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A Novel Formulation of Synbiotics Fortified with Phytoactives for Augmentation of Performance, Meat and Litter Quality, Health, Gut Health and Gut Microflora in Broiler Chickens

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ABSTRACT

Background: Antibiotic resistance raised interest in probiotics, prebiotics and synbiotics as alternatives for improving gut health in chickens. This feeding trial aims to assess the effectiveness of a synbiotic formulation with phytoactives (SFP).

Methods: On the first day of the trial, 160 Cobb 430Y one-day-old chicks were procured from a hatchery and split into groupings: G1, G2, G3 and G4. Each group comprised four replicates of 10 birds. G1 was raised with no antibiotics growth promoter (AGPs) on typical commercial feed. While G2, G3 and G4 were reared on standard commercial feed, they were also given supplements of competitor product I (CP-I) at a rate of 500 g/ton, competitor product II (CP-II) at a rate of 100 g/ton and SFP at a rate of 150 g/ton, respectively.

Result: Dietary SFP supplementation at 150 g/ton improves nutrient absorption, digestion, growth performance, feed efficiency, immunity and overall wellbeing, increasing poultry safety and recommended as an alternative to AGP in commercial broiler production.

Key words: Broiler performance, Gut health, Gut microflora, Health, Synbiotic formulation with phytoactives (SFP).

INTRODUCTION

The poultry industry is rapidly growing due to increased consumption of eggs and meat, which are rich in nutrients and affordable. Antimicrobial resistance is a significant global health concern, with excessive use of antimicrobials promoting gut health (Dhama *et al*., 2015).

Antibiotic growth promoters (AGPs) have been found to improve gut health and reduce subclinical infections in poultry birds. Additionally, antibiotics helps in nutrient absorption by thickening of the gut (Cox and Dalloul, 2015). However, overuse of antibiotics has led to antibiotic resistance, increasing susceptibility to diseases and residues in animal products (Ronquillo and Hernandez, 2017). European countries have banned the use of antibacterials in animal feed, prompting the search for safer substitutes (Diarra and Malouin, 2014). The gastrointestinal tract plays a critical role in the livestock industry and gut health is a critical factor (Oviedo-Rondon, 2019; Kogut *et al*. 2017). Probiotics and prebiotics in poultry diets have shown promising results in controlling bacterial infections Mead (2000), influencing the gut microbiota (Nava *et al*., 2005) and stimulating immune responses (Koenen *et al*., 2004). Synbiotics (Saarela *et al*., 2000), a combination of probiotics and prebiotics (Roberfroid, 1998), have been found to be more effective than single or congruent antibiotic treatments (Gadde *et al*., 2017) and (Tayeri *et al*., 2018).

Dietary synbiotics in broilers have modulated gastrointestinal microbial colonization (Brugellata *et al.,* 2020). Prebiotics are non-digestible carbohydrates that are often added to the combination of probiotics to stimulate host gut-beneficial bacterial growth (Lee *et al.,* 2016). It has been demonstrated that prebiotic supplementation mimics the pathogens' attachment sites, decreases the gut wall attachment of bacterial pathogens, and increases definite helpful bacteria (Igi, 1998).

Phytobiotics, also known as phytogens, are less toxic, residue-free and growth-boosting feed additives. They alter gut microorganisms, improve digestibility and performance, improve intestinal architecture and stimulate intestinal mucus secretion (Alloui *et al*., 2014); (Tabatabaei, 2016). These benefits include enhanced nutrient absorption, digestion, growth enhancement, immune modulation and gut health, ultimately improving the safety of poultry products (Rafiq, 2021).

Driven by the IPC's (2019) call for responsible antibiotic use and recognition of broader industry concerns, poultry science and practice have embraced new feed supplements and biotechnological solutions, offering alternatives to traditional antibiotics (Mogotlane *et al*., 2023).

This study investigates the effectiveness of a novel synbiotic formulation fortified with phytoactives (SFP)

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developed by Himalaya Wellness Company, Bengaluru, Karnataka, India in improving performance parameters, meat and litter quality, health, gut health and broiler gut microflora.

MATERIALS AND METHODS

SFP

SFP is a proprietary poly-herbal feed supplement, called as HimFlora. SFP consists of probiotics *viz. Bacillus subtilis, Bacillus coagulans, Bacillus pumilus and Bacillus polymyxa,* herbal actives, *Zingiber officinale*, extract of *Curcuma longa, herbal prebiotic* and prebiotics powder of yeast β-Glucan.

Ethical approval

The protocol for the current study was approved by the IAEC (Institutional Animal Ethics Committee) and abided by the establishment for the care and use of animals. [IAEC Protocol No.:- AHP/P/12/21].

Study design

The experiment involved 160 Cobb 430Y broilers and feeds from Sriya Farms and Feeds Pvt. Ltd., with four groups: G1, G2, G3 and G4. G1 was raised on commercial feed without AGPs, while G2, G3 and G4 were reared with commercial feed and supplemented with CP-I (Competitor Product I), CP-II (Competitor Product II) and SFP (Table 1). The duration of the study was 6 weeks and the trial was conducted at Makali village, near Himalaya Wellness Company, Makali, Bengaluru.

Experimental setup

The study involved randomly assigning chicks to four dietary groups and replicating them into four subgroups. The corn-soya-based diet was prepared according to the National Research Council. (1994) nutritional requirements. Chicks were housed in semi-enclosed houses with pens and bedding materials. Ad libitum drinking water was provided. Three feed lines were prepared and chicks received pre-starter, starter and finisher feeds (Table 2). An intraocular Newcastle disease vaccine and infectious bursal disease vaccination were administered.

Assessment parameters

Growth performance

Bird mortality and feed consumption were monitored daily for growth performance in broiler chickens. The European efficiency factor (EFF) was assessed by cumulative mortality, body weight, FCR and days reared.

Quality parameters

RTC

Quality parameters included RTC (ready to cook) after 40 days by cervical dislocation sacrifice Yalcin *et al*. (1999). The study followed official guidelines for animal welfare.

Litter quality

Litter characteristics were assessed and scored as per the classification described below at the end of the experiment (De Jong *et al*., 2016) by collecting litter at a minimum of 4 and 6 spots in the poultry house (*i.e*., under feeders and drinkers, close to doorways, along the edges of the house.

Score	Description
Score 0	Totally dry and flaky, *i.e*., moves easily with the foot
Score 1	Dried up, unable to move with the foot
Score 2	Leaves a foot imprint and forms a compacted ball, but poor ball stability
Score 3	Sticks to boots and readily sticks in a ball when impacted
Score 4	The litter cap or compact crust breaks, sticks to boots

Health

Blood collection and serum separation

Blood samples were collected, ultracentrifuge (COOLING centrifuge REMICPR-30 PLUS) for 10 min at 4000 rpm for serum collection, separated and stored at 20°C until biochemical analysis and IBD antibod.

Serum biochemistry assay

Serum biochemical parameters, including protein, albumin, globin, A/G ratio, urea, BUN, creatinine, SGPT and SGOT, were measured using standardized kits from Erba Diagnostics, Mannheim, GmbH, Germany and distributed by Transasia Biomedicals Ltd., Mumbai. Serum levels of creatinine, SGOT, SGPT and blood urea were measured using modified Jaffe, IFCC, GLDH urease and calculation methods, respectively.

$$\text{BUN mg/dl} = \text{Urea mg/dl} \times 0.67$$

Serum IBD antibody titer

The indirect ELISA kit was used to analyze IBDV antibody levels in serum samples at 35 days. To determine the levels of antibodies against Newcastle Disease Virus (NDV) in collected sera, a hemagglutination (HA) test was performed at the Poultry Diagnostic and Research Centre (PDRC) located at VHL Lab, Bengaluru. The IDEXX IBD Ab Test kit was followed and absorbance readings were recorded using an ELISA reader.

Gut health and gut microflora

The study involved two birds from each group sacrificed and their intestines were collected for gut health analysis (Yalcin *et al*., 1999). Gut health parameters scoring system is represented in Table 3. Fecal content was analyzed for bacterial enteritis using compendial microbiological methods for organisms *viz*. *Bacillus sp., Bifidobacterium sp., Lactobacillus sp. and Clostridium sp.* The intestine was assessed for the bacterial enteritis score using the method described by De Gussem (2010).

Intestinal histomorphometry

On day 41, small intestines were collected and eviscerated. A 2 cm ileum piece was excised, fixed in formaldehyde and sections were cut, mounted on glass slides, stained and examined for villi height and crypt depth measurements.

Statistical analysis

Data were analyzed using one-way ANOVA and multiple comparisons, with a p-value of <*0.05* considered statistically significant. Statistical analyzes were performed using the Statistical Package for Social Sciences version 20 software, IBM SPASS Statistics, IBM Corp. published (2011).

RESULTS AND DISCUSSION

Growth performance

At the end of the study duration, the livability (%) was observed, *i.e.,* day 40th, was highest (97.50%) in G4 as compared with G1 (92.50%), G2 (90.00%) and G3 (95.00%). The mean body weight (g) in the finisher phase, *i.e.*, day 40, increased by 2.73% and 2.46% in G2 and G4, respectively, compared with G1. These findings indicated that SFP at the supplementation level of 150 g/ton was the best regarding body weight improvement effects compared with G1 and G3. FCR was improved in all the SFP-added groups, *i.e.*, G2, G3 and G4, compared with G1. Results of FCR showed that birds in G2, G3 and G4 consumed 8 g, 12 g and 26 g less feed per unit body weight gain, respectively, compared with G1. EEF was increased by 4.75%, 8.17% and 18.79% in G2, G3 and G4, compared with G1. These results inferred G4 is the best regarding feed utilization efficiency. The overall growth performance of birds in G4 was better than all other groups (Table 4).

Quality parameters

RTC

The relative RTC was increased in all the SFP supplemented groups, *i.e.*, G2, G3 and G4, compared with G1. However, G4 performed on par with G2 and G3 regarding RTC (%) improvement effects (Table 4).

Litter quality

There was a wet litter observed in G1. However, in SFP supplemented groups, *i.e.*, G2, G3 and G4, no wet litters were observed (Table 5). These findings inferred that SFP performance at the 150 g/ton supplementation level was on par with competitor products regarding litter quality maintenance.

Table 1: Study design.

Groups	Supplementation	Number of replicates	Number of chicks/replicate	Number of chicks/group	Duration of supplementation
G1- Control	-	4	10	40	40 days
G2- CP-I	500 g/ton	4	10	40	
G3- CP-II	100 g/ton	4	10	40	
G4- SFP	150 g/ton	4	10	40	

Table 2: Nutrient composition of commercial broiler feed.

Parameters	Broilerpre-starter feed	Broilerstarter feed	Broilerfinisher feed
Crude protein (%)	22.40	20.26	18.60
Metabolizable energy (kcal/kg)	2800	2975	3025
Crude fiber (%)	3.57	3.28	3.42
Ether extract (%)	4.51	5.61	5.89
Calcium (%)	0.93	0.80	0.66
Methionine (%)	0.60	0.52	0.48
Retained phosphorus (%)	0.44	0.42	0.37
M + C (%)	0.90	0.79	0.74
Lysine (%)	1.24	1.07	0.98
Threonine (%)	0.76	0.66	0.63
Arginine (%)	1.45	1.27	1.14
Tryptophan (%)	0.24	0.22	0.19
Isoleucine (%)	0.84	0.78	0.68
Valine (%)	0.94	0.87	0.80
Sodium (%)	0.16	0.16	0.16
Chloride (%)	0.27	0.26	0.24
Potassium (%)	0.90	0.90	0.79
DEB (MEq/kg)	236	234	214

Health

The liver marker enzyme SGPT decreased in G4 compared with G1 (Table 6). Except for changes in biochemical parameters of all the groups, including the control group, no effect of product supplementation was recorded.

Serum IBD antibody titer

IBD antibody titer is represented in Table 7 (Vaccination index-VI). These findings revealed that the VI of IBD titer was increased by 0.97% in G4 compared with G1. This increase could be attributed to better immune-modulatory effects of SFP (150 g/ton) than CP-I and CP-II on B and T lymphocytes, phagocytic cells, CD+ cells and other immune organs, which are involved in augmenting the immune response and effective in maintaining the broiler chicken health status.

Gut health

The mean gut health score results revealed a significant (p<.05) reduction in the total gut health score of G3 and G4 compared with G1 (Table 8 and Fig 1a, 1b, 1c and 1d). These findings implied that SFP at the supplementation levels of 150 g/ton would play a key role in gut health improvement compared with competitive products.

Mean bacterial enteritis

The mean bacterial enteritis and intestinal histomorphometry scores exhibited by G1, G2, G3 and G4 were 6.80, 7.70, 3.00, 2.3 and 7.62, 7.85, 7.17 and 8.65,

Table 3: Gut health parameters scoring system.

Parameter	Description	Score
A. Overall distension	Distension	1
	No distension	0
B. Significant redness of the bowel's mucosal and/or serosal sides	Redness	1
	No redness	0
C. A macroscopically visible and/or tangible decrease in bowel wall thickness (Cranial)	Reduced thickness	1
	No reduction	0
	No inversion of gut edges	1
D. Cranial bowel wall edges	Inversion of gut edges	0
E. The appearance of the contents in the bowel lumen is abnormal (Cranial)	Slimy, greasy, watery and gas content	1
	Normal contents	0
F. Redness on the guts'serosal and/or mucosal side	Redness	1
	No redness	0
G. Intestinal wall thickness (Caudal)	Reduced thickness	1
	No reduction	0
H. Caudal gut wall edges	No inversion of gut edges	1
	Inversion of gut edges	0
I. The appearance of the contents in the bowel lumen is abnormal (Caudal)	Slimy, greasy, watery and gas content	1
	Normal contents	0
J. Undigested feed particles caudal	Undigested food	1
	No undigested food	0

Table 4: Effect of SFP on production parameters in Cobb 430Y broilers.

	G1	G2	G3	G4
Livability, %	92.50	90.00	95.00	97.50
Body weight, kg	2267.03	2330.56	2251.32	2324.10
FCR	1.86	1.78	1.74	1.64
EEF	281	295	306	346
Relative RTC, %	62.80	65.26	65.80	65.65

Values are expressed as means.

Table 5: Effect of SFP on litter quality in Cobb 430Y broiler chickens.

	G1	G2	G3	G4
Litter quality	Dry, not easy to move	Dry, flaky, easy to move	Dry, flaky, easy to move	Dry, flaky, easy to move

Slight gut ballooning, lack of intestinal tract tonus, foamy intestinal content, macroscopically thin intestinal tract, colon with undigested food particles and inflammation of the gut.

Fig 1a: Gut health parameters in Cobb 400 Y Broiler control group.

Slight gut ballooning, lack of intestinal tract tonus, slight foamy intestinal content, macroscopically thin intestinal tract, colon with undigested particles and inflammation of the gut.

Fig 1b: Effect of CP-1 on gut health parameters in Cobb 400 Y broiler.

No gut ballooning, orange intestinal content, medium to good tonus of the intestinal tract, macroscopically, normal intestinal track thickness, little undigested food particles in the colon and uninflamed gut.

Fig 1c: Effect of CP-2 on gut health parameters in Cobb 400 Y broiler.

Overall, no gut ballooning, mucoid, orange intestinal content and good tonus of the intestinal tract. Macroscopically, normal intestinal track thickness, no undigested particles in the colon and uninflamed gut.

Fig 1d: Effect of SFP on gut health parameters in Cobb 400 Y broiler.

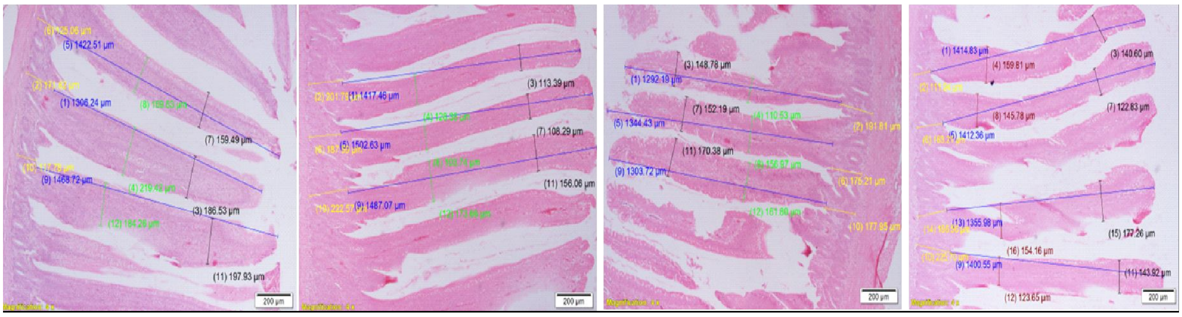

Fig 2: Effect of SFP on the intestinal absorptive surface area (Villi: Crypt ratio).

Table 6: Effect of SFP on serum biochemistry in Cobb 430Y broiler chickens.

Biochemical parameters	G1	G2	G3	G4
Total protein (g/dL)	2.78	2.85	2.70	2.72
Albumin (g/dL)	1.12	1.10	1.11	1.16
Globulin (g/dL)	1.66	1.75	1.59	1.56
A/G ratio (g/dL)	0.69	0.65	0.64	0.76
Urea (mg/dL)	5.45	6.24	6.21	6.66
BUN (mg/dL)	2.55	2.91	2.90	3.11
Creatinine (mg/dL)	0.28	0.26	0.28	0.24
SGPT (IU/L)	34.55	32.41	37.30	30.29
SGOT (IU/L)	267.91	253.64	351.31	269.95

Values are expressed as means.

Table 7: Effect of SFP on IBD antibody titer in Cobb 430Y broiler chickens.

	G1	G2	G3	G4
IBD titer (Vaccination index)	58.47	42.58	35.30	59.04

respectively (Table 9). These findings indicated that SFP supplementation at 150 g/ton provided better protection against bacterial enteritis infection and played a pivotal role in enhancing intestinal health as compared to the control group.

Intestinal histomorphometry

The ratio of villi: Crypt was calculated as 7.62, 7.85, 7.17 and 8.65 in by G1, G2, G3 and G4, respectively (Table 9 and Fig 2). Present study findings implied that supplementation of SFP at 150 g/ton caused an increment in the ratio of villi: crypt indicating that SFP supplementation plays a pivotal role in improving intestinal health as compared to control and other groups through the proliferation of the intestinal surface area and intestinal villus height.

Gut microflora

The analysis results of the fecal flora of individual organisms *viz. Bacillus sp., Lactobacillus sp., Bifidobacterium sp. and Clostridium sp.* are depicted in Fig 3a and Fig 3b. The results implied that the counts of pathogens revealed a significant reduction in G4, *i.e.*, SFP (150 g/ton) formulation when compared to the control group. The probiotic counts were compared with the competitors. Supplementation with SFP (150 g/ton) formulation showed reduced pathogens loads.

The study suggests that SFP at 150 g/ton can improve production performance, protect against bacterial enteritis and enhance gut health in Cobb 430 broiler chickens. This can be attributed to individual herbal ingredients and probiotics like *B. coagulans*, *B. subtilis*, *B. polymyxa*, B. *pumilus* and yeast β-glucan. Research indicates that combining probiotics and prebiotics can yield greater benefits than either alone (Adil and Magray, 2012; Hasanpur *et al*., 2013; Madej *et al*., 2015). Additionally, multi-strain probiotics have been shown to improve production performance, gut health and overall health status in Cobb 430 broiler chickens (Mokhtari *et al*. 2010; Razieh *et al*., 2015).

SFP supplementation likely enhances bacterial enteritis protection and intestinal health by promoting increased villi height and crypt depth. β-glucans possess both probiotic and immunostimulatory properties (Chen *et al*., 2016; Mitsou *et al*., 2010; Lam and Cheung, 2013); influencing leukocyte activity (Chae *et al*., 2006; Volman *et al*., 2008). Furthermore, *Z. officinale* extracts exhibit antimicrobial activity against common poultry pathogens

Table 8: Effect of SFP on gut health parameters in Cobb 430Y broiler chickens.

	G1	G2	G3	G4
Overall gut distension	0.00	0.33	0.00	0.00
Significant redness of the serosal and/or mucosal side of the bowel	0.33	0.83	0.33	0.33
A macroscopic visible and/or tangible reduction of the bowel wall thickness (Cranial)	0.83	0.83	**80.00	*0.17
Cranial bowel wall edges	1.00	1.00	0.50	0.67
Abnormal appearance of the contents in the lumen of the bowel (Cranial)	1.00	1.00	0.83	0.50
Redness of the serosal and/or mucosal side of the gut	1.00	1.00	0.67	*0.17
Gut wall thickness (Caudal)	0.83	0.67	**0.00	**0.00
Caudal gut wall edges	0.83	1.00	0.33	0.33
Abnormal appearance of the contents in the lumen of the gut (Caudal)	0.83	1.00	0.33	*0.17
Undigested feed particles caudal	0.17	0.00	0.00	0.00
Total score	6.83	7.67	*3.00	*2.33

Values are expressed as means.

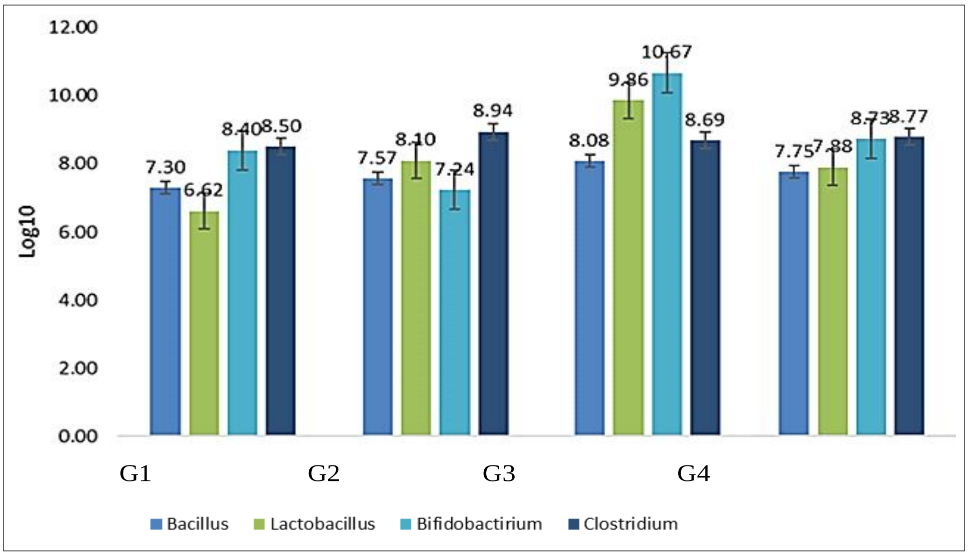

Fig 3a: Effect of SFP on beneficial microflora in Cobb 400 Y broiler.

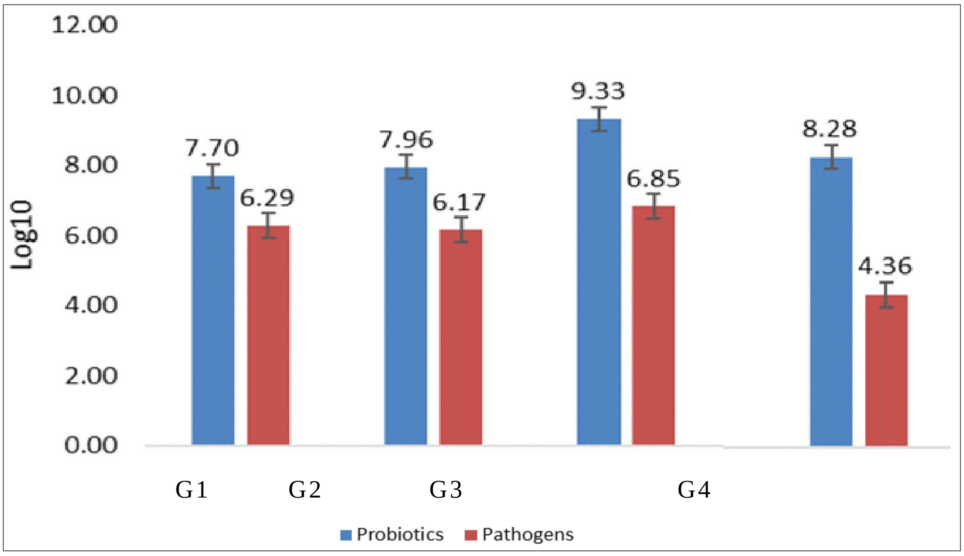

Fig 3b: Effect of SFP on pathogens.

Table 9: Effect of SFP on gut health in Cobb 430Y broiler chickens.

	G1	G2	G3	G4
Mean bacterial enteritis score	6.80	7.70	3.00	2.30
Mean intestinal histomorphometry (µm)	7.62	7.85	7.17	8.65

Values are expressed as means.

like *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella* spp. Dieumou *et al*., 2009; Bhattarai *et al*., 2018).

Studies demonstrate that dietary SFP supplementation with *Z. officinale* and *C. longa* improves production performance in poultry (Mohamed *et al*., 2012; Ademola *et al*., 2009; Onimisi *et al*., 2005). These ingredients stimulate salivary and gastric gland secretions, potentially reducing microbial load and aiding gut digestion (Yamauchi, 2009). Additionally, *Z. officinale* enhances intestinal function by boosting enzyme activities like intestinal and pancreatic lipase (Srinivasan, 2016). Moreover, supplementing with *Z. officinale* has been shown to improve dressing percentage and potentially boost immunity due to its antioxidant and natural aromatic compounds like gingerol and shogaols (Eltazi, 2014; Azhir *et al*., 2012; Nidaullah *et al*., 2010; Khan *et al*., 2012). Curcumin, another component of SFP, exhibits positive effects on nutrient metabolism, reduces oocyst shedding and lesions and protects the gut by enhancing host humoral immunity (Rajput *et al*., 2013; Srinivasan, 2016; Khalafalla *et al*., 2011 and Kim *et al*., 2013).

CONCLUSION

In a world demanding change, SFP rises as a potent answer. It nourishes chickens not just with feed, but with resilience, gut health and an edge against harmful pathogens. This translates to not just growth, but a symphony of positive impacts: optimized feed conversion, improved gut health and ultimately, safer poultry products on our plates. SFP isn't just an alternative; it's a revolution, whispering the promise of a healthier, more responsible future for chickens, farmers and consumers alike.

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Conflict of interest

The authors declare that they have no conflict of interest.

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