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Research Article
Impact of Phyto-Cocci® Powder Supplementation on Broiler Growth Performance, *Eimeria* Oocyst Shedding and Gut Health

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Abstract

Objective: This study was carried out to investigate the effect of supplementing proprietary mixture of herbal extracts (Phyto-Cocci) to the corn-soybean meal diet of broiler chickens without using antibiotics on growth performance, *Eimeria* oocyst shedding and small intestine histology. **Materials and Methods:** A total of 720 one-day old Ross 308 broiler chicks (as hatched) were subjected to a five-week dietary experiment. The chicks were randomly divided into three experimental groups [basal diets supplemented with 0, 100 and 500 g t^{-1} ration Phyto-Cocci powder]. Each treatment comprised 4 replicates (60 chicks per replicate). **Results:** During starter diet stage (the first two weeks of age), chicks that were fed on T1 diet recorded the heaviest body weight compared to other treatments. Whereas, at marketing age, chicks that were fed on T2 or T3 diets recorded the heaviest live body weight compared to the control group. No significant differences were detected among all experimental groups in body weight gain (BWG) during starter and grower periods. The higher and significant BWG values were recorded by chicks which were fed on T2 diet during the finisher and overall periods. No significant differences were detected in feed consumption (FC) among all experimental groups. Nonetheless, the best overall FCR was found in chicks that were fed on T2 ration followed by T3 and T1, respectively. Using Phyto-Cocci in T2 or T3 improved the economic efficiency by 10 and 8%, respectively than control diet. Also, both European performance efficiency index and performance index were improved with Phyto-Cocci addition. Adding Phyto-Cocci increased villus height and width linearly and reduced coccidiosis oocysts count in feces of chicks at different ages. **Conclusion:** The findings of the present study showed that feed with herbals supplementation (Phyto-Cocci) has a positive effect on productive performance, gut morphology and lowest average number of coccidia oocysts.

Key words: Growth performance, poultry diet, herbal extracts, Eimeria oocyst, gut health

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Growth promoters are chemical and biological substances which are added to live stock feed with the aim at improving the utilization of feed and in this way obtain better production and financial results. The antibiotics have been used as growth promoters for decades[1]. However, the use of most antibiotic growth promoters has been banned in many countries because of health concerns about their residues in carcass tissues and the development of antibiotic-resistant bacteria[2]. Phytogenic feed additives or phytogenics consist of mixture of herbs, spices, other plants (wood, fruits and roots) and their extracts with proven efficacy[3]. Presently, phytogenics have received a great attention among several appropriate alternatives[4]. Consequently, it is better to replace these additives with other natural products that have the lowest side effects on both the health of animals and humans.

Diversified secondary metabolites of phytogenics (herbs, spices and essential oils) have been known to exhibit medicinal properties that are digestion-stimulating, appetizing, antioxidant and antimicrobial attributes[5]. Studies on herbal mixtures showed that the positive action of herbs is superior to the effect of single herbs. Since active components of herbal extracts as phytobiotics are known for their synergistic effect and some for their suppressing effects. Taking that into consideration, it is important to evaluate the optimal configuration of herbal extracts composition to get the best results. However, they need to be studied in more details and under standardized conditions.

Phytogenics are considered one of the promising alternatives because of their significant potentiality to promote growth, enhance feed intake and stimulate immunity competence[6]. In addition, they could improve feed intake, feed conversion ratio and carcass yield[7]. In general, gut health could be affected by several factors such as dietary factors, feed additives, immune status and environment.

The objectives of the current study were to evaluate the effects of mixtures of herbal extracts (Phyto-Cocci®) on growth performance, histological aspects of small intestine and coccidiosis oocysts count.

MATERIALS AND METHODS

This trial was carried out at Poultry Production Farm, Applied Feed Research House (AFRH), Orabi Community, Qalyobia Governorate. A total of 720 Ross 308 one-day old broiler chicks (as hatched) with an initial body weight around 44 g were obtained from a local commercial hatchery. The chicks were weighed and randomly distributed into 12 pens to examine the effect of three experimental treatments comprising three feeding regimens: (T1) Basal diet without Phyto-Cocci powder supplement (control group) and basal diet supplemented with either 100 or 500 g t^{-1} Phyto-Cocci powder (T2 and T3, respectively).

Each group contained 240 chicks which were allotted into four replicates and each replicate contained 60 chicks. All the experimental birds were reared in well ventilated shed on soft wood shaving litter used as bedding material in pens with 2.0×3.0 m^2 dimensions and kept under uniform management conditions. Starter (0-14 day), grower (15-24 day) and finisher (25-end) diets were formulated according to the nutritional recommendation of Ross 308 strain catalogue[8]. The composition and calculated analysis of the diets are shown in Table 1. Phyto-Cocci Powder is a dry stabilized preparation manufactured by Kanzy Medipharm, Canada: It is a mixture of herbal extracts (Garlic, Thyme, Oregano, Peppermint, Rosemary) and Calcium carbonate as carrier. Feed and water were available *ad libitum* during the whole experiment. All the birds were weighed at the same time weekly before feeding time. Feed intake was calculated by measuring the amount of feed offered and residue left. Feed conversion ratio (FCR) was calculated by dividing the feed intake by weight gain. House temperature was maintained at 34-31°C for the first week which was gradually reduced by 3°C per week until it reached 24°C. Vaccination and other routine poultry management practices were carried out neatly.

Parameters for productive performance (body weight, body weight gain, feed consumption and feed conversion ratio) were measured on a weekly basis throughout the experimental period. Body weight gain of broilers was measured as a difference of weight between two weighing intervals.

After correction for feed refusals, feed consumption was calculated using the following formula:

$$\text{Feed consumed} = \frac{\text{Feed consumed per pen}}{\text{No. of birds}}$$

Feed conversion ratio (FCR) was estimated using the following expression:

$$\text{Feed conversion ratio} = \frac{\text{Feed consumed in each replicate}}{\text{Total weight gain}}$$

Economic efficiency (EE) and relative economic efficiency (REE) were calculated according to input-output analysis data for live weight was expressed as broiler-production throughout the study and calculated using the following equation:

Table 1: Feed ingredients and chemical composition of diets presented to birds during starter, grower and finisher phases (0-35 day of age)

	Dietary treatment								
	Starter			Grower			Finisher		
Ingredients	T1	T2	T3	T1	T2	T3	T1	T2	T3
Yellow corn (grains)	59.391	59.381	59.471	66.092	66.061	66.014	70.890	70.880	70.840
Corn gluten meal (60%)	1.783	1.783	1.753	4.599	4.600	4.600	6.543	6.543	6.543
Soybean oil	0.500	0.500	0.500	0.500	0.500	0.500	1.000	1.000	1.000
Soybean meal (44%)	34.720	34.720	34.620	25.273	25.293	25.300	18.017	18.017	18.017
Calcium carbonate	1.382	1.382	1.382	1.225	1.225	1.225	1.294	1.294	1.294
Mono-Calcium phosphate	1.030	1.030	1.030	0.961	0.961	0.961	0.924	0.924	0.924
Sodium bicarbonate	0.082	0.082	0.082	0.239	0.239	0.239	0.200	0.200	0.200
Salt (NaCl)	0.218	0.218	0.218	0.113	0.113	0.113	0.144	0.144	0.144
HCl lysine	0.347	0.347	0.347	0.505	0.505	0.505	0.535	0.535	0.535
DL-methionine	0.197	0.197	0.197	0.144	0.144	0.144	0.124	0.124	0.124
L-threonine	0.000	0.000	0.000	0.049	0.049	0.049	0.029	0.029	0.029
Choline chloride 60%	0.050	0.050	0.050	0.000	0.000	0.000	0.000	0.000	0.000
Premix	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300
PHYTO-cocci powder	0.000	0.010	0.050	0.000	0.010	0.050	0.000	0.010	0.050
Total	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000
Calculated chemical composition									
Crude protein (%)	22.000	22.000	22.000	20.000	20.000	20.000	18.200	18.200	18.200
Metabolizable energy (kcal kg^{-1})	3000.000	3000.000	3000.000	3100.000	3100.000	3100.000	3200.000	3200.000	3200.000
Linoleic acid	1.500	1.500	1.500	1.600	1.600	1.600	1.910	1.910	1.910
Calcium (%)	1.000	1.000	1.000	0.900	0.900	0.900	0.900	0.900	0.900
Available phosphorus (%)	0.500	0.500	0.500	0.470	0.470	0.470	0.450	0.450	0.450
Lysine (%)	1.400	1.400	1.400	1.300	1.300	1.300	1.150	1.150	1.150
Methionine (%)	0.560	0.560	0.560	0.500	0.500	0.500	0.470	0.470	0.470
Methionine+cysteine (%)	0.980	0.980	0.980	0.900	0.900	0.900	0.850	0.850	0.850
Price t^{-1} ($)	363.610	364.440	367.780	354.440	355.280	361.620	346.390	347.220	353.500

Each 3 kg of premix contains: Vitamins; A: 12000000 IU, Vit. D3: 2000000 IU, E: 10000 mg, K3: 2000 mg, B1:1000 mg, B2: 5000 mg, B6:1500 mg, B12: 10 mg, Biotin: 50 mg, Pantothenic acid: 10000 mg, Nicotinic acid: 30000 mg, Folic acid: 1000 mg, Minerals: Mn: 60000 mg, Zn: 50000 mg, Fe: 30000 mg, Cu: 10000 mg, I: 1000 mg, Se: 100 mg and Co: 100 mg. T1: Control, T2: Control diet with 100 g t^{-1} Phyto-Cocci, T3: Control diet with 500 g t^{-1} Phyto-Cocci. Phyto Cocci price 6$ kg^{-1}

$$\text{Economic efficiency (\%)} = \frac{\text{Net return}}{\text{Total cost}} \times 100$$

Where:

Net return : Total return-total cost

Performance index (PI) was calculated according to North[9] as given below:

$$PI = \frac{BW\ (kg)}{FCR} \times 100$$

European performance efficiency Index (EPEI) was calculated by the following equation:

$$EPEI = \frac{LBW \times SU}{FCR \times age} \times 100,$$

Where:

LW : Live body weight (kg)

SU : Survival rate (%)

FCR : Feed conversion ratio

Age : Age of slaughter (d)

At the end of trial (five weeks. of age), broiler chicks were individually weighed in each group.

Four chicks from each treatment were randomly slaughtered. Samples of the duodenum from two birds per replicate were taken for each treatment at 17, 24 and 32 days of age; as duodenum was considered the part from the loop along the pancreas up to bile ducts insertion. Samples were cleaned with distilled water to remove the intestinal contents and fixed in 10% neutral buffered formalin solution then dehydrated, cleared and embedded in paraffin wax, then specimens were sectioned to 4 -5 micron thickness, prepared and stained with Hematoxylin and Eosin stain (H and E) and examined microscopically[10]. The height of the intestinal villi was determined by measuring from the base of the lamina propria to the tip of the villi for duodenum 10-13 intestinal villi per group were measured at 100 magnifications using ImageJ Analyzer software[11].

Oocyst count: A sample of 1 g of rectal content was taken from each specimen at 17, 24, 32 days of age, then mixed with 10ml physiological saline, sieved in a 150 micro-mesh to remove debris and poured in test tube and centrifuged at 1500 rpm for 5 min. Heavy material in the dropping stetted at the bottom and any suspension was thrown away. The material that remained in the test tube was mixed with 10 mL Aluminum nitrate and samples are removed with a Pasteur pipette and a McMaster counting chamber is filled. The number of oocysts was counted under the microscope.

$$\text{No. of Oocysts per gram of feces}^{12} = \frac{n \times \text{vol. (10)}}{0.15}$$

where, n is number of oocysts counted, vol. is 10 mL physiological saline that feces is soaked in 0.15 volume of the Mc-Master counting chamber.

Statistical analysis: Statistical analysis was conducted using the General Linear Model (GLM) procedure of SAS[13]. Means were compared using Duncan's Multiple Range Test[14], where the level of significance was set at minimum (*$p \leq 0.05$ or **$p \leq 0.01$).

The statistical model was:

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

Where:

Y_{ij} : An observation
μ : Overall mean
T_i : Effect of treatment
e_{ij} : Random error

RESULTS AND DISCUSSION

The live body weight and average weight gain of broiler chicks as affected by dietary treatments are illustrated in Table 2. Results revealed that chicks that were fed on diets supplemented with Phyto-Cocci Powder (T2 or T3) exhibited significant ($p \leq 0.05$) heavier live body weight at marketing age than those chicks which were fed on the control diet. Moreover, final live body weight of chicks fed T2 or T3 significantly increased ($p \leq 0.05$) by about 5.4 and 2%, respectively compared with the control group (T1).

The results indicated no significant difference due to dietary treatments on body weight gain (BWG) during starter and grower period (0-4 weeks). On the other hand, chicks that were fed on T2 diet showed the highest BWG during finisher and overall period (474 and 2087 g, respectively) compared to other groups.

These results agree with a previous study conducted by Ocak *et al.*[15], who reported significantly higher LBW at 21 and 42 day of age as well as higher BWG from 7-35 day of age in

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

	Treatments				
Parameters	T1	T2	T3	SEM	Sig.
Live body weight (g)					
Initial body weight (g)	44.53	44.53	44.24	0.08	NS
2 weeks	516.00^{a}	504.00^{ab}	483.00^{b}	5.52	*
4 weeks	1642.00	1658.00	1633.00	21.73	NS
5 weeks	2021.00^{b}	2131.00^{a}	2062.00^{ab}	28.21	**
Average weight gain (g)					
0-2 weeks.	236.00	230.00	220.00	4.57	NS
2-4 weeks	563.00	577.00	575.00	11.31	NS
4-5 weeks	379.00^{b}	474.00^{a}	429.00^{ab}	11.00	**
0-5 weeks	1976.00^{c}	2087.00^{a}	2017.00^{b}	26.59	**
Average feed consumption (g)					
0-2 weeks	603.00	613.00	608.00	10.24	NS
2-4 weeks	2128.00	2163.00	2106.00	46.33	NS
4-5 weeks	977.00	1003.00	974.00	18.88	NS
0-5 weeks	3708.00	3779.00	3688.00	45.05	NS
Feed conversion ratio (g feed g^{-1} gain)					
0-2 weeks	1.23	1.27	1.29	0.01	NS
2-4 weeks	1.84	1.83	1.78	0.02	NS
4-5 weeks	2.58^{a}	2.12^{c}	2.29^{b}	0.03	**
0-5 weeks	1.72	1.66	1.69	0.03	NS

$^{a, b}$Means within the same row with different superscripts are significantly different. SEM: Standard error Sig.: Significance, **($p \leq 0.01$), *($p \leq 0.05$). NS: Non-significant

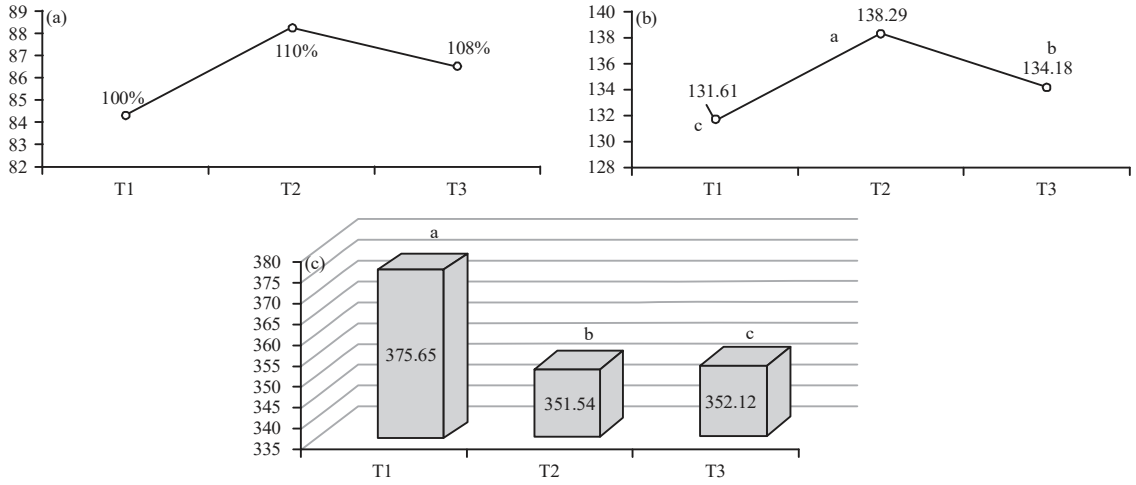

Fig. 1(a-c): Effect of dietary treatments on (a) economic efficiency, (b) Performance index and (c) European performance efficiency index

broilers that were fed on peppermint and thyme compared to the control group. Also, Al-Kassie[16] and Abid[17] reported that chicks fed on diet supplemented with thyme had significantly heavier live body weight and body weight gain compared with those that were fed on the control diet. Another study showed that an addition of 5 g kg^{-1} thyme herb improved BWG by about 6% when compared to the corresponding control group[18]. On the contrary, another study showed that using 5 g kg^{-1} thyme caused a substantial decrease in BWG approaching almost a level of significance[19].

With respect to feed consumption trait, it could be observed that chicks fed on T2 ration consumed more feed compared with those that were fed on other diets (T1or T3) at all phases of trial (starter, grower, finisher and overall). Generally, during studied period (0-5 weeks), there was no significant difference among treatments in feed consumption trait. Moreover, birds fed on T3 consumed less quantity of feed during the whole fattening period compared to others.

Another study showed that the presence of active compounds such as essential oil in the herbals stimulate appetite and improve the digestion and mineral absorption and increase feed efficiency in chicks[20]. The feed conversion ratio (FCR) describes the relation of feed intake and BW gain. More precisely, it is the animal's overall efficiency in converting feed mass into body mass over a specific period of time. Table 2 shows that during starter or grower periods, there was no significant difference in FCR among treatments. Whereas, during finisher period, chicks which were fed on T2 diet were more efficient by about17.8 and 7.4% in converting their feed into body weight gain compared with those which were fed on other dietary treatments (T1 or T3, respectively). The same trend was realized for overall FCR, where, T2 chicks had the best FCR followed by T3 and T1, respectively. The improvement in protein utilization, absorption and suppression of gram-negative bacteria and Clostridium that cause growth depression might be the reason for the improvement in FCR[21].

The non-significant effect of different dietary treatments on overall FCR of broilers was in line with the findings of Lee *et al.*[21] and Demir *et al.*[22]. On the other hand, Ali[23] and Alagawany *et al.*[24] found that supplementation of broiler diet with thyme had a significant positive effect on FCR. However, several commercial herbal blends were included in trials but only two studies confirmed a significant improvement in FCR. The addition of 100 mg kg^{-1} of RepaXol®optimized FCR by 4%[25], while the supplementation of 100 mg kg^{-1} of XTRACT™ enhanced FCR by 4.5%[19,26]. Furthermore, using oregano herbs in broiler diets improved body weight and feed intake at 1,000[27], 500[28], 100 and 250 ppm[29].

The data related to economic efficiency (EE), performance index (PI) and European performance efficiency index (EPEI) of broiler birds fed on a Phyto-Cocci feed additive were presented in Fig. 1a-c. On scanning the data of benefit-cost ratio, it was found that chicks fed on T2 had significantly the highest ($p \leq 0.05$) economic efficiency than other treatments. Also, the Performance Index (PI) was the highest in T2 compared to others. Similarly, the value of EE and PI was also significantly higher ($p \leq 0.01$) in T2 than other treatments. The results of the present study were in line with the findings of Alagawany *et al.*[24]. In concurrence with our findings, EL-Faham *et al.*[30] reported better economic efficiency and relative economic efficiency in birds fed on diet supplemented with herbal substance as compared with control group.

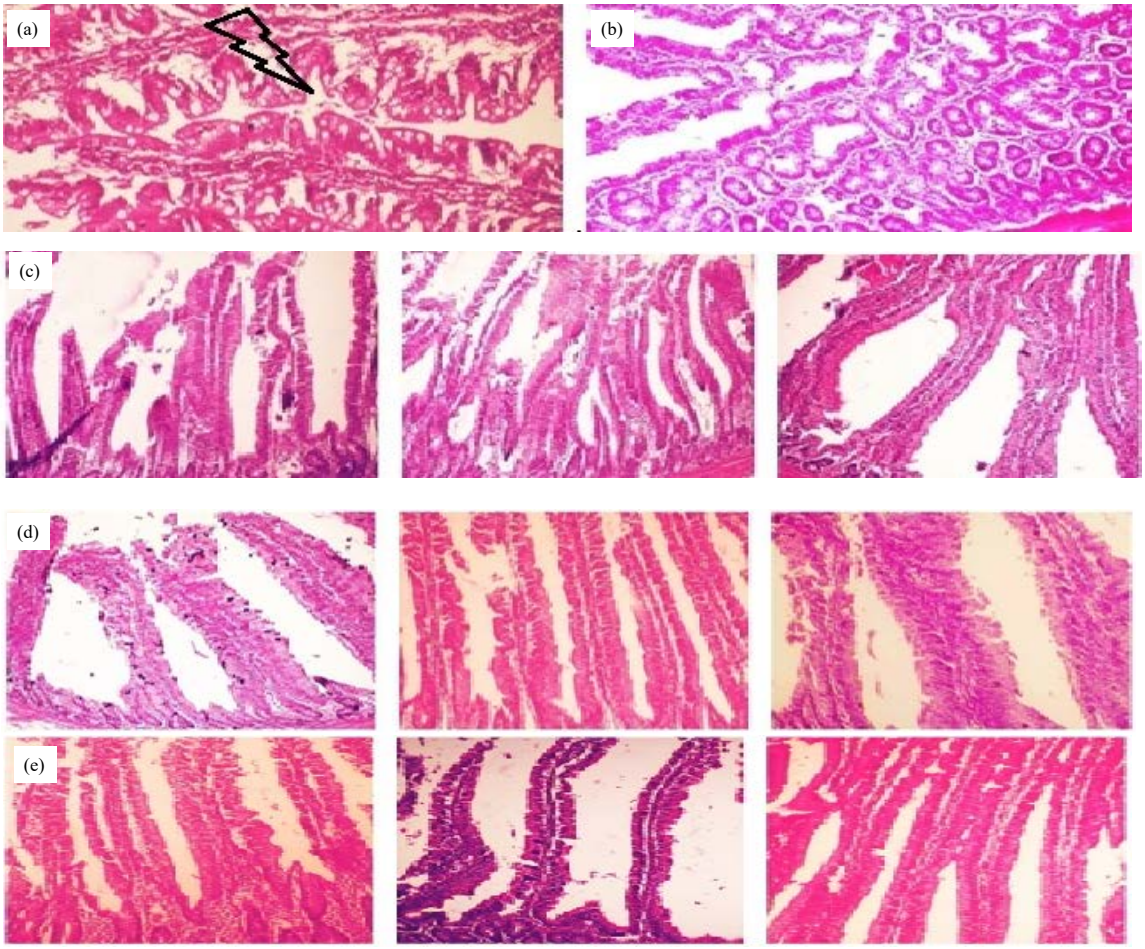

Fig. 2(a-c): Intestinal villi of chicks treated with Phyto-Cocci showed hyperplasia of (a) goblet cells. (HXE 200), (b) glandular lining epithelial cells. (HXE 200), (c) Intestine of control group showing the lowest values of villus length when tested at different times, (d) Intestinal villi of chicks treated with Phyto-Cocci at a dose of 100 g t^{-1} showing significant increase in villus width at 17 and 35 days of age and (e) Intestinal villi of chicks treated with Phyto-Cocci at a dose of 500 g t^{-1} showing significant increase in villus length when tested at different times

Table 3: Means (μm) of intestinal villi height and width at different ages (17, 24 and 35 days of age) as affected by treatments

	Villi height			Villi width		
Groups	17 Days	24 Days	35 Days	17 Days	24 Days	35 Days
T1	912^{c}	978.2^{b}	1244.5^{c}	196^{c}	240	247^{ab}
T2	1283^{b}	1424^{ab}	1561^{b}	320^{a}	213	341^{a}
T3	1518^{a}	1630^{a}	1985^{a}	202^{b}	177	162^{b}
Sig.	0	0	**	0	NS	0

Sig.: Significance, **($p \leq 0.01$), *($p \leq 0.05$). NS: Non-significant

Regarding histological aspects of the gut, the hyperplasia in goblet cells of the villi in T3 and T2 groups was detected (Fig. 2a) and also hyperplasia was observed in glandular epithelial cells of the villi in T3 and T2 (Fig. 2b). These results are in agreement with Eid *et al.*[31] who studied the activity of the thyme in commercial broiler rations and found more numerous effects on goblet cells.

Table 3 and Fig. 2c-e shows the villus height and width of the experimental groups. Findings showed that villus height was significantly longer in T3 group followed by T2 group compared to control group.

Regarding villus width, T2 group had significantly higher estimated values compared to other two groups as shown in Fig. 2d. The intestinal villi height depends on the balance

Table 4: Effect of dietary treatments on oocyst count at different ages (17, 24 and 35 days of age)

	Oocyst in 1 g feces		
Group	Count in 17th day	Count in 24th day	Count in 35th day
T1	0	700±30.25[a]	3275±188.73[a]
T2	0	425±25.66[b]	2600±05.55[b]
T3	0	350±11.70[c]	1450±70.30[c]
Sig.	NS	*	**

Sig.: Significance, **($p \leq 0.01$), *($p \leq 0.05$). NS: Non-significant

between proliferation, migration and cell apoptosis. The main function of the villi is the absorption of nutrients, an activity which is partially dependent on its size[32]. Villi can be considered as an intestinal health indicator. These results may be due to antimicrobial agents which are known to reduce the intestinal microbial load, which in turn reduces the presence of toxins at this level that are associated with changes in intestinal morphology, such as shorter villi and deeper crypts[31]. Similar observations are demonstrated by Garcíaa Francisco Escalera Valentea *et al.*[33], who reported that the height of intestinal villi increased with the level of oregano oil supplementation. Natural additives can improve the epithelium surface structure and increase the enterocytes size[34]. Olnood *et al.*[35] reported that phytogenic additives may be favorable for the development of bacteria and improve growth of the anatomical structures. However, the variation in oregano oil components and diet are factors that can change these effects. Chiang *et al.*[36] and Sayrafi *et al.*[37] reported that the villi height increased with the amount of oregano oil in the feed, an effect that was also observed in the experiment both for three and six week-old chicks.

Table 4 shows the effect of Phyto-Cocci on oocyst count at different ages (17, 24 and 35 day of age). Chicks fed T3 and T2 diet respectively had a positive and significant effect on oocyst count (reduce oocyst count) compared to T1 group. Therefore, it is also observed that Phyto-Cocci was more effective against coccidiosis with high dosage.

Chicks that were fed on phytogenic feed additives showed longer ileal villi with excellent gut health, high absorptive efficiency and healthier intestinal tract[38]. The recent studies reported that dietary supplementation of thymol and carvacrol reduced intestinal lesions, improved the intestinal histomorphology and enhanced the specific immune response[39].

In general, Akyurek and A. Yel[40] mentioned that carvacrol diet from one day to seven days of age, improved weight gain, reduced gut lesions and the oocyst shedding lowered pro-inflammatory cytokine gene expression during coccidiosis in birds challenged with *Eimeria acervulina*. Further carvacrol regulated lipid metabolism in intestinal intra epithelial lymphocytes of broilers. In another study, altered expression of 74 genes (26 up regulated, 48 down regulated intestinal intra epithelial lymphocytes) was observed in chicks that were fed on carvacrol[24].

CONCLUSION

Supplementation of Phyto-Cocci Powder increased the final LBW in 35 days and improved the FCR in 28-35 days, economic efficiency, performance index and gut parameters and health. Overall, the present study demonstrated the efficacy of Phyto-Cocci Powder utilization and confirms its importance in poultry diets.

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