

## RESEARCH ARTICLE

## INFLUENCE OF PHYTOBIOTIC AND SYNBIOTIC-ACIDIFIER BLEND SUPPLEMENTS ON HEMATO-BIOCHEMICAL INDICES, ANTIOXIDANT ENZYMES AND GUT MICROFLORA OF BROILER CHICKEN

Andrew B. Falowo\*, Olugbenga D. Oloruntola, Olumuyiwa J. Olorotimi

Department of Animal Science, Faculty of Agriculture, Adekunle Ajasin University, P.M.B. 001, Akungba-Akoko, Ondo State, Nigeria.

\*Corresponding Author Email: [anddele2013@gmail.com](mailto:anddele2013@gmail.com) / [andrew.falowo@aaau.edu.ng](mailto:andrew.falowo@aaau.edu.ng)

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## ARTICLE DETAILS

## ABSTRACT

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This study examined the effect of phytobiotic (Superliv powder®, SP) and symbiotic-acidifier blend (Bacflora-F®, BF) feed additives on hemato-biochemical indices, antioxidant enzymes, and gut microflora of broiler chicken. A total of 120-day-old Arbor Acres broiler chicks were randomly allotted to four dietary treatments, with three replicates of ten birds per treatment as follows, Diet 1 (basal/control diet), Diet 2 (basal + 0.05 g/kg SP), Diet 3 (basal + 1g/kg BF) and Diet 4 (basal + 0.05g/kg SP and 1g/kg BF). The result revealed significant differences ( $P < 0.05$ ) in values of all haematological parameters except monocyte counts. Broiler chickens fed a diet supplemented with SP and BF had higher packed cell volume, red blood cell, and haemoglobin concentration but lower white blood cells and lymphocyte count compared to the control group. The dietary supplementation of SP and BF did not influence the concentration of alanine aminotransferase, creatinine, total protein, and globulin contents ( $P > 0.05$ ) but significantly reduced cholesterol levels of the broiler chicken compared to the control group. The result further showed that broiler chickens fed the diet supplemented with SP and BF had higher ( $P < 0.05$ ) concentration of total glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) compared to the control group. There was no significant difference in aerobic bacteria, lactic acid-producing bacteria and coliform bacteria ( $p > 0.05$ ) count across the experimental treatments. In conclusion, the dietary supplementation of SP and BF can be used to enhance the antioxidative status, reduce cholesterol levels and maintain the gut health of broiler chickens during production.

## KEYWORDS

Antioxidant Enzymes, Chicken, Health, Phytobiotic, Serum, Symbiotic-Acidifier Blend

## 1. INTRODUCTION

Feed additives are essential ingredients that are required in small quantities to improve the quality of feed and animal welfare and performance during production. However, with the ban of antibiotic growth promoters (AGP) as feed additives in poultry production by the European Union in 2006 due to the occurrence of drug-resistant bacteria, antimicrobial residue in carcass, and disorders of the gastrointestinal tract, several management and nutritional strategies have been proposed to boost the productivity, healthiness, and welfare of broiler chickens (Gadde et al., 2017; Żbikowski, et al., 2020; Shittu et al., 2022; Manuelian et al., 2021). Among the nutritional strategies which are widely accepted by consumers and considered natural, cheap and safe as feed additives in animal diets are phytobiotics, probiotics, prebiotic and acidifier (Żbikowski, et al., 2020).

Phytobiotics are a group of plants (herbs, spice and their extracts) that possess high secondary metabolites. They are rich in antioxidant and antimicrobial compounds. They can be used as digestive enhancers, nutraceuticals, growth and health-promoting substances in animal production (Abdelli et al., 2021). In addition, they can be utilized to modify the gut microflora by reducing the number of pathogenic organisms and decreasing the microbial burden on the animal's immune status (Abdelli et al., 2021). Probiotics are feed supplements that contain live strains of selected beneficial microorganisms or growth stimulants of microbial,

animal, plant origin that help to improve the health of the host (Markowiak and Ślizewska, 2018; Poberezhets and Kupchuk, 2021). They are useful in improving the microbiological balance of the intestines and strengthening the intestinal barrier against the influence of harmful factors (Vineetha et al., 2017, Żbikowski, et al., 2020).

While prebiotics are preparations that contain indigestible nutrients that favorably affect the growth and/or activity of one or more intestinal bacterial species (Patterson and Burkholder 2003; Żbikowski, et al., 2020). Acidifiers are acids that are used to lower the pH of the feed, gut, and microbial cytoplasm to inhibit the growth of pathogenic intestinal microflora or reduce subclinical infections and improve the performance of animals (Yadav et al. 2016; Vinolya et al., 2021). The combination of probiotics, prebiotics and acidifier in a blend can create a synergy that will ensure intestinal integrity, limit the growth of pathogenic bacteria, and regulate the absorption of nutrients, immune system and health status of broiler chicken during production.

However, for a feed ingredient to be considered safe for animal health, its effect on blood parameters must be studied, since the evaluation of haematological traits is an important indicator of the nutritional status of the animals (Attia et al, 2017; Oloruntola et al., 2018). Several studies have revealed that the type of diet offered to animals can influence the blood biochemical and haematological components during production (Olafedehan et al., 2010; Oloruntola et al., 2019; Adu et al., 2020). Similarly,

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the examination of gut health and microbiome is important in knowing the rate of gut development and immunity to nutrition and physiological functions (Shang et al., 2018). Any adverse effects on gut health could partially or wholly affect poultry health and impede nutrient uptake and utilization, which invariably may undermine productivity (Aruwa et al., 2021). Thus, the objective of this study was to examine the effect of phytobiotic (Superliv powder®) and synbiotic-acidifier blend (Bacflora-F®) supplements on hemato-biochemical indices, antioxidant enzymes and gut microflora of broiler chicken.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Site

The study was conducted at the poultry unit of Teaching and Research Farm, Adekunle Ajasin University, Akungba Akoko, Nigeria. Akungba-Akoko is situated at 326m above sea level and lies at a latitude of 07° 28' 11" North and a longitude of 5° 44'10" East (NGIA, 2020). It has a mean minimum and maximum temperature of 21 and 32 °C, and precipitation of 1500 - 2000 mm per annum. The annual relative humidity range is 41 - 91% (Olabode 2014). The entire study was carried out for eight weeks following the research ethics and guidelines of the Animal Science Department of the institution.

### 2.2 Experimental Diets and Animals

**Table 1:** Composition and Nutrient Contents of Experimental Basal Diets

Ingredients (%)	Starter (1 to 28 days)	Grower (29 to 56 days)
Maize	52.35	59.35
Wheat Offal	7.0	0
Rice Bran	0	6.00
Soybean Meal	30	24.00
Fish Meal	3.00	3.00
Vegetable Oil	3.00	3.00
Bone Meal	3.00	3.00
Limestone	0.50	0.50
Premix	0.30	0.30
Methionine	0.30	0.30
Lysine	0.25	0.25
Salt	0.3	0.3
Total	100	100
<i>Chemical Analysis (% DM)</i>		
Crude protein	22.18	20.03
Crude fibre	3.35	3.17
<i>Calculated Analysis (%DM)</i>		
Metabolizable Energy (kcal/kg)	3018.10	3108.89
Ca	1.01	0.99
Available P	0.70	0.73
Methionine	0.68	0.66
Lysine	1.36	1.24

Kcal/Kg-Kilocal/Kilogram Ca= Calcium, P= Phosphorus

The feed additives, Superliv powder® and Bacflora-F®, were obtained from Farm Support Services Ltd, Akure, Nigeria. The Superliv powder® (SP) contains a mixture of eighteen (18) different plant leaves while Bacflora-F (BF) contains a mixture of probiotic, prebiotic and acidifier blends. Two basal diets (starter (0-28 days) and finisher phase (28-56 days)) were formulated to meet the broiler's manual requirement (Table 1). At each phase, the experimental diets were divided into four (4) treatments and designated as Diet 1 (basal/control diet), Diet 2 (basal + 0.05 g/kg SP), Diet 3 (basal + 1g/kg BF) and Diet 4 (basal + 0.05g/kg SP and 1g/kg BF). One hundred and twenty (120) 1-day-old Arbor Acres broiler chicks were randomly distributed to four dietary treatments. Each treatment was replicated three (3) times. Thirty (30) birds were assigned to each treatment (10 birds/replicate) in a completely randomized design (CRD).

The birds were housed in their respective pen (200 x 100 cm) with the floor covered with wood shavings. The temperature of the house was

maintained within 31°C ± 2 for the first 7 days and reduced by 2°C after each consecutive 7 days until the house temperature was 26°C±2. The light was provided 23 hours/day while feed and water were provided ad libitum throughout the eight weeks feeding trial.

### 2.3 Experimental Procedures

On the last day of the experiment, 18 birds were randomly selected from each dietary treatment (3 birds/replicate) and humanely slaughtered. Before slaughter, feeds were withdrawn from the birds overnight. During slaughtering, blood samples were obtained from jugular veins into an EDTA bottle for haematological parameters and also into a plain blood sample bottle for serum biochemistry (creatinine, alanine aminotransferase, and cholesterol) and antioxidant enzymes (catalase, superoxide dismutase and, glutathione peroxidase). The blood sample in each of the plain bottles was centrifuged for 10 min at 3000 rpm to obtain clean supernatant serum. The harvested supernatant serum was kept at -20 °C before analysis. The haematological indices were determined by the method described (Shastri, 1983).

The content of serum biochemistry was determined on a Reflectron® Plus 8C79 (Roche Diagnostic, GombH Mannheim, Germany), using kits. The serum antioxidant enzymes (catalase, superoxide and glutathione peroxidase) were determined by the method described (Aebi, 1974; Misra and Fridovich, 1972; Rotruck et al., 1973). The contents of the caeca from broiler chickens (1 bird/replicate) were collected for bacterial population analysis by serial dilution. The agar plates for the determination of the caecal content were aseptically prepared a day before collection. Nutrient agar, Rogosa agar, and MacConkey agr were used to culture aerobic bacteria, lactic acid-producing bacteria, and coliforms and intestinal negative lactose bacteria, respectively (Dibaji et al., 2014; Seidavi and Simoes, 2015).

### 2.4 Statistical Analysis

All data obtained on hemato-biochemical indices, antioxidant enzymes, and gut microflora were subjected to one-way analysis of variance (ANOVA) using SPSS version 20 for a Completely Randomised Design. The differences between treatment means were examined by Duncan's multiple range test of the same package. For all statistical tests, significance was determined at  $p < 0.05$ .

## 3. RESULTS

The result of the blood parameters of broiler chickens fed diets supplemented with Superliv powder® (SP) and Bacflora-F® (BF) is presented in Table 2. The result revealed significant differences ( $P < 0.05$ ) in values of all haematological parameters except monocyte counts. Specifically, the broiler chickens fed supplemented with SP (Diet 2) had the highest packed cell volume ( $38.00 \pm 0.89\%$ ) compared to those on control ( $35.00 \pm 0.89\%$ ), BF ( $34.00 \pm 0.89\%$ ), and SP+BF ( $30.50 \pm 0.89\%$ ) diets. The value of red blood cells was significantly higher in broiler chickens supplemented with SP ( $2.32 \pm 0.11 \times 10^{12}/l$ ), BF (diet 3,  $2.50 \pm 0.11 \times 10^{12}/l$ ), and SP+BF ( $2.21 \pm 0.11 \times 10^{12}/l$ ) compared to control group ( $1.66 \pm 0.11 \times 10^{12}/l$ ). Also, broiler chickens supplemented with SP ( $12.60 \pm 0.29$  g/dl) recorded the highest haemoglobin concentration compared to control ( $10.17 \pm 0.29$  g/dl) and other treatments. The value of the white blood cells and lymphocyte count of the broiler chickens on the control diet were significantly higher than those supplemented with SP, BF, and mixtures (SP+BF). However, no significant differences were observed in the value of monocytes across the experimental treatments ( $P > 0.05$ ).

The result of serum biochemistry indices of broiler chickens fed the supplemented diets is presented in Table 3. The supplementation of SP and BF feed additives significantly influenced the concentration of all the serum biochemistry indices of experimental birds across the dietary treatments ( $P < 0.05$ ), except alanine aminotransferase, creatinine, total protein, and globulin contents ( $P > 0.05$ ). Broiler chicken fed diet supplemented with SP, BF, and SP+BF recorded the lowest cholesterol levels as well as highest albumin content compared to broiler chickens on control diets. The serum antioxidant enzymes of broiler chickens fed diets supplemented with SP, BF, and SP+BF are presented in Figure 1-3. The dietary SP, BF, and SP+BF supplementation revealed a significant increase ( $P < 0.05$ ) in the concentration of total serum glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) ( $P < 0.05$ ) across the dietary treatments.

Broiler chicken fed a diet containing SP, BF, and SP+BF recorded higher concentrations of serum glutathione peroxidase at 230.65, 315.90, and 343.55 mg/ml, respectively, compared to control (191.45mg/ml). Similarly, the concentration of catalase was higher in broiler chickens fed diets containing SP, BF, and SP+BF at 21.71, 15.55 and 18.47mg/g,

respectively, compared to the control group. The superoxide dismutase of broiler chickens supplemented with SP, BF, and SP+BF recorded higher concentrations at 82.21, 97.83, and 84.82%, respectively than those on control treatment. Table 4 shows the result of the gut health of the broiler

chickens. The results showed that supplementation of SP, BF, and SP+BF feed additives did not significantly affect ( $P > 0.05$ ) the aerobic bacteria, lactic acid-producing bacteria, and coliform bacteria ( $P > 0.05$ ) count across the experimental treatments.

**Table 2: Haematological Indices of Broiler Chicken's Broiler Chicken Fed Diets Supplemented with Superliv Powder® and Bacflora-F® Feed Additives.**

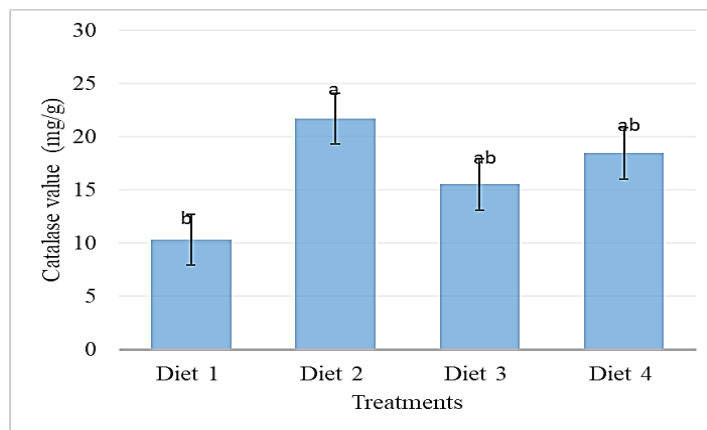
Parameters	Diet 1 Control	Diet 2 0.05g/kg SP	Diet 3 1g/kg BF	Diet 4 0.05g/kgSP+ 1g/kg BF	SEM	P value
Packed cell volume (%)	30.50 <sup>b</sup>	38.00 <sup>a</sup>	34.00 <sup>b</sup>	35.00 <sup>b</sup>	0.89	0.02
Red blood cells (x10 <sup>12</sup> /l)	1.66 <sup>b</sup>	2.32 <sup>a</sup>	2.50 <sup>a</sup>	2.21 <sup>a</sup>	0.11	0.05
Haemoglobin conc. (g/dl)	10.17 <sup>c</sup>	12.66 <sup>a</sup>	11.33 <sup>b</sup>	11.66 <sup>b</sup>	0.29	0.02
White blood cells (x10 <sup>9</sup> /l)	3.13 <sup>a</sup>	2.51 <sup>b</sup>	2.42 <sup>b</sup>	2.08 <sup>c</sup>	0.12	0.00
Lymphocytes (x10 <sup>9</sup> /l)	1.18 <sup>a</sup>	1.05 <sup>a</sup>	1.17 <sup>a</sup>	0.52 <sup>b</sup>	0.09	0.02
Monocytes (x10 <sup>9</sup> /l)	0.27	0.17	0.05	0.28	0.04	0.14

Means within a row with different letters and significantly different ( $p < 0.05$ ). SEM Standard error. SP = Superliv powder®, BF = Bacflora-F®, SP+BF = Superliv powder + Bacflora-F® feed additives

**Table 3: Serum Metabolites of Broiler Chickens Fed Diets Supplemented with Superliv Powder® and Bacflora-F® Feed Additives.**

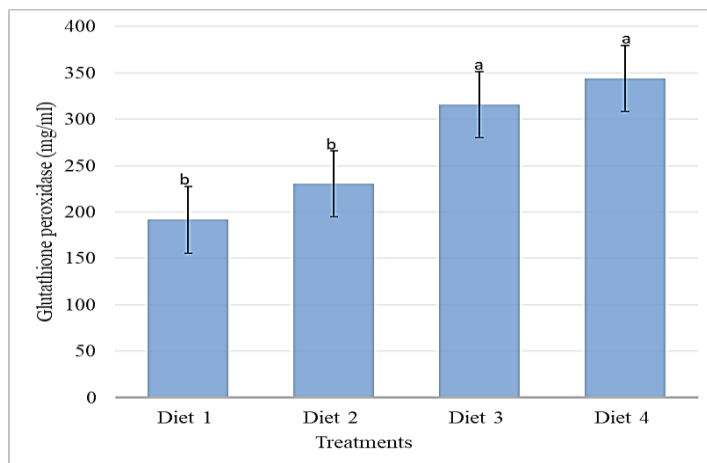
Parameters	Diet 1 Control	Diet 2 0.05g/kg SP	Diet 3 1g/kg BF	Diet 4 0.05g/kgSP+ 1g/kg BF	SEM	P value
Alanine aminotransferase (U/L)	29.6	30.44	30.45	28.87	1.26	0.976
Creatinine (mmol/l)	24.19	26.62	22.52	22.69	2.60	0.183
Cholesterol (mmol/l)	2.87 <sup>a</sup>	1.71 <sup>b</sup>	2.10 <sup>ab</sup>	1.72 <sup>b</sup>	0.18	0.05
Total protein (g/l)	59.71	54.24	54.56	49.60	2.47	0.615
Albumin (g/l)	3.45 <sup>c</sup>	8.05 <sup>b</sup>	10.00 <sup>ab</sup>	12.35 <sup>a</sup>	1.10	0.04
Globulin (g/l)	56.26	46.19	44.56	44.55	2.41	0.26

Means within a row with different letters and significantly different ( $p < 0.05$ ). SEM Standard error. SP = Superliv powder®, BF = Bacflora-F®, SP+BF = Superliv powder®+ Bacflora-F® feed additives



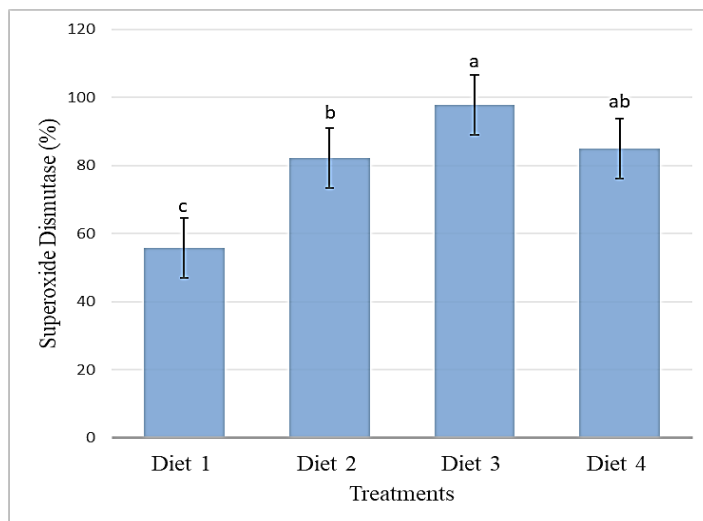
**Figure 1:** Catalase content of broiler chicken fed diets supplemented with Superliv powder® and Bacflora-F® feed additives.

Where Diet 1= Control, Diet 2= 0.05g/kg SP, Diet 3=1g/kg BF, and Diet 4= 0.05g/kgSP+ 1g/kg BF. SP = Superliv powder®, BF = Bacflora-F®, SP+BF = Superliv powder®+ Bacflora-F® feed additives.



**Figure 2:** Glutathione peroxidase content of broiler chicken fed diets supplemented with Superliv powder® and Bacflora-F® feed additives.

Where Diet 1= Control, Diet 2= 0.05g/kg SP, Diet 3=1g/kg BF, and Diet 4= 0.05g/kgSP+ 1g/kg BF. SP = Superliv powder®, BF = Bacflora-F®, SP+BF = Superliv powder®+ Bacflora-F® feed additives.



**Figure 3:** Superoxide dismutase content of broiler chicken fed diets supplemented with Superliv powder® and Bacflora-F® feed additives.

Where Diet 1= Control, Diet 2= 0.05g/kg SP, Diet 3=1g/kg BF, and Diet 4= 0.05g/kgSP+ 1g/kg BF. SP = Superliv powder®, BF = Bacflora-F®, SP+BF = Superliv powder®+ Bacflora-F® feed additives

Table 4: Gut Microflora Content of Broiler Chicken Fed Diets Supplemented with Superliv Powder® and Bacflora-F® Feed Additives						
Parameters (log10 CFU/g)	Diet 1 Control	Diet 2 0.05g/kg SP	Diet 3 1g/kg BF	Diet 4 0.05g/kg SP + 1g/kg BF	SEM	P value
Aerobic bacteria	7.89	8.11	8.30	8.22	0.09	0.47
Coliform bacteria	8.33	8.47	8.71	8.67	0.21	0.93
Lactic acid-producing bacteria	7.75	7.96	6.96	7.71	0.23	0.51

Means within a row with different letters and significantly different ( $p < 0.05$ ). SEM Standard error. SP = Superliv powder®, BF = Bacflora-F®, SP+BF = Superliv powder®+ Bacflora-F® feed additives.

#### 4. DISCUSSION

The assessment of hematological, serum biochemical, and antioxidant parameters is considered an important tool for evaluating the status of animal health and welfare in response to nutrition, diseases, and other environmental challenges. In this study, the supplementation of SP and BF significantly increased the concentration of PCV, RBCs, and HbC in broiler chickens compared to the control group. This suggests that dietary inclusion of SP and BF promotes the formation of blood cellular components (blood haemopoiesis) in the broiler chickens compared to those on the control diet. Also, the increase in PCV, Hb, and RBCs is an indication of improved oxygen carrying capacity of the cells which aids better availability of nutrients to the birds consequently affecting their well-being (Oleforuh-okoleh et al., 2015). This result is in agreement with the report of Rawat et al. (2016) who found that broiler chicken fed Superliv powder at 0.1, 0.2, and 0.3 % had higher PCV, RBCs, and HbC compared to control. Also, some researchers in their study found that the RBCs and HbC content of broilers fed diets supplemented with phytobiotic (*Azadirachata indica* dried leaf meal at 1.25, 2.5, and 5.0g/kg of feed) were higher compared to control group (Ansari et al., 2012).

Conversely, supplementation of SP and BF decreased the concentration of WBCs, lymphocytes, and monocytes compared to the control group, which disagrees with the findings of who fed broiler diet Superliv 0.1, 0.2, and 0.3 % (Rawat et al., 2016). On the other hand, these results are in agreement with the report of who found that broiler chickens fed the diet supplemented with commercial enzymes (Alquerzim at 1gm/L), probiotics (Protexin at 1gm/L) and liver tonic (Lilavit at 1gm/L) decrease the concentration of WBCs, lymphocytes, and monocyte in broiler chicken (Siddiqui et al., 2017). A group researcher also reported that broiler chickens fed diet containing sybiotics (prebiotic+probiotics) had lower WBCs, lymphocytes, and monocytes compared to control group (Mohammed et al., 2016).

Additionally, in their study found that broiler chicken fed diets supplemented with phytobiotic (thymol, carvacrol and cinnamic) had reduced WBCs, lymphocytes and monocytes compared to the control group (Reis et al., 2018). Moreover, the observed lower WBC, lymphocytes and monocytes values in this study showed that supplementation of SP and BF may not have increased the production of these blood components against the residual metabolites in the additives. Hence, this indicates that broiler chickens fed diet containing SP and BF were not immunologically

challenged. WBCs, lymphocytes, granulocytes and monocytes are important cells components that possess phagocytic function and biomarkers for immune functions against infectious organisms in animals (Blumenreich, 1990).

As expected, the observed higher increase in catalase, glutathione peroxidase, and superoxide dismutase content in broiler chickens fed the diet supplemented with SP compared to control could be attributed to the presence of antioxidant and other bioactive compounds inherent in the additives. Studies have shown that diets supplemented with phytobiotics help to stimulate the production of endogenous antioxidant enzymes due to their inherent bioactive and antioxidant compounds (Adeyeye et al., 2020). Similarly, dietary inclusion of symbiotics and acidifiers has been reported to induce the production of higher endogenous antioxidant enzymes in broiler chickens compared to control (Sunu et al., 2021).

As a function, endogenous antioxidant enzymes are essential in protecting cells from the harmful effects of free radicals or scavenging excess free radicals' production and reactive oxygen species (ROS) in the body (Falowo et al., 2014). Particularly, catalase has been reported as important antioxidant enzyme that inactivate, scavenge and remove free radical and other reactive species in tissue to protect the body against oxidative stress (Delles et al., 2014, Kurutas, 2015; Eddaikra and Eddaikra, 2020). Glutathione peroxidase (GSH-Px) on the other hand acts to catalyze the degradation of various peroxides to protect the cells against the damaging effect of oxidation (Kurutas, 2015; Eddaikra and Eddaikra, 2020). While superoxide dismutase helps to scavenge initiated superoxide ion in the system (Kurutas, 2015; Eddaikra and Eddaikra, 2020).

However, the decrease in cholesterol level of broiler chicken fed SP and BF diets in the broiler diets suggest their hypocholesterolemic activity which could be due to their ability to delay the absorption of dietary fat in the intestine by inhibiting the activity of pancreatic lipase when applied in the poultry diet. Cholesterol is a type of lipid that usually occurs as part of lipoproteins in the body. Excessive deposition of cholesterol in the body has been linked with cardiovascular disease, with evidence of blocking of artery walls and reducing the flow of blood to the heart (Oloruntola et al., 2018). This result is in agreement with the findings of who reported that broiler chickens fed diet containing phytobiotics (Chlorophyllum root and Camellia seed) and synbiotic (prebiotics and probiotics), respectively, had lower serum cholesterol level compared to control (Gaurav, 2015; Sunu et al., 2021). Also, the observed similarity in values of alanine

aminotransferase, creatinine, and total protein across treatments showed that dietary inclusion of SP and BF did not have a negative influence on the function of the internal organs of the broiler chicken. This result is in contrast with the finding of who found no significant difference in aspartate aminotransferase content of broiler chickens fed diet containing phytobiotics and probiotics compared to control group (Gholami-Ahangaran et al., 2021).

On the other hand, the lack of significant difference in the result of the total aerobic bacteria, coliform bacteria, and lactic acid-producing bacteria across the treatments is suggesting that dietary supplementation of SP and BF could maintain the amount of gut microbiome of the broiler chickens throughout the experimental period. Earlier reports have shown that the maintenance of non-pathogenic gut bacteria is essential for inhibiting proliferation of pathogen, improvement of growth rate, and reduction of morbidity and mortality of broiler during production (Seidavi and Simoes 2015; Oloruntola et al., 2019). This result is in agreement with the result of who found that the addition of phytobiotic did not significantly affect the gut microflora of boiler chicken compared to the control group (Oloruntola et al., 2019). On the contrary, found that broiler chickens supplemented with probiotic and synbiotics had reduced total coliform number and increased numbers of lactobacilli in the gut of broiler chickens compared to control (Dibaji et al., 2014).

## 5. CONCLUSION

Findings from this study have shown that dietary inclusion of Superliv powder and Bacflora-F feed additives could be used to enhance the antioxidative status, reduce the cholesterol level and maintain the gut health of broiler chickens during production. The results also showed that supplementation of SP and BF as potential feed additives in broiler diets would not impair their health and welfare during production.

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