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Research Article
Synergistic Effects of Synbiotics and Essential Oil on Coccidian Control in Broiler Chickens

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Abstract

Background and Objective: The use of antibiotic growth promoters and coccidiostats drugs in poultry feed led to human health hazard, it has become necessary to develop a natural alternative. This study discovers the possible synergistic effect of probiotics, prebiotics, butyrate and essential oil (All-Pro feed) that can be beneficial for improving the growth performance of broiler chickens and coccidian control. **Materials and Methods:** A total of 480 one-day-old Ross 308 broiler chicks (as hatched) were subjected to a 5 weeks dietary experiment. The chicks were randomly divided into 2 experimental groups, group 1 fed basal diets only (T_1), group 2 fed basal diets supplemented with 750 g t^{-1} (All-Pro feed) T_2. Each treatment comprised 4 replicates (60 chicks per replicate). It includes parameters for production performance as body weight gain feed consumed and feed conversion ratio. Immunological, histomorphology and coccidian study. Statistical analysis was conducted using the student's t-test. **Results:** There was a significant increase in Body Weight Gain (BWG) in the group treated with All-Pro feed compared with the control group in the hall period of the experiment. There was a significant improvement in Feed Conversion Ratio (FCR) between the control and treated group except for the 3rd week. Adding All-Pro feed increased villus height and width linearly, increased humoral immunity and reduced coccidian oocyte shedding in faeces of chickens at different ages. **Conclusion:** From the above study can prove the synergistic action of herbal extract with synbiotic to control coccidiosis in chickens in addition to its role as growth promoters.

Key words: Probiotics, prebiotics, butyrate, essential oil, histomorphology, coccidiosis, immunity

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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 that are added to livestock feed to improve the utilization of feed and in this way realize better production and financial results. 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 public concerns about their residues in animal products and the development of antibiotic-resistant bacteria[2]. As a consequence, it has become necessary to develop alternatives using either beneficial microorganisms (probiotics) or non-digestible ingredients (prebiotics) that enhance microbial growth. A way of potentiating the efficacy of probiotics preparation may be the combination of both probiotics and prebiotics (synbiotic) that beneficially affects the host by improving the survival and implantation of live dietary microbial supplements in the gastrointestinal tract. Those effects are due to activating the metabolism of one or a limited number of health-promoting bacteria or by selectively stimulating their growth, which improved the welfare of the host or both[3].

Phylogenic considered one of the promising alternatives because of its significant potentiality to promote growth, enhance feed intake and stimulate immunity[4]. Besides, they could improve feed intake, feed conversion ratio and carcass yield[5].

Butyrate is important as food for cells lining the colon (colonocytes). Without butyrate for energy, colon cells undergo autophagy (self-digestion) and die[6]. Short Chain Fatty Acids (SCFAs), which include butyrate are produced by beneficial colonic bacteria (probiotics) that feed on or ferment prebiotics, which are plant products that contain adequate amounts of dietary fibre. These SCFAs benefit the colonocytes (cells of the colon) by increasing energy production and cell proliferation. When butyrate is present in the bloodstream or the proximal parts of the intestinal tract, it induces the production of host defence peptides[7]. These peptides stimulate the development and repair of the intestinal tract through an increase in cell proliferation[8]. Recently it has been shown that butyrate, when present in the blood, stimulates a peptide that increases the absorption of glucose from the intestine. Indications that a similar mode of action can be expected in poultry, is shown by previous study[9], which found an increased development of the villi when sodium-butyrate was added to the diet. Butyrate also has been shown to stimulate several functions in the lower part of the intestinal tract. Studies have identified specific G-protein-coupled receptors, specifically G-Protein-Coupled Receptor 41 (GPR 41) and GPR 43, on gut epithelial cells in the epithelium of particularly the ileum, caeca and colon[10]. When butyrate is attached to these receptors the production of several different peptides is stimulated[11,12]. Some of these peptides have a positive effect on the development of the immune system and improve the functioning of the immune system in case of a health challenge[11]. Other peptides have been shown to optimize gut motility, by reducing the rate of feed passage[1]. In poultry, the emptying of the feed out of the gizzard into the small intestine is slowed down. Thus, it seems that butyrate is inducing a similar effect to passage rate as coarse particles such as an oyster shell.

Indications that butyrate also stimulates the immune system in poultry were obtained by Leeson *et al.*[13] in that, birds previously fed butyrate showed more ability to withstand the stress of coccidial challenge at 21 days of age. Weber and Kesr[14] found that when pigs were challenged with *Escherichia coli* Lipopolysaccharide (LPS), sodium butyrate increased the magnitude of the cortisol response and increased skeletal muscle IL-6 mRNA expression, also indicating that dietary butyrate affects the response to inflammatory stimuli.

The objectives of the current study were to evaluate the effects of a mixture of prebiotics, probiotics, butyrate and herbal extracts (All-Pro feed®) on growth performance, small intestine histopathology, coccidian oocytes excretion and humoral immunity.

MATERIALS AND METHODS

Study area: This trial was carried out at Poultry Production Farm, Feed Research House, Orabi Community, Qalyobia Governorate Egypt during 2019-2020. The laboratories study was carried out at the Animal Health Research Institute of Pharmacology and Parasitology Laboratory, Giza, Egypt.

Experimentation: A total of 480 Ross 308 broiler chicks (as hatched) at one-day-old with an initial body weight of around 44 g were obtained from a local commercial hatchery. The chicks were weighed and randomly distributed in 8 pens to examine the effect of 2 experimental treatments comprising two feeding regimens: (T_1) Basal diet without All-Pro feed supplement (control group) and a basal diet supplemented with 750 g t^{-1} All-Pro feed (T_2).

Each group contained 240 chicks which were allotted into 4 replicates and each replicate contained 60 chicks. All the experimental birds were reared in a well-ventilated shed on softwood shaving litter used as bedding material in pens with 2.0×3.0 m^2 dimensions and kept under uniform management

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

	Dietary treatments					
	Starter		Grower		Finisher	
Ingredient	T_1	T_2	T_1	T_2	T_1	T_2
Yellow corn (grains)	59.391	59.381	66.092	66.061	70.890	70.880
Corn gluten meal (60%)	1.783	1.783	4.599	4.600	6.543	6.543
Soybean oil	0.500	0.500	0.500	0.500	1.000	1.000
Soybean meal (44%)	34.720	34.720	25.273	25.293	18.017	18.017
Calcium carbonate	1.382	1.382	1.225	1.225	1.294	1.294
Mono-Calcium phosphate	1.030	1.030	0.961	0.961	0.924	0.924
Sodium bicarbonate	0.082	0.082	0.239	0.239	0.200	0.200
Salt (NaCl)	0.218	0.218	0.113	0.113	0.144	0.144
HCl lysine	0.347	0.347	0.505	0.505	0.535	0.535
DL-Methionine	0.197	0.197	0.144	0.144	0.124	0.124
L-Threonine	0.000	0.000	0.049	0.049	0.029	0.029
Choline chloride 60%	0.050	0.050	0.000	0.000	0.000	0.000
Premix	0.300	0.300	0.300	0.300	0.300	0.300
ALL-PRO FEED	0.000	0.750	0.000	0.750	0.000	0.750
Total	100.000	100.000	100.000	100.000	100.000	100.000
Calculated chemical composition						
Crude protein (%)	22.00	22.00	20.00	20.00	18.20	18.20
Metabolizable energy (Kcal kg^{-1})	3000	3000.00	3100	3100	3200	3200
Linoleic acid	1.50	1.50	1.60	1.60	1.91	1.91
Calcium (%)	1.00	1.00	0.90	0.90	0.90	0.90
Available phosphorus (%)	0.50	0.50	0.47	0.47	0.45	0.45
Lysine (%)	1.40	1.40	1.30	1.30	1.15	1.15
Methionine (%)	0.56	0.56	0.50	0.50	0.47	0.47
Methionine+Cysteine (%)	0.98	0.98	0.90	0.90	0.85	0.85

Each 3 kg of premix contains: Vitamins: A: 12000000 IU; Vit. D_3 2000000 IU; E: 10000 mg; K_3: 2000 mg; B_1:1000 mg; B_2: 5000 mg; B_6:1500 mg; B_{12}: 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. T_1: Control, T_2: Control diets with750 g t^{-1} All-Pro feed

conditions. Basal starter (0-14 days), grower (15-24 days) and finisher (25 day-end) diets were formulated according to the nutritional recommendation for broilers[15], their composition and calculated analysis are shown in Table 1. All-Pro feed Powder is a dry stabilized preparation manufactured by (Kanzy Medipharm, Canada) it is a mixture of probiotics, prebiotics, butyrate, essential oil and silicate as (carrier). Feed and water were available *ad-libitum* during the experiment. All the birds were weighed at the same time weekly before feeding.

Feed intake calculation: Feed intake was calculated by measuring the amount of feed offered and residue left. The Feed Conversion Ratio (FCR) was calculated by dividing the feed intake by weight gain.

Parameters for productive performance (body weight, body weight gain, feed consumption and feed conversion ratio) were measured on weekly basis throughout the experimental period. The 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:

- Feed consumed = feed consumed per pen/ (number of surviving birds×days of the period)+days of died birds alive
- Feed Conversion Ratio (FCR) was estimated using the following expression:

Feed conversion ratio = feed consumed in each replicate/total weight gain (with a weight gain of died or culled chickens).

Vaccination and other routine poultry management practices were carried out neatly.

The groups were experimentally infected with coccidiosis. For this purpose, a suspension containing *E. tenella* oocytes in 2.5% potassium dichromate solution a 100,000 oocysts per 1 mL suspension. The groups of chickens were infected for 14 days by giving 1 mL suspension containing *E. tenella* oocytes by gavages.

Histopathological study: For histopathology, slides were prepared for duodenum samples of the dissected birds on day 17, 24 and 32 of age as the duodenum was considered the part from loop along with 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. Morphometric measurements of duodenum villi were performed including villus length and villus width 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 9-10 intestinal villi per sample were measured for each intestinal section and each bird was measured at 40 magnifications using ImageJ Analyzer software[16].

Oocyst counting: A sample of 1 g of rectal content was taken from each specimen at 17, 24 and 32 days of age, then mixed with 10 mL physiological saline, sieved in a 150 micro-mesh to remove debris and poured in a test tube then centrifuge 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 a microscope (Long *et al.*[17]).

$$\text{Number of oocysts per gram of faces} = \frac{n \times \text{Vol. (10)}}{0.15}$$

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

Immunity assessment: To investigate the possible effect of All-Pro feed on humoral immunity, an immunoassay was adopted. For this purpose, blood samples were collected from wing veins from 5 randomly selected birds at weekly intervals (1-5 weeks of age) from each group. The serum samples were subjected to HI test for determining antibody titers against Newcastle Disease (ND) vaccine employing 8 HA units as described by Swayne *et al.*[18].

Statistical analysis: Statistical analysis was conducted using the student's t-test[19].

RESULTS AND DISCUSSION

The live body weight and average weight gain of broiler as affected by dietary treatments are illustrated in Table 2. Results revealed that chickens fed diets supplemented with All-Pro feed Powder (T_2) exhibited significant ($p \leq 0.05$) heavier live body weight (2098±29.48) at marketing age than chickens fed the control diet (1983.51±19.44).

The results indicated a significant difference due to the main effects of dietary treatments on Body Weight Gain (BWG)during the breeding period. These results are in the same line with the results published by Ocak *et al.*[20], who

Table 2: Effect of dietary treatment on the productive performance of broiler chickens

	Groups						
			LBW				
Treatments	Mean±SE	IBW	$Week_1$	$Week_2$	$Week_3$	$Week_4$	$Week_5$
Live body weight							
T_1	Mean±SE	44.74±0.17	175.21±3.53	443.14±7.69	890.57±10.197	1535.9±10.98	1983.5±19.44
T_2	Mean±SE	44.53±0.31	197.86±1.45	515.66±3.94	970.85±12.36	1641.7±8.14	2098.2±29.48
Sig.		N.S	***	***	**	***	*
Body weight gain		0					
T_1	Mean±SE		130.47±3.63	267.93±8.56	447.43±3.74	645.36±9.65	447.57±14.26
T_2	Mean±SE		153.33±3.75	317.79±5.06	455.2±8.86	670.82±9.66	466.17±27.21
Sig.			**	**	N.S	*	*
Feed intake							
T_1	Mean±SE		152.16±1.49	426.23±2.40	668.14±5.53	1339.3±14.82	956.23±11.60
T_2	Mean±SE		157.41±1.25	441.41±4.31	672.04±3.21	1354±33.82	964.48±21.55
			*	*	N.S	N.S	N.S
FCR							
T_1	Mean±SE		1.17±0.03	1.6±0.05	1.49±0.01	2.08±0.03	2.14±0.05
T_2	Mean±SE		1.03±0.02	1.39±0.03	1.42±0.03	1.98±0.04	1.9±0.15
			*	**	N. s	*	0

N.S: Non-Significant, * Sig.: Significance, ($p \leq 0.05$), **Highly Significant at $p<0.01$, *** Very highly significant at $p<0.001$, FCR: Feed conversion ratio, Wk: Week, T_1: Control, T_2: Control diets with750 g t^{-1} All-Pro feed

reported significantly higher LBW at 21 and 42 days of ages as well as higher BWG from 7-35 days of age in broilers fed peppermint and thyme compared to the control group. Also, Al-Kassie[21] and Abid[22] showed that chickens fed with thyme supplement had significantly heavier live body weight and body weight gain compared with those fed 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[23]. On the contrary, other studies showed that using 5 g kg^{-1} thyme caused a substantial decrease in BWG approaching almost a level of significance[24]. Studies on the beneficial impact on chicken's performance have indicated that probiotic supplementation can have positive effects. It is evident from the result of Kabir *et al.*[25] that the live weight gains were significantly ($p<0.01$) higher in the experimental birds as compared to control ones during the period of 2nd, 4th, 5th and 6th weeks of age. Besides Torres-Rodriguez *et al.*[26] reported that administration of the selected probiotic (FM-B11) to turkeys increased the average daily gain and marked body weight (BIV) representing an economic alternative to improve turkey production. Mechanisms by which probiotics improve feed conversion efficiency include alteration in the intestinal flora, enhancement of growth of nonpathogenic facultative anaerobic and gram-positive bacteria forming lactic acid and hydrogen peroxide, suppression of growth of intestinal pathogens and enhancement of digestion and utilization of nutrients[27]. Using probiotic microorganisms shorten the period needed to stabilize the microflora. This microflora regulation may serve to improve feed conversion, weight gain and also improve the intestinal health and immune competence of the chickens[28]. For feed consumption trait, it could be observed that during the studied period (0-5 weeks), there was no statistically significant difference among treatments in feed consumption trait except starter period there was a mild significant difference between T_2 (441.41±4.31) and T_1 (426.23± 2.40). Accordingly, Lee *et al.*[29] the positive effects can be evaluated based on different perspectives as thyme improves diet palatability by influencing the main components of the thymus that stimulate and improve the appetite and the digestive process. The Feed Conversion Ratio (FCR) describes the relationship between feed intake and Body Weight (BW) gain. More precisely, it is the animal's overall efficiency in converting feed mass into body mass over a specific time. It was obvious from Table 2 that during starter or grower periods, there was a significant effect on FCR among treatments except for 3rd week. Whereas, during the finisher period, chickens fed (T_2) diet were more efficient (1.90±0.15) in converting their feed into body weight gain compared with control group T_1 (2.14±0.05). Accordingly, the improved FCR could be due to enhancing the efficiency of energy and nutrient utilization or altered carcass composition[30]. On the other hand, Alagawany *et al.*[31] and Zhang *et al.*[32] found that supplementation of broiler diet with thyme had a significant positive effect on the FCR of broilers. However, several commercial herbal blends were included in trials but only two studies confirmed a significant improvement of FCR. The addition of 100 mg kg^{-1} of RepaXol® optimized FCR by 4%[33]. Furthermore, using oregano herbs tested in broiler diets improved body weight and feed intake at 1,000[34], 500[35], 100 and 250 ppm[36]. Global financial losses due to coccidiosis in broiler flocks are estimated to be more than 3 billion annually[37]. The addition of coccidiostats to poultry feed can control the diseases but the emergence of drug resistance as well as possible future ban restricting coccidiostat use mean that there is an urgent need for an alternative method of reducing the burden of disease in poultry[38]. Effect of All-Pro feed on oocytes count at different ages (21 and 32 days of age) T_2, comparing with the control groups T_1 was obvious in Table 3. All-Pro feed treated groups had a significant decrease in the number of shaded oocysts after 21 days (454,000±38807) compared to the T_1 group (698,000±33643). While there was a highly significant difference between T_1 (822,200±32456) and T_2 (322400 ±33643) on day 32. Essential oils contain known active ingredients including, phenols, aldehydes, terpenes and oxides which have a direct anti-parasitic effect. The majority of the antimicrobial prowess of carvacrol and thymol can be attributed to creates membrane permeability problems for the organisms[39]. Carvacrol has been shown to activate and desensitize receptors in calcium channels[40]. Carvacrol may to the observed inhibition of sporozoites invasion by disrupting calcium-mediated signalling in the sporozoites. Gianmenas *et al.*[41] reported that carvacrol and thymol increased broilers weight gain and lower oocyst counts compared to infected, untreated controls. Finally, Ovideo-Rondon *et al.*[42] showed that treatment of *Eimeria* spp. infected broilers with two specific EO blends (containing thymol, eugenol, curcumin, piperine) prevented major shifts in the intestinal microbial communities as compared to non-treated

Table 3: Oocytes count in 1 g faeces in control and treated group at 21 and 32 days

Groups	21 days	32 days
T_1 Control group	698000±4979	822200±32456
T_2 All-Pro feed 750 g t^{-1}	454000±38807	332400±33643
Sig	**	***

**Highly significant at $p<0.01$, ***Very highly significant at $p<0.001$, (T_1): Control, (T_2): Control diets with750 g t^{-1} All-Pro feed

Table 4: Effect of all-pro feed on Hemagglutination Inhibition (HI) antibody titer against Newcastle disease vaccine of control and treated group

		Groups			
Age treatments		1 week	2 weeks	3 weeks	4 weeks
T_1	Mean±SE	5.32±0.136	2.14±0.238	7.16±0.181	6.9±0.071
T_2	Mean±SE	6.0±0.158*	4.56±0.186	7.42±0.213	7.86±0.121
Sig.		*	**	N.S	*

N.S: Non-Significant *Sig.: Significance, ($p \leq 0.05$), **Highly Significant at $p<0.01$, T_1: Control, T_2: Control diets with750 g t^{-1} all-pro feed

Table 5: Means (μm)±SE of intestinal villi length and width at different ages (17, 24 and 32 days of age) as affected by treatments

	Villi height			Villi width		
Groups	17	24	32	17	24	32
T_1	495.4±33.65	548.2±4.71	582.6±19.14	83.4±4.40	84.3±3.58	87.1±7.46
T_2	655.6±12.06	698.8±18.06	811.2±10.04	80.2±4.33	81.1±3.61	65.3±2.88
Sig.	**	***	***	N.S	N.S	*

N.S: Non-Significant, *Sig.: Significance, at ($p \leq 0.05$), **Highly Significant at $p<0.01$, ***Very highly significant at $p<0.001$, T_1: Control, T_2: Control diets with750 g t^{-1} All-Pro feed

controls, leading to less severe clinical signs. The results of these studies suggested that in broilers, essential oils could be an alternative to drugs and/or vaccination for protection against *E. tenella* infection. The results of the humoral immune response are presented in Table 4 which indicated a significant increase in the All-Pro feed treated group (7.86±0.121) over the control group (6.9±0.071) in the HI titers against ND vaccine at different examined intervals. Brander *et al.*[43] reported that immunomodulators administered simultaneously with antigens might potentiate specific immune responses, particularly to vaccines. Chau *et al.*[44] concluded that β-Glucan can be efficacious as an oral adjuvant to enhance immunoglobulin production in response to vaccination. Williams *et al.*[45] and Lowry *et al.*[46] suggested that the protective effect of β-glucans might be due to the antioxidant capacity, antimicrobial activities as well as the inhibition of early activation of tissue muscular Nuclear Factor-KB (NF-KB) and NF-IL6. In the present investigation, the results of villus height and width of the control and treated groups are given in Table 5. Findings of the intestinal villi to the control group fed basal diet showing normal length with lining epithelial simple columnar absorptive cell and goblet cells with slightly increasing of the average length of the villi with times after slaughter 17, 24 and 32 days by scale bar measurement 495.4±33.65, 548.2±4.71 and 582.6±19.14 respectively and also slightly increasing of the average width of the villi with times after slaughter 17, 24 and 32 days by scale bar measurement 83.4±4.40, 84.4±3.58 and 87.1±7.46 um respectively. The luminal side of the intestinal wall is lined with absorptive columnar epithelial cells, needed for water and nutrient uptake and mucin-producing goblet cells which are important in innate defences that form a semi-permeable barrier. This semi-permeable formed by the cell membranes of the epithelial cells and tight junctions that connect neighbouring epithelial cells[47]. The permeability of the intestinal epithelial cell layer can be affected by epithelial cell death and also by luminal signals that increase the epithelial layer permeability by affecting the tight junctions and thus cause loss of integrity of this important barrier[48]. Regarding intestinal histomorphology of the chickens in the treated group fed basal diet and 750 g t^{-1} of All-Pro feed showing increasing the length of the intestinal villi with increasing the time after slaughter 17, 24 and 32 days with average length 655.6±1, 698.8±1 and 811.2±1 respectively and average width 80.2±1, 81.1±1 and 65.3±1 respectively. The villus height was significantly longer in the T_2 group which was given All-Pro feed in a dose of 750 g t^{-1}, besides increasing the length of villi with age, so villi were longer at day 32 than 24 and 17 days old of chickens, respectively. However, villus width did not differ significantly in the experimental groups as well as in the control. These results may be due to higher levels of intestinal probiotics in birds fed with Mannan Oligosaccharides (MOS) that resulted in the improvement of gut health status. Mannan oligosaccharides have been reported to increase villus height, surface area and decrease crypt depth[49,50]. Similarly, Cheled-Shoval *et al.*[51] reported that administration of MOS enhanced the villus area. When butyrate is present in the bloodstream or the proximal parts of the intestinal tract it induces the production of host defence peptides[7] these peptides stimulate the development and repair of the intestinal tract through an increase in cell proliferation[8] recently it has been shown that butyrate, when present in the blood, stimulates a peptide that increases the absorption of glucose from the intestine. Hu and Guo[9] found an increased development of the villi when sodium butyrate was added to the diet. When butyrate is attached to specific G-protein- coupled receptors specifically GPR 41 and GPR 43 on ileum epithelial cells, the production of several different

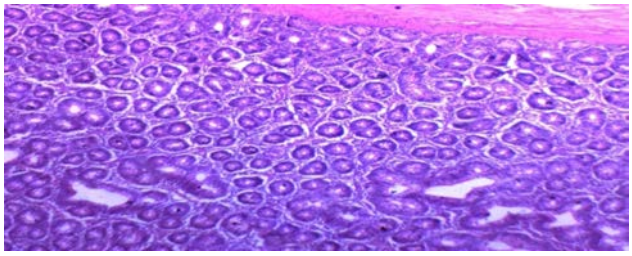

Fig. 1: Intestinal gland of chickens treated with all pro feed 750 g t^{-1} showing proliferation in glandular lining epithelial cells. (H and E)

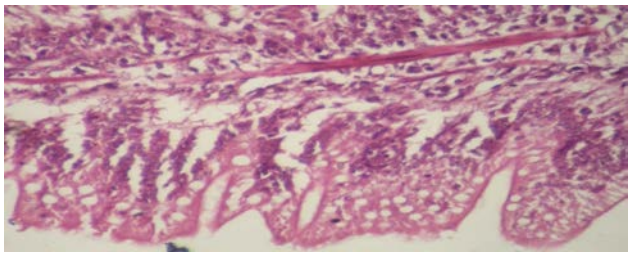

Fig. 2: Intestinal villi of chickens treated with all pro feed 750 g t^{-1}

Image is showing simple columnar epithelium containing hyperplasia of goblet cells and the core of the villi containing loose connective tissue (H and E400)

peptides is stimulated, some of these peptides have a positive effect on the development of the immune system[11] other peptides have been shown to optimize gut motility, by reducing the rate of feed passage[12]. Also, Alfaro *et al.*[52] and Alaeldein *et al.*[53] mentioned that chickens fed phytogenics feed additives were showed longer ileal villi with excellent gut health, high absorptive efficiency and healthier intestinal tract. The recent studies reported that dietary supplementation of thymol and carvacrol reduced intestinal lesions, improved the intestinal histomorphology and enhanced the specific immune response[54]. In the present study chickens treated with basal diets supplemented with 750 g t^{-1} (All-Pro feed) in the 2nd group showed proliferation in glandular lining epithelial cells of the intestinal villi (Fig. 1) and simple columnar epithelium containing hyperplasia of goblet cells and the core of the villi containing loose connective tissue in 2nd group (Fig. 2). These results are agreed with the previous researchers[55-57] as they mentioned that Mannan oligosaccharides have been reported to induce numbers of sulphated-acidic goblet cells. It has been reported that sulphated-acidic goblet cells are less degradable by the pathogen's glycosides[58,59]. Therefore, they can provide stronger protection against pathogens for the host. Similarly, Cheled *et al.*[51] reported that administration of MOS enhanced the proliferation of goblet cells, synthesizing and secreting more mucin, which plays an important role as the first line of defence. Mucin can trap pathogens or impede them from invading epithelial cells[60]. Besides, higher levels of intestinal prebiotics and probiotics in birds feed may further result in the improvement of gut health status, the increase of probiotics may improve intestinal development, whereas mucin produced by goblet cells can conversely limit attachment of pathogens to epithelial cells and prevent its invasion so improving health and productivity of broilers[61]. Also, Supplementation of butyrate in the feed can beneficially influence growth performance and intestinal villus structure in broiler chickens[49] which play role in the control of pathogens such as *Salmonella enteritidis* and Clostridium perfringens[62,63] as well as a decrease of necrotic lesions induced by *C. perfringens* in the small intestine[64]. Generally, when butyrate-producing bacteria are present in a sufficiently high concentration, the epithelial barrier integrity will be stronger, the epithelial cells will proliferate more and thus the villi will be longer. Moreover, inflammatory reactions will be reduced, while the stimulation of regulatory T-lymphocytes will yield a state of tolerance toward non-harmful bacteria[65]. The present finding suggested using butyrate with synbiotics to improve body weight gain and use essential oil in the control of coccidian in the recommended dose, above dose may cause a negative result and need to study in another research article.

CONCLUSION

The present finding provides evidence of the encouraging role of All-Pro feed as coccidian control in chickens without using anti-coccidian drugs. Besides its role in improving bird growth performance and intestinal histomorphology. More studies are needed to explore its capabilities to support this study.

SIGNIFICANCE STATEMENT

This study discovered the possible synergistic effects of symbiotic, butyrate and essential oil combination to control coccidiosis and improve growth rate in broiler chickens. This study will help the researcher to uncover the critical area of how butyrate works in GIT and improve the histomorphology picture of the intestine that many researchers were not able to explore.

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