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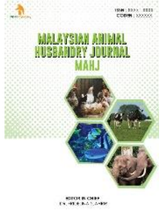

RESEARCH ARTICLE

ASSESSING THE IMPACT OF CASHEW LEAF POWDER AND A BLEND OF PROBIOTIC, PREBIOTIC, AND ACIDIFIER ON THE PERFORMANCE OF BROILER CHICKENS AND ANTIOXIDANT ENZYME ACTIVITY UNDER TROPICAL CONDITIONS

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ABSTRACT

The poultry industry continually seeks innovative strategies to optimize broiler performance and address the challenges posed by tropical climates. This study evaluated the influence of Cashew Leaf Powder and a blend of Probiotic, Prebiotic, and Acidifier on the performance metrics of broiler chickens, focusing on parameters such as body weight gain, feed efficiency, and antioxidant enzyme activity, under the specific environmental conditions of tropical climates. A total of 240 day-old Cobb 500 broiler chickens (6 replicate/diet; 10 birds/replicate) were distributed across 4 dietary treatments: control/no supplement, 250 mg/kg blend of Probiotic, Prebiotic, and Acidifier, 2,500 mg/kg Cashew Leaf Powder and 250 mg/kg BPPA+2,500mg/kg Cashew Leaf Powder and labelled diets 1, 2, 3 and 4, respectively. The broiler chickens' body weight gain in supplemented diets (diets 2, 3, and 4) was significantly ($P<0.05$) greater than in diet 1 during the finishing phase (22-42 days) and overall growing period (1-42 days). The serum albumin concentrations were considerately ($P<0.05$) higher in diets 2, 3 and 4 than in diet 1, while aspartate aminotransferase levels were lower ($P<0.05$) in 2, 3 and 4 than in diet 1. The birds' glutathione peroxidase and superoxide dismutase levels were greater ($P<0.05$) in the 2, 3 and 4 than in diet 1. The 250 mg/kg blend of Probiotic, Prebiotic, and Acidifier and 500 mg/kg Cashew Leaf Powder dietary supplementation improved the birds' body weight gain and antioxidant standing.

KEYWORDS

Broiler health, Dietary treatments, Innovative strategies, Performance metrics, Poultry industry challenges.

1. INTRODUCTION

In current decades, the human populace withinside the tropics has grown, ensuing in extra animal protein production (Kpomasse et al., 2021). Furthermore, broiler production, mainly in tropical and subtropical countries, has been acknowledged as a method of solving the problems of animal protein scarcity (Kpomasse et al., 2021). High ambient temperatures, on the alternative hand, impair the overall growth rate of commercial broiler birds because of heat stress (Rahimi et al., 2019). Predictions that the common temperature in western Africa will climb from 2°C to 6°C through 2100, for instance, provide a widespread hurdle to profit-orientated broiler production due to the fact the birds develop optimally in the temperature variety of 18.00°C-24.00°C (Oke et al., 2020). Reduced hunger, reduced immunologic responses, decrease feed consumption, physiologic abnormalities, behind-schedule growth, intestine microbiome modification, and oxidative harm have all been connected to heat strain in broiler chickens (Sugiharto, 2020).

The adjustment of birds' diets to the climatic circumstance and supplementation of antioxidants are many of the suggestions for ameliorating the consequences of warmth strain on the birds (Kpomasse et al., 2021). Acidifiers, phytobiotics, prebiotics, and probiotics have all been investigated as nutritional dietary supplements to help mitigate the detrimental results of growing ambience warmth and thermal strain (Awaad et al, 2018; Awad et al., 2020). Taking phytosupplements or botanicals has currently turned out to be famous as a manner to lessen the terrible consequences of thermal strain on broiler birds (Oloruntola et al., 2018; Adeyeye et al., 2020; Sugiharto, 2020). Various natural plant dietary supplements have demonstrated antioxidant activity by supplying hydrogen to metals, blocking their pro-oxidative activity, or by dousing O_2, diminishing O_2 concentrations, preventing peroxide generation, and activation of antioxidant enzymes (Manuelian et al., 2021). By suppressing linoleic acid peroxidation and scavenging hydroxyl ions and superoxide ions, probiotics lessen antioxidative strain in animals; even as prebiotics have been connected to growing the useful microbial populace, that could have resulted withinside the launch of pharmacological materials or factors that might lessen the expression of heat stress protein through mitigating oxidative strain and oxidative harm (Hoffmann et al., 2019; Awad et al., 2020). Dietary acidifiers have been taken into consideration as antioxidants due to the fact they prohibited the formation of oxygen

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radicals (Awaad et al., 2018). Cashew (*Anacardium occidentale*) L, a potential plant whose leaves may be processed and used in animal production, grows to a height of as much as forty-nine ft and is located in lots of tropical countries. Cashew leaf powder has bioactive components such as tannins, flavonoids, and phenols among others and antioxidant properties, establishing it as a natural bioactive or phytogenic supplement or additive in an animal feeding simulation (Oloruntola, 2021).

Consequently, the objective of this study is to peer how including Cashew leaf powder and a commixture of acidifiers, probiotics, and prebiotics in the feed of broiler chickens influences their performances, serum biochemistry indices, and antioxidant enzymes.

2. Materials and Methods

2.1 Animal protocol approval and dietary supplements

The experiment's requirements and standards for animals and animal procedures were approved by the Research and Ethics Committee of the Animal Science Department at Adekunle Ajasin University in Akungba Akoko town, Ondo State, Nigeria.

Oloruntola meticulously described the process of collecting and converting cashew leaves into cashew leaf powder (CLP), along with the evaluation of CLP (Oloruntola, 2021). Fresh cashew leaves sourced from Adekunle Ajasin University's Akungba Teaching and Research Farm underwent a systematic procedure. They were air-dried for a period of 12 days in the shade and subsequently milled with a 0.5 mm screen to produce CLP. This CLP underwent comprehensive testing, including proximate composition analysis (crude protein, nitrogen-free extract, crude fibre, ash, crude fat, and moisture), following the AOAC method (AOAC, 2016). Additionally, phytochemical composition assessment (tannins, flavonoids, phenols, total saponins, alkaloids, and phytate) was conducted (Oloruntola, 2021). The antioxidant properties of CLP were evaluated using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method, a technique for radical degradation activity (Baliyan et al., 2022). The mineral composition, encompassing zinc, calcium, phosphorus, and magnesium, was also determined (Oloruntola, 2021).

As indicated in a preliminary study on CLP, the proximate composition includes crude fat (10.8%), crude protein (14.7%), crude fibre (17%), ash (3.7%), and nitrogen-free extract (49.6%). Furthermore, the phytochemical profile consists of phenol (6.1 mg/g), flavonoids (8.6 mg/g), alkaloids (75.6 mg/g), phytate (2 mg/g), and tannins (0.4 mg/g) (Oloruntola, 2021). The antioxidant properties, measured through DPPH, indicated a radical degradation activity of 42.2%. The mineral composition revealed magnesium (1.9 mg/kg), zinc (6.7 mg/kg), phosphorus (6.2 mg/kg), and calcium (5.8 mg/kg). The blend of probiotic, prebiotic, and acidifier (BPPA) was prepared by Xvet, GMBH, located in 22529 Hamburg, Germany. The BPPA was procured from commercial animal feed ingredient dealers in Akure, Nigeria. The composition of BPPA includes *Saccharomyces cerevisiae* (40.00%); *Enterococcus faecium* (1x4x109 CFU); *Bacillus licheniformis* + *Bacillus subtilis* (4x109 CFU); *Lactobacillus acidophilus* (5x4x109 CFU); Magnesium (5.00%); Formic acid (9,000mg); Orthophosphoric acid (3,000 mg); Citric acid (2,000 mg); and Lactic acid (3,000 mg).

2.2 Experimental diets, site, and design

During the starter phase (1-21 days) and finisher phase (22-42 days) of broiler chicken production, two distinct basal diets (Table 1) were formulated and compounded, considering the specific nutritional requirements of the birds at each stage. The proximate composition, including crude fiber, crude fat, and crude protein, of the basic diets was determined according to the AOAC method (AOAC, 2016). Subsequently, the fundamental or standard diet for each phase was divided into four equal portions and labeled as diets 1 through 4, each with its specific composition:

Diet 1: No supplement

Diet 2: 250mg/kg BPPA

Diet 3: 2,500mg/kg CLP

Diet 4: 250mg/kg BPPA+2,500mg/kg CLP

Table 1: Composition of the experimental basal diets

Experimental basal diets		
Ingredients (%)	**Starter**	**Finisher**
Maize	52.33	59.32
Maize bran	7.02	0.00
Rice bran	0.00	6.03
Fish meal	3.00	3.00
Soybean meal	30.00	24.00
Premix	0.30	0.30
Bone meal	3.00	3.00
Soy oil	3.00	3.00
Methionine	0.30	0.30
Limestone	0.50	0.50
Salt	0.30	0.30
Lysine	0.25	0.25
Analyzed composition (%)		
Crude fibre	3.52	3.61
Crude fat	4.44	3.98
Crude protein	22.13	20.06
Calculated composition (%)		
Calcium	1.01	0.99
Available phosphorus	0.45	0.40
Methionine	0.69	0.66
Lysine	1.38	1.26
Metabolizable energy (Kcal/kg)	3018.91	3108.14

The feeding trial was conducted at the Teaching and Research Farm (RTF) of Adekunle Ajasin University (AAUA), located in Akungba Akoko town, Nigeria, from February to March 2021. The geographical coordinates of the location are approximately between 7°28' and 7°0' N latitudes and 5°44' and 5°0' E longitudes of the Greenwich meridian. The average environmental conditions during the trial period were 30.13°C temperature and 68.27% relative humidity.

A total of 240 Cobb 500 broiler chicks, one day old and weighing 34.98±1.18 g, were randomly assigned to four experimental diets using a completely randomized design, with six replicates per dietary treatment and ten experimental birds per replicate. Each experimental pen, measuring 2m x 1m, was covered with dry wood shavings to a depth of 3.5cm. The temperature was initially maintained at 31±3 degrees Celsius for the first seven days and gradually reduced by 2 degrees Celsius each week until reaching 26±3 degrees Celsius. During the feeding study, adherence to the European Union's animal welfare guidelines was maintained. The experimental pen implemented a daily lighting cycle, incorporating 6 hours of darkness within every 24-hour period, and hand-operated drinkers and feeders were installed in the experimental pens. The dietary supplements, Blend of Probiotic, Prebiotic, and Acidifier (BPPA), and Cashew Leaf Powder (CLP), were added to the diets and fed to the experimental birds.

In addition, a combination of electrolyte and vitamin solution was added to the drinking water, provided 24 hours before and after vaccination, to alleviate the stress associated with the vaccination process. Vaccination programs for Newcastle disease (at 1 and 19 days), infectious bronchitis virus, and avian influenza (at 1 day), as well as infectious bursa disease virus (at 19 days), were implemented during the 42-day feeding trial.

2.3 Blood samples collection, analysis and data analysis

Throughout the study, feed intake (FI) and body weight (BW) were systematically monitored and recorded every seven days. The average body weight gain (BWG) was calculated by comparing the initial and final weights of the birds. The feed conversion ratio (FCR) was determined by dividing the amount of feed consumed by the weight gained.

At the conclusion of the six-week feeding study, four birds were randomly chosen from each replicate. Blood samples were collected via the brachial vein using a syringe and needle. A portion (4ml) of each blood sample was dispensed into a plain blood sample bottle for the analysis of serum biochemicals, enzymes, electrolytes (including total protein, albumin, globulin, aspartate aminotransferase, alanine aminotransferase, creatinine, cholesterol, potassium, chlorine, and sodium), as well as antioxidant enzymes (catalase, glutathione peroxidase, and superoxide dismutase). The blood samples in each plain bottle were spun, and the serum was decanted into another plain bottle before being frozen at -20°C for subsequent analysis. Serum biochemical and enzyme concentrations were analyzed using a Reflectron® Plus 8C79 and associated kits. Specifically, serum catalase (CAT), and superoxide dismutase (SOD) were assessed (Tekin and Seven, 2022). While the glutathione peroxidase (GPx) was determined following the procedures reported (Moretti et al., 2017). For the determination of potassium, chlorine, and sodium, the OPTI CCA-TS2 Blood Gas and Electrolyte Analyzer from OPTI Medical Systems, Inc. (235 Hembree Park Drive, Georgia 30076, USA) were utilized.

The model: $D\xi y = \mu + a\xi + b\xi y$, was used in this experiment, where $D\xi y$ is the response variable; ξ = the overall average; $a\xi$ = the ξth dietary effect (D= diets 1, 2, 3, and 4); and $b\xi xy$ = random error due to the investigation. All of the data was treated to one-way ANOVA in SPSS. The SPSS Duncan multiple range test was used to find discrepancies between the treatment means ($P<0.05$).

3. Results

Table 2 illustrates the impact of Cashew Leaf Powder (CLP) and Blend of Probiotic, Prebiotic, and Acidifier (BPPA) on the performance of broiler chickens. Throughout the starting phase, performance indicators such as Body Weight Gain (BWG), Feed Intake (FI), and Feed Conversion Ratio (FCR) remained consistent ($P>0.05$) across the dietary regimens. However, during the finishing period (22-42 days) and the overall growing period (1-42 days), the BWG of broiler chickens receiving the supplemented diets (diets 2, 3, and 4) was significantly ($P<0.05$) greater than that of diet 1.

Table 2: The effects of cashew leaf powder and BPPA on the performance of broiler chickens

	Diet 1	Diet 2	Diet 3	Diet 4	SEM	P value
Starter phase (1-21day)						
IBW (g/bird)	35.00	35.7	34.30	34.90	1.18	0.66
BWG (g/bird)	804.00	852.00	839.00	817.00	43.5	0.71
FI (g/bird)	1660.00	1410.00	1330.00	1490.00	214	0.51
FCR	2.05	1.65	1.59	1.82	0.25	0.31
Grower phase (22-42day)						
BWG (g/bird)	1700.00^{b}	1890.00^{a}	1900.00^{a}	1940.00^{a}	58.6	0.01
FI (g/bird)	3100.00	2910.00	3150.00	3060.00	489	0.96
FCR	1.82	1.54	1.67	1.57	0.26	0.71
Overall (1-42 day)						
BWG (g/bird)	2500.00^{b}	2740.00^{a}	2740.00^{a}	2760.00^{a}	53.3	0.01
FI (g/bird)	4760.00	4320.00	4490.00	4550.00	648	0.92
FCR	1.90	1.57	1.64	1.64	0.23	0.52

Means in a row without a common superscript letter differ ($P<0.05$); BPPA: Blend of probiotic, prebiotic, and acidifier; IBW:Initial body weight; BWG: Body weight gain; FI: Feed intake; FCR: Feed conversion ratio; Diet 1: Control, Diet 2: 250mg/kg BPPA; Diet 3: 2,500mg/kg cashew leaf powder; Diet 4: 2,500mg/kg cashew leaf powder + 250mg/kg BPPA; SEM Standard error of the mean.

Table 3 reveals that dietary treatments did not have a significant impact on serum total protein, globulin, albumin-globulin ratio, alkaline transferase, creatinine, and cholesterol. Nevertheless, serum albumin concentrations were notably ($P<0.05$) higher in diets 1, 2, and 3 compared to those in diet 1, while aspartate aminotransferase levels were lower ($P<0.05$) in diets 2, 3, and 4 compared to those in diet 1.

Table 3: The effects of cashew leaf powder and BPPA on serum biochemical indices of broiler chickens.

	Diet 1	Diet 2	Diet 3	Diet 4	SEM	P value
Total protein (g/l)	59.70	54.20	60.40	59.60	4.29	0.48
Albumin (g/l)	3.45^{b}	8.05^{a}	7.80^{a}	11.40^{a}	1.85	0.02
Globulin (g/l)	56.30	46.20	52.60	48.20	5.72	0.35
Albumin globulin ratio	0.06	0.19	0.15	0.25	0.06	0.08
Aspartate aminotransferase (IU /L)	122.00^{a}	88.00^{b}	95.80^{b}	90.10^{b}	4.18	0.01
Alkaline transferase (IU/L)	29.60	30.40	28.10	27.10	3.78	0.82
Creatinine (mmol/l)	24.20	26.60	27.00	27.40	1.19	0.10
Cholesterol (µmol/l)	2.88	1.71	2.10	1.92	0.59	0.29

Means in a row without a common superscript letter differ ($P<0.05$); BPPA: Blend of probiotic, prebiotic, and acidifier; Diet 1: Control, Diet 2: 250mg/kg BPPA; Diet 3: 2,500mg/kg cashew leaf powder; Diet 4: 2,500mg/kg cashew leaf powder + 250mg/kg BPPA; SEM Standard error of the mean.

The effects of BPPA and CLP on serum antioxidant enzymes in broiler chickens are presented in Table 4. The concentration of catalase (CAT) tended to be influenced by dietary supplements ($P=0.08$). Glutathione peroxidase (GPx) levels were greater ($P<0.05$) in the BPPA and CLP supplemented diet birds than in the control diet birds. Similarly, superoxide dismutase (SOD) levels were higher ($P<0.05$) in diets 2, 3, and 4 compared to diet 1, with diet 3 (2,500mg/kg CLP) exhibiting the highest SOD concentration. Dietary supplementations had no significant effect ($P>0.05$) on blood potassium, chlorine, or sodium (Figure 1).

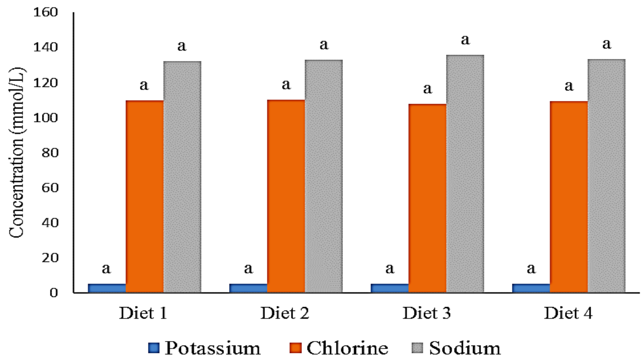

Figure 1: The effects of cashew leaf powder and BPPA on the serum potassium, chlorine and sodium of broiler chickens.

BPPA: Blend of probiotic, prebiotic, and acidifier; Diet 1: Control, Diet 2: 250mg/kg BPPA; Diet 3: 2,500mg/kg cashew leaf powder; Diet 4: 2,500mg/kg cashew leaf powder + 250mg/kg BPPA.

Table 4: The effects of cashew leaf powder and BPPA on serum antioxidant enzymes of broiler chickens

	Diet 1	Diet 2	Diet 3	Diet 4	SEM	P value
Catalase (kU)	9.77	22.86	13.92	16.94	1.95	0.08
Glutathione peroxidase (mg/ml)	190.89[b]	231.80[a]	231.06[a]	324.60[a]	19.11	0.04
Superoxide dismutase (%)	55.31[d]	83.36[b]	99.78[a]	69.10[c]	5.23	0.01

Means in a row without a common superscript letter differ ($P<0.05$); BPPA: Blend of probiotic, prebiotic, and acidifier; Diet 1: Control, Diet 2: 250mg/kg BPPA; Diet 3: 2,500mg/kg cashew leaf powder; Diet 4: 2,500mg/kg cashew leaf powder + 250mg/kg BPPA; SEM Standard error of the mean.

4. Discussion

The observed variations in Body Weight Gain (BWG) among birds in different dietary supplementation treatments during the starter and finisher phases in this study may be attributed to divergent management practices and nutritional requirements adopted in these distinct phases (Oloruntola et al., 2021). This trend aligns with earlier research by the author in 2021, where discrepancies in the performance characteristics of birds fed diets supplemented with wild mango kernel powder and clove basil leaf powder were reported, particularly becoming evident in the finisher phase rather than the initial phase (Oloruntola et al., 2021). Birds often exhibit depressed growth performance, reduced protein gain, and increased fat gain in response to heat stress and oxidative stress (Kpomasse et al., 2021). However, the enhanced Body Weight Gain (BWG) observed in this study among birds fed diets supplemented with a blend of Probiotic, Prebiotic, and Acidifier (BPPA) and Cashew Leaf Powder (CLP) suggests the presence of antioxidant and growth-improving properties in these dietary supplements (Oloruntola, 2021). This implies that BPPA and CLP supplementation could potentially mitigate the negative consequences of thermal and oxidative stress in birds, consequently improving their overall body weight gain.

Moreover, phytobiotics, such as BPPA and CLP, are recognized for their potential in aiding animal growth by enhancing the nutrient digestion process and boosting intestinal functions (Valenzuela-Grijalva et al., 2017). Enhanced intestinal function resulting from phytobiotic supplementation prevents the infiltration of toxic constituents, such as lipopolysaccharides, infections, or disease-causing organisms into the circulatory system. This also curtails the development of inflammatory processes by increasing cytokine and glucocorticoid output. Consequently, the stabilization of animals' metabolism, lowered metabolism expenditure, and fostered growth are observed (Kikusato, 2021).

Furthermore, prebiotic and probiotic supplementation positively influences body weight by stimulating digestibility and influencing gut microbes (Emili Vinolya et al., 2021). The synergistic action of probiotics, prebiotics, and acidifiers has also been reported to enhance body weight gain. Previous studies such as Oloruntola et al., with pawpaw leaf and seed meals and Adeyeye et al., with wild sunflower and goat weed, have reported improvements in the body weight gain of broiler chickens through the supplementation of phyto-supplements (Oloruntola et al. 2020; Adeyeye et al., 2020). Serum biochemistry indicators are valuable tools for understanding the origins and progression of diseases, as well as assessing overall health (Gbore et al., 2021). Previous research has demonstrated that dietary treatments can influence specific serum biochemistry indices (Oloruntola et al., 2018; Adeyeye et al., 2020). However, in the current investigation, the concentrations of serum total protein, globulin, albumin-globulin ratio, alkaline transferase, creatinine, and cholesterol in broiler chickens remained consistent across all diets, indicating the nutritional safety of the supplements.

The analysis of blood proteins is fundamental in general biochemistry, serving as crucial markers for identifying health conditions, production characteristics, and metabolic changes. The higher albumin concentration observed in broiler chickens fed diets 2, 3, and 4 in this study could be attributed to their increased body weight gain and the subsequent demand for higher albumin concentration to maintain metabolic balance (Piotrowska et al., 2011). This finding aligns with the elevated serum albumin values reported by Piotrowska et al., in broiler chickens as their weight increased with age (Piotrowska et al., 2011). The liver and skeletal muscle exhibit the highest levels of aspartate aminotransferase (AST), and elevated serum AST is commonly associated with bruising, trauma, necrosis, infection, or liver/muscle neoplasia. Therefore, the reduced AST levels observed in birds fed diets supplemented with a blend of Probiotic, Prebiotic, and Acidifier (BPPA) and Cashew Leaf Powder (CLP) indicate a health benefit, suggesting hepatoprotective properties of the supplements (Oloruntola et al., 2018). This hepatoprotective effect may be attributed to the antioxidant properties of BPPA and CLP (Awad et al., 2020; Zang et al., 2011; Oloruntola, 2021). The consumption of antioxidant-rich natural compounds enhances the scavenging of free radicals, maintains a healthy oxidative-antioxidant balance in the liver, and, consequently, offers liver protection (Casas-Grajales and Muriel, 2015).

Moreover, in this study, the observed increase in serum concentrations of glutathione peroxidase and superoxide dismutase in diets 2, 3, and 4 may be linked to the antioxidant properties of these supplements. Phytobiotics, probiotics, prebiotics, and acidifiers have all been associated with enhancing the antioxidant status in broiler chickens, reflecting their potential in promoting a robust first-line defense against oxidative stress (Ma et al., 2021). Electrolytes play a crucial role in regulating key bodily functions, necessitating a delicate balance for proper body function. Specifically, sodium, potassium, and chloride ions significantly impact the acid-base balance of blood and tissues (Gałęska et al., 2022). The consistently maintained serum levels of potassium, chloride, and sodium in broiler chickens throughout this study further underscore the nutritional suitability of Probiotic, Prebiotic, and Acidifier (BPPA) and Cashew Leaf Powder (CLP) in broiler chicken nutrition.

5. Conclusion

The dietary supplementation of 250mg/kg BPPA and 2,500mg/kg CLP resulted in improved Body Weight Gain (BWG) and antioxidant status in broiler chickens. Importantly, these supplements did not exert negative effects on the biochemical indices or the concentrations of potassium, chloride, and sodium ions in the broiler chickens. This suggests the potential health benefits and nutritional safety of BPPA and CLP in enhancing broiler performance and overall well-being.

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