinal tract.

Phytobiotics are medical herbs and spices and their derivatives including essential oils and powders. There are scientific evidences indicated those compounds substantially improved feed intake and feed conversion, nutrient digestibility to weight gain of broiler chicks (Isikwenu, 2015) and represents a desirable and recommended feed additives from many research workers due to those being natural extracts, safely, environment friendly and doesn't have any side effects.

Citrus fruits (orange, lemon and tangerine) and their essential oil extracted of peel are important source of bioactive compounds including ascorbic acid, flavonoids, phenolics and pectins. Those compounds have antioxidant effect on intestinal microvilli and antibacterial activities against pathogenic bacteria causing higher ability for absorption of essential nutrients and necessary for healthy intestinal gut (Slaughter, 2013).

Geranium oil is an essential oil derived from the Geranium flower and contains basically on geraniol compound. Geraniol is a monoterpense (alcoholic compounds) which used as perfumery, cosmetics, phytotherapeutics, aromatherapy and improving flavor (Wang *et al*., 2014) additionally, plays an important role in the prevention of food-borne diseases due to their antimicrobial activity (Singh *et al*., 2012).

Many research workers have been compare between those GP's on broiler chicks either individually or as a combination of different GP's types (mostly commercial preparation) against antibiotics (antibiotic alternative investigation) or as anti infectious microorganisms. Besides, many publications investigated the comparison between some sources of GP's for broiler performance such as prebiotic, organic acids and their blend (Fallah and Rezaei, 2013), phytobiotic powder, prebiotic, probiotic and synbiotic (Mokhtari *et al*., 2010), while, incorporation of essential oils (as a single form, not as blend preparation), phytobiotc forms or synbiotic preparations in broiler diets and those influence on chick performance haven't been sufficiently investigated, especially, under a wide variation in those marketing price.

Our study aims to investigate the effect of supplementation of diet with two sources of essential oils, phytogenic powder mixture or blend of synbiotic including residues of other GP's types as feed additives on performance, carcass traits and blood constituents of broiler chicks.

MATERIALS AND METHODS

Birds management: During the experimental period which lasted 5 weeks, a total number of 150 days old of unsexed broiler chicks (Avian strain) were randomly distributed into 5 starter dietary treatments from 0-21 days old and 5 grower dietary treatments from 22-35 days old. Starting of 0 day old, chicks were reared on 10 floor pens and randomly allocated to 5 treatments of 30 chicks each in two replicates (15 chicks per replicate) until the end of experiment at 35 days old. Feed and drinking water were available *ad libitum* for chicks which subjected daily to 22 h. light and 2 h. darkness along the experimental period. Periodical vaccinations of chicks were done according to the recommendation of guide book of the strain.

Experimental Growth Promoter compounds (GP's): Four sources of GP's used in present study are described below:

- **Geranium oil:** It extracted from *Geranium trilopum* flower by ethanol extraction method. The essential oil was added to experimental diets by 166 mg kg^{-1} diet where, it well mixed with dietary soybean oil as a carrier to inhibit its volatilization during the mixing of experimental diets
- **Tangerine oil:** This essential oil was extracted from tangerine peels using ethanol solvent procedure and well mixed with dietary soybean oil as a carrier by 166 mg kg^{-1} diet during mixing of experimental diets

Amio-flash® (synbiotic): It is a commercial product, manufactured by IBEX Int. Ltd Company, Egypt. It consists basically of synbiotic (live *Lactobacillus* bacteria, *Aspergillus Oryzae* and *Torellolisis Aotis* yeast with fructo oligosaccharides and mannan oligosaccharides) and fewer molecules of β

glucans, amino acids (methionine+lysine), some vitamins, betaine and L-carnetin. It was added to experimental diets by 2 g kg^{-1} diet according to the product guide book recommendation.

Bio-strong®510 (phytobiotic preparation): It is a blend of different plant derivatives as feed additive in poultry feeds and produced commercially by Delacon, PROVAX Company. It is comprised of high quality, proven active ingredients that improve digestion, enhance metabolic function and increase nutrient retention, so, it optimizes performance, production and profitability. The active ingredients of Bio-Strong are essential oils (not reported in composition list of the product), bitter substances, pungent substances and saponins derived from herbs, spices and their extracts. According to the product book guide, the recommended level is 150 mg kg^{-1} diet.

Experimental diets and treatments: All groups received iso-caloric (starter = 3000 Kcal ME kg^{-1} and grower = 3183 Kcal ME kg^{-1}), iso-nitrogenous (starter = 23.01% and grower = 21.06%) yellow corn-soybean meal starter diet from 0-21 days old then grower diet from 22-35 days old. The experimental treatments represented, Con. = un supplemented basal diet, T*gro* = Con. +Geranium oil (166 mg kg^{-1} diet), T*tno* = Con. +tangerine oil (166 mg kg^{-1} diet), T*amf* = Con. +Amio-Flash® (2 g kg^{-1} diet) and T*bis* = Con.+Bio-Strong® (150 mg kg^{-1} diet). The composition and calculated analysis of basal diets are showed in Table 1.

Experimental measurements

Chick performance: For all groups, feed intake and body weight were weekly recorded and corrected to mortality rate. Then Body Weight Gain (BWG) g/chick and Feed Intake (FI) g/chick then Feed Conversion Ratio (FCR) g FI/g BWG were mathematically calculated for starter, grower periods and overall period.

Carcass traits and blood sampling: At 35 days old, 5 chicks were randomly taken from each treatment group, then slaughtered to determine the common plasma constituents, weight of carcass, some organs, calculated as a percent of live body weight.

Blood constituent determination: Individual blood samples were collected from jugular vein in dry clean centrifuge tubes from the slaughtered birds and plasma was separated by centrifugation at 3000 rpm for 15 min and assigned for subsequent determination. plasma samples were harvested after centrifugation of the clotted blood, stored at -20°C in the deep freezer until the time of chemical determinations. Biochemical analyses of blood plasma were conducted in Poultry Physiological Laboratory, Faculty of Agriculture, Ain Shams University, Egypt. Quantitative determination of plasma included; total protein, albumin, globulin, total cholesterol, ALT and AST were determined calorimetrically using commercial KITS which produced by Bio-Diagnostics Company, Egypt.

Table 1: Composition and calculated analysis of experimental basal diets

	Basal diets	
Ingredients (%)	Starter (0-21 days old)	Grower (22-35 days old)
Yellow corn	55.0	58.0
Soybean meal (44%)	33.0	28.0
Corn gluten meal (62%)	6.15	6.15
Soybean oil	2.00	4.00
Mono-calcium phosphate	1.80	1.80
Calcium carbonate	1.15	1.15
Premix*	0.30	0.30
Salt (NaCl)	0.20	0.20
DL-methionine	0.20	0.20
HCl lysine	0.20	0.20
Calculated chemical analysis		
Crude protein (%)	23.01	21.06
ME (Kcal kg^{-1})	3000	3183
Calcium (%)	0.84	0.83
Available phosphorus (%)	0.50	0.49
Lysine (%)	1.29	1.17
Methionine+cystine (%)	0.95	0.90

*Each 3 kg of the premix contains the followings: Vit A: 12000000 I.U, Vit D_3: 2000000, Vit E: 10000 mg, Vit K3: 2000 mg, Vit B1: 1000 mg, Vit B2: 5000 mg, Vit B6: 1500 mg, Vit B12: 10 mg, Ca D-Pantothenate: 10000 mg, Niacin: 30000 mg, Folic acid: 1000 mg, Biotin: 50 mg, Choline chloride: 250000 g, Mn: 60000 mg, Zn: 50000 mg, Iron: 30000 mg, Cu: 10000 mg, Iodine:1000 mg, Se: 100 mg, where, calcium carbonate taken as a carrier

Statistical analysis: Data of performance, carcass traits and plasma constituents were statistically analyzed by SAS software (SAS., 1995) with one way analysis procedure. The difference between means was separated using Duncan's multiple range test (Duncan, 1955) at 0.05 significance level. The statistical model was performed as follow:

$$Y_{ij} = \mu + T_i + E_{ij}$$

where, Y_{ij} is the experimental observation, μ is overall mean, T_i is the effect of ith dietary treatment and E_{ij} is random error.

RESULTS AND DISCUSSION

Effect of different experimental treatments on chick performance: Effect of different treatments on chick performance through different experimental periods is showed in Table 2, which revealed that, comparing between unsupplemented (Con.) and supplemented diets with experimental GP's: the final live body weight and BWG through overall period didn't significantly affect by GP's supplementation. While, BWG was significantly improved by dietary GP's inclusion only at starter period, but not significantly differed between treatments during growing and overall period. This improvement in weight gain associated with GP's supplementation may be resulted of the positive effect of GP's as a consequence of that broiler chick more sensitive to the presence of any growth promotors in diet through the early age of fattening period, considering the digestive system during the first three weeks of age is anatomically complete but its functional digestion and absorption capacities are still immature with low secretion of endogenous enzymes especially pancreatic enzymes and rise as feed intake and bird age increase. Consequently, the chicks during this stage need to compounds like exogenous enzymes or any other GP's those enhance the digestive tract to increases feed utilization.

Even though, the non significant difference between the unsupplemented treatments for BWG at overall period but Amio-Flash recorded marked value (T*amf* = 1407 vs. 1353, 1272 and 1331 g. for T*gro*, T*tno* and T*bis*, respectively) at the same time it is not differed than control group (1417 g).

Mortality rate ranged between 3.33 and 6.67% along overall period and the GP's supplementation in diet significantly increased FI at starter period, while, this difference have not been significant at grower and overall periods. Moreover, that the difference between supplemented groups for FI averages was obviously marked at different periods, whereas, during starter period, no significant difference have been showed in FI values between supplemented treatments but, surprisingly, Geranium oil supplementation caused a significant higher feed consumed by chicks and exhibited the higher feed intake through grower period and consequently, overall period. This may be resulted from the potent effect of geraniol compound found in Geranium oil, on taste and favor sense for chicks causing higher feed intake. This result in agreement with Wang *et al.* (2014) whereas, they settled that geraniol compound (major compound of Geranium oil) have aromatherapy, flavoring properties and used as flavoring agent in human food industries. Even though, essential oils derived of citrus family including tangerine have a flavoring property and extensively used as a flavor product in human food (Ahmad and Rahman, 2006) but didn't significantly

Table 2: Effect of different dietary treatments on performance of broiler chicks

	Dietary treatments					
Items	Con.	T*gro*	T*tno*	T*amf*	T*bis*	Significant
Finale live body weight (g)	1461^{a}	1397^{ab}	1315^{b}	1451^{a}	1375^{ab}	*
Body weight gain (g)						
Starter 0-21days old	647.0^{d}	698.9^{ab}	707.5^{a}	690.2^{b}	673.4^{c}	*
Grower 22-35 days old	770.2^{a}	654.8^{bc}	564.5^{c}	717.5^{ab}	658.3^{bc}	*
Overall period	1417^{a}	1353^{ab}	1272^{b}	1407^{a}	1331^{ab}	*
Feed intake (g)						
Starter 0-21 days old	1058^{d}	1101^{c}	1120^{b}	1121^{b}	1153^{a}	*
Grower 22-35 days old	1578^{ab}	1713^{a}	1515^{b}	1528^{b}	1542^{b}	*
Overall period	2636^{b}	2814^{a}	2635^{b}	2649^{b}	2695^{b}	*
Feed conversion ratio (g feed/g gain)						
Starter 0-21 days old	1.636^{b}	1.575^{c}	1.583^{c}	1.624^{b}	1.712^{a}	*
Grower 22-35 days old	2.049^{c}	2.618^{a}	2.684^{a}	2.149^{c}	2.345^{b}	*
Overall period	1.860^{b}	2.079^{a}	2.071^{a}	1.885^{b}	2.023^{a}	*
Mortality rate (%)	6.67	6.67	3.33	3.33	3.33	

$^{a-d}$Means within the same row with different superscript letters are significant1y differed, *p<0.05, Con: Unsupplemented basal diet, T*gro*: Con+Germinium oil, T*tno*: Con.+tangerine oil, T*amf*: Con.+Amino flash®, T*ibs*: Con.+Bio-strong®

increase feed intake as showed with Geranium oil, referring to that Geranium oil may be have a flavoring property superior to tangerine oil for broiler chicks.

Although, both T*amf* and T*tno* treatments exhibited moderate and non significant differences in averages of FI during different periods but no certain trend was showed for both groups received diets contained tangerine oil or Bio-Strong® for different performance parameters through all experimental periods.

Both Con. and T*amf* recorded the best FCR at all experimental periods, compared with other treatments. This indicated to that GP's supplementation didn't improve FCR and the Amio-Flash was the most effective growth promoters between tested ones for improving feed conversion.

The non significant difference between supplemented and un supplemented treatments on most performance traits is agreed with that documented by Alavi *et al.* (2012) with inclusion of probiotic preparation, acidifier, prebiotic mixture and synbiotic in broiler diets. But disagreed with Zakeri and Kashefi (2011) using commercial probiotic and prebiotic (four products) despite of feed intake didn't significantly differ between treatments.

Previous studies interested to the comparison between various GP's inclusion in broiler diet related to those effects on chick performance gave contradict results and final recommendations, for instance, an improvement in feed conversion associated with GP's supplementation was showed by Toghyani *et al*. (2011b) using phytobiotics, probiotics, prebiotics or synbiotics and they postulated that probiotics and prebiotics are better GP's for broiler performance than phytobiotics. Bozkurt *et al*. (2009) recommended that the synbiotics were more sustainable for FCR of broiler chicks compared with probiotic, prebiotic or organic acid, while, Mokhtari *et al*. (2010) mentioned that BWG didn't significantly differ between broiler chicks received either unsupplemented diet, probiotic, prebiotic, phytobiotic or synbiotic supplemented diets, furthermore that, chicks fed probiotic diet showed the better FCR than control or other treatments. They summarized to, probiotic or synbiotic was the best feed additives could be supplied for broiler diet compared with the other tested GP's. Fallah and Rezaei (2013) recognized that, blend of prebiotic and organic acids is a suitable for broiler performance than prebiotic or organic acids as alone feed additive for each while, Afsharmanesh and Sadaghi (2014) documented a superior effect of probiotics to broiler performance than phytobiotics.

However, current study showed that the Amio-Flash (synbiotic) was the best growth promoter for BWG and FCR compared to Geranium oil, tangerine oil or phytobiotic mixture preparation but it was not statistically differed than control treatment.

Effect of experimental treatments on carcass characteristics: The averages of carcass characteristics as a percent of live body weight for different experimental treatments at 35 days old are showed in Table 3. It is clear that, neither supplementation of diets with GP's nor GP's source significantly affect different carcass characteristic percents. This result is contradict with Toghyani *et al*. (2011a) and Mehdipour *et al*. (2013) where, they reported a significant improvement in most tested carcass yield parameters resulted from dietary supplementation of different sources of phytobiotics (essential oils or herbs derivatives) including capsaicin oil, cinnamaldehyde oil, carvacrol oil, garlic powder or thymol powder. A similar result was revealed by Mokhtari *et al.* (2010), where they reported a best carcass percent with control broiler diet compared with GP's supplemented groups representing probiotic, probiotic, synbiotic or phytobiotics. Notably, Fallah and Rezaei (2013) noted a significant lower percent of abdominal fat and higher carcass weight with prebiotic, acidifier or their blend in broiler diet.

The non significant effect of GP's inclusion or GP's source for carcass trait percents showed in our study is completely agreed with Bozkurt *et al*. (2009) as a result of incorporate experimental diets with prebiotic, formic acid, probiotic, synbiotic and their blends as well as with Toghyani *et al*. (2011b) using diets supplemented by phytobiotic compound (FlavoPhosphoLipol), prebiotic, prebiotic or

Table 3: Effect of different dietary treatments on carcass characteristics (%) of broiler chicks at 35 days old

	Dietary treatments					
Carcass characteristics of live body weight (%)	Con.	T*gro*	T*tno*	T*amf*	T*bis*	Significant
Carcass	70.72	68.80	70.50	70.81	70.29	NS
Abdominal fat	0.88	1.45	0.85	1.24	1.32	NS
Liver	2.14	2.47	2.31	2.02	2.26	NS
Gizzard	1.74	1.41	1.42	1.52	1.23	NS
Heart	0.43	0.53	0.44	0.51	0.45	NS
Giblets*	4.32	4.42	4.17	4.06	3.95	NS
RTC**	75.05	73.23	74.68	74.87	74.24	NS

NS: Non significant difference, *Giblets = Liver+gizzard+heart, **RTC (ready to cook) = Carcass+giblets

Table 4: Effect of different dietary treatments blood plasma parameters of broiler chicks at 35 days old

	Dietary treatments					
Blood component	Con.	T*gro*	T*tno*	T*amf*	T*bis*	Significance
Total protein (g dL^{-1})	5.84	6.20	6.64	5.96	6.70	NS
Albumin g (dL^{-1})	4.30	3.96	4.13	3.97	3.81	NS
Globulin (g dL^{-1})	1.54^{b}	2.24^{ab}	2.51^{ab}	1.99^{ab}	2.89^{a}	*
Albumin/Globulin ratio (A/G ratio)	2.85^{a}	2.00^{ab}	1.69^{b}	2.03^{ab}	1.35^{b}	*
Total cholesterol (mg dL^{-1})	196.50	168.00	197.50	176.00	191.50	NS
ALT RFU (dL^{-1})	31.15	26.30	31.57	32.82	38.71	NS
AST RFU (dL^{-1})	46.02	50.36	37.22	44.50	52.86	NS

^{a,b}Means within the same row with different superscript letters are significant1y differed, *$p \leq 0.05$, NS: Non significant difference

synbiotic preparations (except abdominal fat which reduced with probiotic supplementation). From our standpoint, the non significant differences between experimental treatments may be attributed to that recommended levels of tested GP's may be insufficient to give a potent trend for this trait.

Effect of experimental treatments on plasma constituents: Many scientific evidences were confirmed that some plasma constituents used as indicators for healthy and functional effectiveness of some organs, immune efficiency of birds, among those plasma constituents; total protein that positively related to protein synthesis, healthy of many tissues as well as growth rate of bird (Kapelanski *et al*., 2004), additionally, higher plasma globulin and lower albumin/globulin ratio used as indicator for good immune efficiency of body (Elagib *et al*., 2012), furthermore that, in most cases, malfunction of lipid metabolism are positively related to the level of plasma total cholesterol (Abdulkarimi *et al*., 2011), moreover, the plasma ALT and AST content positively related to hepatic malfunction as settled by Kim *et al*. (2008). Table 4 concerning to some plasma constituents of broiler chicks at 35 days old as resulted of experimental treatments. It referred to that, GP's supplementation or source of GP's didn't significantly affect most plasma components except Albumin (A), Globulin (G) and A/G ratio, which recorded a higher albumin, lower globulin values and consequently lower A/G ratio compared with un supplemented treatment. Although this difference are apparently slight but somewhat, referred to that all tested growth promoters have a desirable effect on immune efficiency indicators of broiler plasma although concerned study didn't show any healthy problems or mortality rate (3.33-6.67%) of chicks along experimental period. This result is in accordance with Hosseini *et al*. (2013) using phytobiotic powders, probiotic preparation and Abd El-Ghany and Ismail (2014) by adding different sources of essential oils in broiler diets.

Concerning to the other plasma constituents those appeared a non significant effect either by GP's supplementation or source; our result is agreed with Hedayati *et al*. (2014) but differed for some blood components especially total cholesterol with some publications in this manner. Fallah and Rezaei (2013) pointed out a significant reduction in total cholesterol of chick groups fed diets supplemented with different GP's included prebiotic, acidifier or their blend as well as a significant reduction recorded for synbiotic group on plasma total cholesterol and the same result referred by Al-Saad *et al*. (2014) when they tested probiotic, organic acid or their blend as feed additive in broiler diets especially for probiotic or organic acid groups. Afsharmanesh and Sadaghi (2014) reported significant lower plasma cholesterol with phytobiotic than probiotic preparations. In addition that, some research workers give a recommendations for a favorable effect of certain GP's source on essential blood components, where probiotics were more superior than phytobiotic powders as showed by Hosseini *et al*. (2013), or prebiotics as documented by Shahir *et al*. (2014), while the essential oils have been enhanced beneficial blood components compared with phytobiotic powders as showed by Abd El-Ghany and Ismail (2014). Although, most plasma parameters statistically were not significant but tangerine oil and phytobiotic preparation (Bio-Strong) beneficially enhanced the total protein (protein metabolism indicator) and lowered A/G ratio (immunity response indicator) content of broiler plasma. Besides, the higher value of ALT for Bio-Strong and AST for both Geranium oil and Bio-Strong supplemented groups let us to suggest that Geranium oil and Bio-Strong (synbiotic) may have a negative effect on liver function but those two points needs to more investigations.

CONCLUSION AND RECOMMENDATIONS

Broiler chicks may be sensitive to the presence of growth promoter forms in diet through the starter period compared with grower period. There were a potent effect of Geranium oil for taste and flavor sense for broiler chicks and superior to tangerine oil. The Amio-Flash (as a synbiotic product) was

more active growth promoters in broiler diet by improving body weight gain and/or feed conversion ratio in broiler chicks than phytogenics preparation and some essential oils, although it was not significantly differ than control treatment. All tested growth promoters improved indicators of plasma immunity, moreover that, tangerine oil and Bio-Strong have worthwhile effect on liver enzymes content of plasma. Recommended levels of tested growth promoter compounds in supplemented diets may be insufficient to express explicit trend for some experimental measurements.

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