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Dietary Supplementation of Synbiotic Formulation with Phytoactives on Broiler Performance, Relative Ready-to-Cook Weight, Health, Nutrient Digestibility, Gut Health, and Litter Characteristics

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ABSTRACT

This study investigated the effects of dietary synbiotic formulation with phytoactives (SFP) on growth performance, health, ready-to-cook (RTC) weight, gut health, nutrient digestibility, and litter characteristics in broiler chickens. Nine hundred one-day-old Cobb 430Y broiler chicks were randomly assigned to five groups: T0, T1, T2, T3, and T4, with two replicates of 90 birds each on day one of the feeding trials. T0 was raised on regular feed without antibiotic growth promoters (AGPs). In contrast, T1, T2, T3, and T4 were raised on regular feed and supplemented per ton of feed with Enramycin at 100 g, SFP at 100 g, 150 g, and competitor probiotic product (CPP) at 100 g. Supplementation of SFP at 150 g/ton of feed affected the performance, RTC weight, gut health, health, nutrient digestibility, litter characteristics, and overall health of Cobb 430Y broiler chickens. SFP is a possible antibiotics alternative in broiler chickens' diets. SFP supplementation at 100 g/ton (T2) and 150 g/ton (T3) performed similarly to Enramycin (T1) and CPP (T4) in terms of litter characteristics. It caused a reduction in fecal water-soluble phosphorus (WSP) of 16.67%, 16.67%, and 23.08%, respectively, when compared to control (T0), Enramycin (T1), and CPP (T4).

1. INTRODUCTION

Antibiotic growth promoter (AGP) use in the poultry industry has significantly increased poultry production worldwide [1]. Chickens fed antibiotic supplementation showed noticeable changes in their gut flora. It affected their immune systems and improved their ability to fight illnesses [1]. However, uncontrolled distribution and overuse of antibiotics result in bacterial antibiotic resistance, increasing the host's susceptibility to infection. Additionally, the overuse of antibiotics increases the possibility that animal products will contain antibiotic residues, which is harmful to human health as well as the health of animals [2]. Because of this, antibiotics in animal feed are prohibited in all developed nations, including Europe. As a result, safer alternatives to AGP's with comparable or superior effects on animal growth are required [3].

When consumed in sufficient amounts, probiotics, or live microbes, help the host's health [4]. Probiotics support gut maturation and integrity, boost the immune system, lower inflammation, and maintain a healthy population of beneficial bacteria in the digestive tract. Additionally, they improve feed intake and digestion by increasing the activity of digestive enzymes. They reduce the activity of bacterial enzymes produced by harmful bacteria, thereby benefiting chickens of all ages and classes. Additionally, probiotics help lower ammonia production, neutralize enterotoxins, and enhance birds' immunological functions [5,6]. Animal products (meat, milk, and eggs) do not include probiotic residues, which improve the health and performance of animals [7].

Probiotics alter the intestinal ecology by delivering digestive enzymes, lowering pH, and enhancing the activity of digestive enzymes in the gastrointestinal tract [8]. They effectively combat Salmonella to prevent avian illness and improve the bird's performance by positively impacting the gut microbiota [9]. The intestinal microbiota is modulated, and pathogens are reduced, improving broiler meat's quality and sensory properties [10]. Probiotic supplementation in feed significantly affects birds' live weight gain, carcass yield, and immunity [6]. In addition to enhancing litter quality, probiotics impact cecal microbial activity. They may further reduce the quantity of undigested phytate in the gut, leading to more significant non-phytate phosphorus (P) levels in the feces [11].

Prebiotics has been proposed as possible antibiotic substitutes. Prebiotics are a source of food for the good bacteria in the gut. Prebiotics act by modulating microbiota composition and controlling pathogenic infections. They improve intestinal morphology, immunity, and production. Plants containing non-digestible carbohydrates are natural prebiotics that promote beneficial gut microbes' growth and are suitable substrates for developing probiotics [12].

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According to research, the birds' gastrointestinal tract has been found to benefit from both probiotics and prebiotics. Probiotic and prebiotic combination is synbiotic [13]. Given that its specialized substrate is accessible for fermentation, this combination may increase the persistence and survival of the bacterium that promotes a healthy gut in birds. Numerous studies [14,15] have demonstrated the potential benefits of synbiotics for chickens' immune systems and intestinal microbial ecosystems. When administered in conjunction rather than separately, synbiotics are more effective. Broiler chicken appears to perform better because of the improvement in intestinal architecture and nutrient absorption by symbiotic feeding [16].

Using herbs and herbal extracts to supplement poultry feed rapidly increases following the antibiotic ban to augment production performance and health and prevent infections. Therefore, the polyherbal formulation SFP was developed by the M/s. Himalaya Wellness Company in Bengaluru, Karnataka, India. It is formulated with phytoactives, synbiotics, and herbal prebiotics. SFP claims to possess augmentation effects on growth parameters, carcass quality, gut health, and immunity enhancement in commercial broilers. This study was planned to assess SFP efficacy on performance, ready-to-cook (RTC) weight, health, gut health, nutrient digestibility, and litter characteristics in commercial broiler chickens compared to antibiotic growth promoters (AGPs) and competitor probiotic products (CPPs).

2. MATERIALS AND METHODS

2.1. Synbiotic Formulation with Phytoactives (SFP)

SFP is a proprietary polyherbal preparation called HimFlora, developed through the M/s. Himalaya Wellness Company in Bengaluru, Karnataka, India. HimFlora comprises the probiotics *Bacillus subtilis, Bacillus pumilus, Bacillus coagulans, Bacillus polymyxa,* herbal actives, *Zingiber officinale*, extract of *Curcuma longa,* and prebiotic powder of yeast β-glucan.

2.2. Experimental Setup

Five dietary supplementation groups were established for the 1-day-old nine hundred 430Y chicks. There were two subgroups of 90 chicks replicated from each group. A corn and soybean meal diet was developed to meet the nutritional requirements of Cobb 430Y commercial broiler chickens (NRC, 1994). Table 1 depicts the nutrient composition of the base diet. The chicks were kept in pens with 96 square feet of floor space in a semi-closed structure. In terms of length, width, and height, the individual pens were approximately 8 feet by 4 feet by 3 feet. Each pen contained a brooder, drinkers, and feeders. Each chick was given a floor space of one square foot. Paddy husk is used as litter, and fresh litter is added once a week or as needed. Newspapers were spread on top of the litter to keep rice husks out of the feeders and drinkers for the first 5 days. Chlorinated drinking water was made available for chicks continually throughout the study. Broiler pre-starter, starter, and finisher feed were prepared as mash feed at Sriya Feed in Kolar, Karnataka, India. In a mixer, investigational feed additives are mixed first with a small amount of feed and then with each feed in its entirety.

Table 1: Nutrient composition of commercial broiler feed.

Particulars	Broiler pre-starter	Broiler starter	Broiler finisher
Protein (%)	22.20	21.30	19.40
Metabolizable energy (Kcal/kg)	2868	2875	3063
Crude fiber (%)	4.03	4.38	3.87
Ether extract (%)	3.41	3.42	6.31
Calcium (%)	1.04	1.04	0.80
Available phosphorus (%)	0.40	0.40	0.34
Methionine (%)	0.61	0.60	0.48
Lysine (%)	1.37	1.31	1.10
M + C (%)	0.95	0.93	0.78
Threonine (%)	0.86	0.83	0.75
Sodium (%)	0.18	0.18	0.18
Chloride (%)	0.27	0.26	0.24
Potassium (%)	0.90	0.90	0.79
DEB (mEq. kg)	236	234	214

Chicks were fed with the pre-starter feed from days 1–14, the starter feed from days 15–25, and the finisher feed from days 26–35. On days 7 and 22 of chick, a Newcastle disease (ND) vaccine was administered intraocularly. A live lentogenic strain from Venkateshwara Hatcheries Ltd. (VHL) on day 7, and on day 22, a live IP VH strain vaccine was used. On day 15, the invasive intermediate Indovax strain B2K was used to vaccinate against infectious bursal disease (IBD). Lighting, humidity, and temperature were monitored per standard farm management practices. Provided the light all day and night during 1st-week broiler rearing and 20 h after that. Chicks were given unlimited access to experimental diets.

2.3. Study Design

Nine hundred 1-day-old Cobb 430Y broiler chicks were procured from M/s Sriya Farms and Feeds Pvt. Ltd., Kolar, Karnataka, India, and were randomly divided into five groups of T0, T1, T2, T3, and T4, with two replicates of 90 birds each. T0 was raised on regular broiler feed without any AGPs. In contrast, T1, T2, T3, and T4 were raised on regular broiler feed and were simultaneously supplemented per ton of feed with enramycin at 100 g, SFP at 100 g, SFP at 150 g, and competitor probiotic product (CPP) at 100 g [Table 2].

2.4. Assessment Parameters

2.4.1. Growth performance parameters

On day one, the body weight of the chicks was recorded, and daily mortality rates were monitored. Body weight and feed consumption were recorded weekly. To determine how SFP-supplemented Cobb 430Y broiler chickens' growth performance parameters, the body weight, food conversion ratio (FCR), cumulative mortality, and European production efficiency factor (EEF) were calculated and evaluated.

2.4.2. Carcass characteristics

On day 36, the RTC weight of three randomly selected birds from each replicate was determined. The birds were killed by cervical dislocation and immersed in hot water (51–55°C) for 120 s. Samples of liver and abdominal fat were taken, weighed, and recorded.

2.5. Nutrient Digestibility

Three birds from each replicate were moved to individual metabolism cages measuring 39 × 20 × 36 cm (length by breadth by height) after the 5th week of the experiment for digestibility testing. After a 3-day acclimatization phase in the cages, the birds underwent 5 days of feces

Table 2: Study design.

Groups	Treatment (g/ton)	Number of Replicates	Number of chicks/replicate	Number of chicks/group	Treatment duration (days)
T0-control	-	2	90	180	35
T1-Eenramycin	100	2	90	180	
T2-SFP-1	100	2	90	180	
T3-SFP-2	150	2	90	180	
T4-CPP	100	2	90	180	

CPP: Competitor probiotic product, SFP: Synbiotic formulation with phytoactive

sample collection. Feathers and other impurities were removed from the fecal samples after air drying and cleaning. The feces of each bird were pooled for a period of all 5 days, homogenized, and delivered to the laboratory for proximate analysis. Protein and energy digestibility was calculated by proximate analysis (AOAC, 1990) of feed samples and fecal samples [17].

2.5.1. Blood collection and serum separation

On day 35, 2 ml blood was collected from the wing vein of eight birds from each group (4 birds from each replicate) for biochemical analysis and evaluation of ND and IBD antibody titers.

2.5.1.1. Assay of serum biochemical parameters

Birds' blood samples were centrifuged in ultracentrifuge (COOLING centrifuge REMICPR-30 PLUS) for 10 min at 4000 rpm for serum collection. Separated serum samples added respective biochemical parameters reagents kept in autoanalyzer (ERBA EM-360) instrument manufactured by Transasia Biomedicals, Mumbai. Samples were analyzed by automated autoanalyzer (EM360) using Erba reagent kits made by Erba Diagnostics in Mannheim, Germany, and sold by Transasia Biomedicals Ltd. Baddi, Dist. Solan (HP)-173205.

For the determination of biochemical parameters (n = 8), standardized kit-based analysis methods were used. The creatinine was determined by the modified Jaffe method, SGOT, and SGPT by IFCC method, blood urea by the GLDH urease method, BUN by the calculation method. (BUN mg/dl = urea mg/dl x 0. 67).

2.5.1.1.1. Estimation of creatinine by modified Jaffe's, no deproteinization method by autoanalyzer

Creatine reacts with alkaline picrate to produce a reddish color (Jaffe's reaction).

2.5.1.1.2. Estimation of SGOT by fully automatic analyzer (IFCC method)

IFCC-recommended reagent without pyridoxal phosphate is used in the assay system. The following series of reaction are involved in assay system:

$$\text{L- Aspartate + 2-Oxoglutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate + L-Glutamate}$$

$$\text{Oxaloacetate + NADH} \xrightarrow{\text{MDH}} \text{Malate + NAD}$$

$$\text{Sample pyruvate + NADH} \xrightarrow{\text{LDH}} \text{L-lactate + NAD}$$

AST catalyzes the transfer of the amino group from L-aspartate to 2-oxoglutarate forming oxaloacetate and L-glutamate. Oxaloacetate in the presence of NADH and MDH is reduced to L-malate. NADH oxidized to NAD. The reaction should be monitored by measuring the rate of decrease in absorbance at 340 nm due to oxidation of NADH to NAD. Lactate dehydrogenase (LDH) was added to reagent to prevent interference from endogenous pyruvate so that it does not interfere with the assay.

2.5.1.1.3. Estimation of SGPT by fully automatic analyzer (IFCC method)

This ALT reagent is based on the recommendations of the IFCC without pyridoxal phosphate.

The following series of reactions are involved in the assay:

$$\text{L-Alanine + 2-Oxoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate + L-Glutamate}$$

$$\text{Pyruvate + NADH} \xrightarrow{\text{LDH}} \text{L-Lactate + NAD}$$

$$\text{Sample pyruvate + NADH} \xrightarrow{\text{LDH}} \text{L-Lactate + NAD}$$

ALT transfers the amino group from alanine to α-oxoglutarate to yield pyruvate and L-glutamate. Pyruvate is reduced to lactate by LDH in reagent with simultaneous oxidation of NADH to NAD. This reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due to NADH oxidation. During initial incubation period to avoid interference during the assay, endogenous pyruvate sample is rapidly and completely reduced by LDH.

2.5.1.1.4. Estimation of urea by fully automatic analyzer ureases (glutamate dehydrogenase method [GLDH])

In the reaction, urea is hydrolyzed by urease to ammonia and carbon dioxide. Ammonia combines with α-ketoglutarate to produce L-glutamate in the presence of GLDH and reduced NADH.

The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm as NADH is converted to NAD.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2\,NH_3 + CO_2$$

$$NH_3 + \alpha\text{-KG} + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-Glutamate + NAD+}$$

2.5.1.1.5. BUN is calculated by the formula

BUN=Urea × 0.467.

2.5.1.2. Assay of serum ND and IBD antibody titers

Using the hemagglutination test (HA) (HI), the collected sera were examined to determine the antibodies' titer against NDV. Hemagglutination test was performed at the Poultry Diagnostic and Research Centre (PDRC), VHL Lab, Bengaluru.

2.5.1.2.1. Procedure for HA

1. Place 50 µL of PBS in 12 wells of 1st column of the microtitration plate.
2. Add 50 µL of antigen and PBS serial dilution.
3. Add 50 µL of 1% RBC to each well.
4. Room temperature – 20 min. After 20 min take reading.
5. Divide the HA titer of antigen by 8 to make and hemagglutination

unit (HAU).
6. The highest dilution of virus showing complete agglutination of RBC should be in well no. 3.

2.5.1.2.2. Procedures for hemagglutination inhibition (HI)

1. Place 25 µL PBS in all wells.
2. 25 µL of serum test samples mix the contents in the first well and then serial dilution.
3. 25 µL of antigen (RD virus) in all wells, room temperature 30 min.
4. After 30 min add 25 microliters of 1% RBC in all wells, room temperature 15 min.

Interpretation:

1. The highest dilution showing HI button formation is taken as HI titer.
2. HI titer is expressed as the reciprocal of the highest dilution of serum inhibiting agglutination of RBC.

Antibody level for IBDV by indirect ELISA kit was analyzed at Poultry Diagnostic and Research Centre (PDRC), VHL Lab, Bengaluru. Antibody level for IBDV, the indirect ELISA kit, was developed in-house by the Poultry Diagnostic and Research Centre (PDRC), Pune, India, and was used to measure the Ab titers against IBDV in serum samples obtained at 35 days. The ELISA reader (Biochek, Holland) was used to read the absorbance readings at 492 nm while applying an interference filter. Readings were taken after the wells containing only substrate chromogen and HCl were depleted to zero.

2.5.1.2.3. Enzyme-linked immunosorbent assay (ELISA) procedures

Materials required:

1. Pre-coated ELISA plates
2. Washing buffer
3. Specific Elisa kits

Procedure:

1. Obtain antigen-coated plates and record the samples' position
2. Dispense 100 µL of undiluted negative and positive control
3. Dispense 245 µL of phosphate-buffered solution with Tween (PBST) and add 5 mL serum in all wells
4. Dispense 90 µL PBST in test plate and add 10 dispense 90 µL PBST in test plate and add 10 µL of diluted sample into appropriate wells of diluted sample into appropriate wells
5. Incubate for 1 h. Wash with buffer solution
6. Conjugate – 1-h incubation and wash
7. Substrate
8. Stop with HCL
9. Reading with ELISA reader (Biochek, Holland).

2.5.2. Assay of litter characteristics

Poultry house litter quality was evaluated by scoring criteria listed below. Poor litter quality could be an indication of litter management issues, which could manifest as skin and foot lesions. Poor litter quality could be an indication of litter management issues, which could manifest as skin and foot lesions. Sample collection: Minimum 4 and maximum 6 spots in the poultry house (i.e., under drinkers and feeders, along the edges of the house, close to doorways).

Scoring:

1. Completely dry and flaky, i.e., moves easily with the foot.
2. Dry but not easy to move with the foot.
3. Leaves imprint of foot and will form a ball if compacted, but the ball does not stay together well.
4. Sticks to boots and sticks readily in a ball if compacted.
5. Sticks to boots once the cap.
6. Litter characteristics were assessed as per Welfare Quality®, 2009 [18], and water-soluble phosphorus (WSP) was analyzed by standard AOAC, 2005 [19].

2.6. Statistical Analysis

The average and standard deviation of the data are shown. Control and treatment groups compared by statistical data analysis using Dunnett's multiple comparison post hoc test and one-way analysis of variance. The study was deemed statistically significant with a p-value below 0.05. IBM SPASS statistics (IBM corp.) software, version 20, of the Statistical Package for the Social Sciences released in 2011 was utilized for statistical analysis.

3. RESULTS

T3 had the highest livability (percentage) after the study on day 35 (98.40%). The mean body weight (g) after the finisher phase on day 35 compared to T0 increased by 9.83%, 2.05%, 10.78%, and 12.88% in T1, T2, T3, and T4, respectively. These findings depicted a dose-dependent improvement in T2 and T3. When compared to the control group, the FCR increased in all treatment groups. Furthermore, compared to T0, the FCR results showed that groups T1, T2, T3, and T4 consumed 22 g, 22 g, 27 g, and 23 g less feed/unit of body weight gain, respectively. Moreover, EEF increased by 24.29%, 22.88%, 31.69%, and 26.41% in T1, T2, T3, and T4 compared to T0. Results indicated that T3 had the most efficient feed utilization. Considering data on livability, body weight gain, feed consumption, and EEF, the birds in T3 showed increased growth and overall performance. When compared to T0, the RTC increased in all treatment groups. T3, however, saw an improvement in RTC (percent) followed by T4, T2, and T1 [Table 3].

Table 3: Effect of synbiotic formulation with phytoactive on Cobb 430Y broiler performance.

Group	T0	T1	T2	T3	T4
Livability (%)	96.70	96.70	96.20	98.40	95.10
Body weight (g)	1902	2089	1941	2107	2147
FCR	1.85	1.63	1.63	1.58	1.62
cFCR	1.87	1.61	1.61	1.56	1.59
EEF	284	353	349	374	359
Relative RTC (%)	55.89	60.22	59.94	62.55	61.61

Mean is used to describe values. FCR: Food conversion ratio, cFCR: Corrected food conversion ratio, EEF: European production efficiency factor, RTC: Ready-to-cook

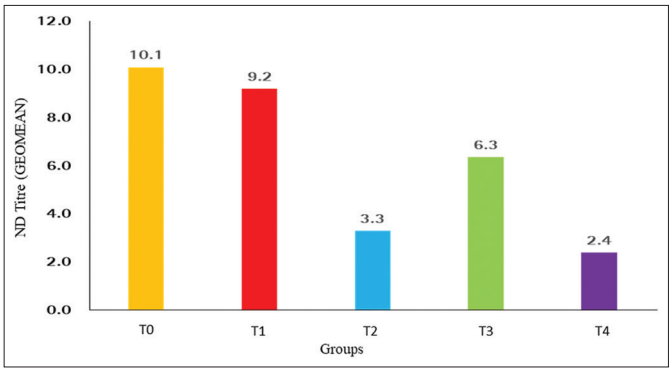

Figure 1: Effect of SEP on Newcastle disease antibody titer (geometric mean) in Cobb 430Y broiler chickens.

The geometric mean of the ND antibody titer was 10.1, 9.2, 3.3, 6.3, and 2.4 in T0, T1, T2, T3, and T4, respectively [Figure 1]. The IBD vaccination index was 29.54, 40.32, 43.28, 42.79, and 44.13 in Y0, T1, T2, T3, and T4, respectively [Figure 2]. IBD vaccination index increased in all treatment groups when compared to the control group. Treatment SFP had better effects on T- and B-lymphocytes, phagocytic cells, CD+ cells, and other immune organs as an immune modulator than enramycin. Broiler chickens' overall health is effectively maintained by these, which help boost immunity.

Table 4 depicts the effects of SFP on the biochemical parameters of the serum. These findings show that liver and kidney marker enzymes were well within the normal reference range limits of broiler chickens, indicating that enramycin, SFP, or CPP does not impact the liver functions of broiler chickens. However, there was a numerical reduction in blood urea and BUN in all treatment groups as compared to the control group.

The results of the effects of SFP on Cobb 430Y broiler chickens' ability to digest nutrients are presented in Table 5. These findings indicate that the protein and metabolic energy digestibility (%) was highest at 84% and 91% in T3 compared with T0, T1, T2, and T4.

The effects of SFP on Cobb 430Y broiler chickens' gut health are shown in Table 6. These findings show that supplementation per ton of feed of SFP at 100 g (T2) and 150 g (T3) was on par with the performance of enramycin (T1) and CPP (T4) for effects on gut health improvement.

Table 7 shows the effect of SFP on litter characteristics in broiler chickens. The wet litter was not observed in the treatment groups, and WSP was slightly higher in CPP (T4) as compared to control (T0) and other treatment groups, namely enramycin (T1), SFP at 100 g/ton (T2), SFP at 150 g/ton (T3) indicated that supplementation of SFP at 100 g/ton (T2) and SFP at 150 g/ton (T3) was at par with the performance of enramycin (T1), and CPP (T4) in terms of maintaining litter characteristics. Whereas WSP was reduced by 16.67%, 16.67%, and 23.08% following supplementation of SFP at 100 g/ton (T2) and SFP at 150 g/ton (T3) as compared to control (T0), enramycin (T1), and CPP (T4), respectively.

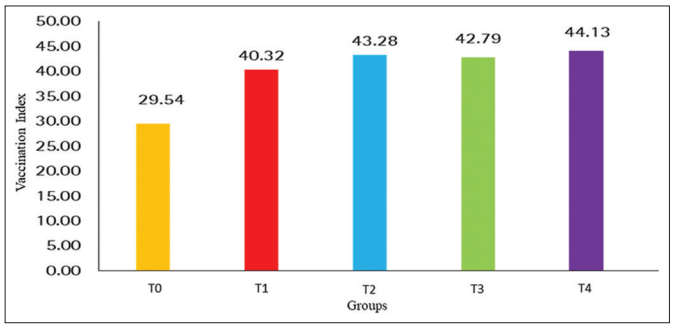

Figure 2: Effect of SEP on infectious bursal disease antibody titer (vaccination index) in Cobb 430Y broiler chickens.

Table 4: Effect of synbiotic formulation with phytoactive on broilers serum biochemical parameters.

Group	T0	T1	T2	T3	T4
SGOT (IU/L)	179.75	176.93	186.73	169.34	194.22
SGOT (IU/L)	12.00	10.10	10.50	11.00	14.70
Creatinine (mg/dL)	0.28	0.27	0.30	0.32	0.34
Blood urea (mg/dL)	3.85	3.56	3.30	3.30	3.40
BUN (mg/dL)	2.80	1.31	0.13	0.06	0.03

Mean is used to describe values. SGOT: Serum glutamic-oxaloacetic transaminase

Table 5: Effect of synbiotic formulation with phytoactive on nutrient digestibility in Cobb 430Y broiler chickens.

Group	T0	T1	T2	T3	T4
Protein digestibility (%)	68	79	65	84	65
ME digestibility (%)	84	89	81	91	83

Mean is used to describe values

4. DISCUSSION

The poultry industry's profitability depends solely upon broiler chickens' growth performance. Thus, researchers invent products that help augment production performance without adverse effects on broiler chickens' health [20]. Over the past 60 years, the primary reasons for dietary AGPs in poultry production have been to improve growth, feed efficiency, and the prevention of subclinical diseases. However, their long-term, low-dose use causes bacteria to become resistant, leaving antibiotic residues in animal products [21]. Despite significant progress, there are concerns about the safety and quality of chicken products because of the risk of these antibiotic-resistant bacteria spreading to humans through the consumption of poultry [22]. The ecological issue of pathogens that are resistant to antibiotics as a result of the spread of antibiotic-resistance genes is exacerbated by the widespread use of antibiotics in livestock, veterinary, and human medicine. As a direct result of this, nations such as the United States of America and the European Union have joined the global effort to limit the subtherapeutic use of feed antibiotics [23]. Debates in India promote withdrawing antibiotics from poultry feed and replacing them with alternatives that would improve the healthy production traits of chickens and safety for human consumers. This situation compelled researchers to investigate other non-therapeutic alternatives to poultry antibiotics so that this industry's ever-increasing growth remains unaffected. Herbs and herbal extracts used as poultry feed additives have continued to increase since antibiotics were banned from improving the health, immunity, and production efficiency of poultry. The purpose of this study was to compare SFP to AGPs and competing probiotic products in terms of growth performance, carcass traits (RTC), gut health, and health and immunity parameters in commercial broiler chickens.

This study demonstrated that 150 g SFP per ton of feed could augment the production performance for body weight, FCR, EEF, RTC, and nutrient digestibility, with enhanced gut health and overall health of Cobb 430Y broiler chickens compared with AGPs and competitor probiotic products. The synergistic effect of *Z. officinale* and *C. longa*

Table 6: PHF on gut health in Cobb 430Y broiler chickens.

Group	T0	T1	T2	T3	T4
Gut health	Poor gut health	Pink gut surface	Pink gut surface	Pink gut surface	Pink gut surface
	Inflamed gut surface	Good gut muscle tone	Good gut muscle tone	Good gut muscle tone	Good gut muscle tone
	Poor gut muscle tone	Gut wall folding back itself	Gut wall folding back itself	Gut wall folding back itself	Gut wall folding back itself
	Mucus and excess fluid in the gut	Good digestion of food	Good digestion of food	Good digestion of food	Good digestion of food

Table 7: Effect of SFP on WSP and litter characteristics in Cobb 430Y broiler chickens.

Group	T0	T1	T2	T3	T4
WSP	0.12	0.12	0.10	0.10	0.13
Litter score	Dry, not easy to move	Dry and flaky, easy to move	Dry and easy to move	Dry and flaky, easy to move	Dry and flaky, easy to move

WSP: Water-soluble phosphorus

herbal ingredients, probiotic strains of *B. subtilis*, *B. coagulans*, *B. pumilus*, and *B. polymyxa*, herbal prebiotic *Z. officinale*, and prebiotic yeast β-glucan, could be the only explanation for these results.

Z. officinale has the natural potential to promote growth and showed a good response compared to AGPs [24]. Adding *Z. officinale* powder to poultry feed improved weight gain and performance [25]. Furthermore, various researchers have reported higher BWG in broiler chickens with a diet including *Z. officinale* [26,27]. Incharoen and Yamauchi, 2009, reported improved FCR in 0.1% and 0.2% *Z. officinale*-supplemented groups [28]. *Z. officinale* increased gastric and salivary gland secretions, lowering the chickens' microbial count and enhancing their digestive processes. [25]. *Z. officinale* enhances pancreatic lipase activity, disaccharides, intestinal lipase, and maltase and sucrose activities in rats [29], which have been reported to influence gut function favorably. This primary mode of action for growth-promoting feed additives demonstrates that the addition of *Z. officinale* increased the average dressing percentage [30]. Additionally, the literature demonstrated that broiler chickens' immune systems are strengthened when they consume *Z. officinale* supplements [31,32]. This could be because *Z. officinale's* natural aromatic active constituents, such as gingerol and Shogaols, have anti-oxidant properties [33].

C. longa, also known as turmeric, is the plant from which curcumin is extracted [34]. In laboratory animals, curcuma demonstrated anti-inflammatory, neuroprotective, anti-proliferative, anti-arthritic, and gastroprotective properties [35]. Rajput *et al.*, 2013, reported that curcumin improves performance and fat metabolism while also making nutrients easier to digest [36]. Curcumin accelerated digestion and absorption by boosting bile acid production and gastric enzyme activity, according to another study [29]. Curcumin may also potentially eliminate *Eimeria tenella* sporozoites, reduce oocyst shedding, and reduce gut lesions [37]. It boosts the humoral immunity of the host, thereby preserving gut integrity [38].

The present study saw improved production performance, nutrient digestibility, enhanced gut health, improved litter quality, and overall health of Cobb 430Y broiler chickens. This could also be ascribed to the multi-strain probiotics of *B. subtilis, B. coagulans, B. pumilus,* and *B. polymyxa,* herbal prebiotics, and phytoactives present in SFP. There are several ways that dietary probiotics may boost bird immunity: (1) Attaching to a host receptor and acting as an immunomodulator to elicit an immune response; (2) directly promoting the immune system's effect through active groups' competition for nutrients with the pathogen; and (3) preventing specific pathogens from colonizing chicken intestines. Increased diffused lymphohistiocytic infiltration, solitary lymphoid follicles in the mucosa, and a larger response all point to an enhanced immune response in chickens fed probiotic-supplemented diets [39]. According to Yurong *et al*., including probiotics in animal diets boosts the immunity. To accomplish this, viable cells traverse the intestinal wall, resulting in the production of immunogenic compounds, mediated downregulation of particular signaling pathways, and limited cell proliferation [40]. Stimulated immunity may then manifest as enhanced macrophage activity and a systemic antibody response through increased production of immune globulins, interferons, IgA levels at mucosal surfaces, and the expression of various pro- and anti-inflammatory cytokines [41].

5. CONCLUSION

According to the findings of this study, the performance of Cobb 430Y broiler chickens, RTC weight, health, nutrient digestibility, gut health, litter characteristics, and overall health status were positively impacted by SFP supplementation at 150 g/ton of feed. Additionally, this study reveals that the SFP may be able to replace antibiotics in broiler chicken diets.

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7. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit it to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be authors per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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9. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

10. ETHICAL APPROVAL

The present study was performed in compliance with guidelines laid down for the care and use of animals, and protocol approval was provided by the IAEC (Institutional Animal Ethics Committee) (IAEC Protocol No.: - AHP/P/11/19).

11. DATA AVAILABILITY

All data generated during the current study are included in this article.

12. PUBLISHER'S NOTE

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