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Nutraceutical Effects of *Justicia carnea* Leaf Powder Supplementations on Performance, Blood Indices, Heat Shock Protein 70, Oxidative Deoxyribonucleic Acid Damage Biomarkers and Intestinal Microbes of Broiler Chickens, Under Tropical Condition

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ABSTRACT

The main reason preventing broiler chickens from reaching their genetic potential and hurting their performance in the tropics is heat stress. This study aimed to ascertain how *Justicia carnea* leaf powder (JLP) supplementation affects broiler chickens' performance, blood indices, antioxidant status, and gut microflora in tropical environments. A completely randomized method was used to assign 240 Cobb 500 broiler chicks to the experimental diets (6 replicates per diet, 10 birds per replication). Diet 1 included no supplement (negative control), diet 2 included 200 mg/kg vitamin C (positive control), diet 3 included 2,500 mg/kg JLP, and diet 4 included 5,000 mg/kg JLP. On day 42, the body weight gain (BWG) of the birds fed on diet 4 was significantly higher than those on diet 1. The packed cell volume, red blood cell count, and hemoglobin concentration of the birds fed on diets 3 and 4 were significantly higher than those of the control ($P<0.05$). The serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and cholesterol were lower in birds fed on diets 3 and 4, compared to those on diet 1 ($P<0.05$). The serum heat shock protein 70 (HSP 70) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) were lower in birds fed on diets 3 and 4, compared to those on diet 1 ($P<0.05$). The lactic acid-producing bacteria (LAB) population was higher in birds fed on diets 3 and 4, compared to those on diet 1($P<0.05$). However, the *Coliform* bacteria population was reduced in birds fed on diets 3 and 4, compared to those on diet 1. The 2,500 and 5,000 mg/kg JLP dietary supplementations enhanced BWG, improved erythrogram indices, and reduced blood AST, ALT, cholesterol, HSP 70, 8-OHdG, and caeca *Coliform* population but increased the caeca LAB population.

Keywords: Antioxidants, Avian species, Feed additives, Performance, Phyto-biotics

1. Introduction

Due to the expansion of the human population and the resulting rise in the demand for animal protein, broiler production has expanded across the tropics and subtropics over the past two decades. However, it has been determined that the effects of climate change, high ambient temperatures, and heat stress incidents are the main factors preventing broiler chickens from realizing their genetic potential and thus harming their performance (1).

Furthermore, broiler chickens can only function at their peak levels in their thermoneutral zone (18°C to 24°C). Some antioxidant enzymes are affected by heat stress, which also increases the levels of reactive oxygen species and leads to DNA damage, protein denaturation, as well as lipid peroxidation (2). Gut health is regulated by reactive nitrogen species and the reactive oxygen produced by the gut epithelia cells as a result of oxygen metabolism and enteric commensal bacteria. In general, the increased synthesis of reactive oxygen species causes oxidative stress by increasing the formation of free radicals and antioxidant insults (3).

As a result, genetic, management, and feeding approaches have been used to reduce the effects of climate change and heat stress on broiler production (1). However, in the tropics, feeding options and the dietary modification approach, which incorporates antioxidant supplements to mitigate the effects of oxidative and heat stress on broiler productivity, are less expensive and easier to adjust to (4).

Animal performance and product quality are currently improved by adding dietary supplements of synthetic antioxidants (5). However, the controversy surrounding the use of artificial supplements in animal agriculture has drawn attention to natural antioxidants or phytochemicals. Particularly, it has been observed that phytochemicals influence the animal's endogenous antioxidant systems, performance, and product quality (5). When employed as dietary components, phytochemicals, phytogens, or other plant-derived compounds play significant roles in the physiology of the animal body and can prevent or treat diseases. They are plentiful, relatively less expensive, and less harmful (6).

Previous research has revealed the potential health benefits of certain botanicals, particularly goat weed, wild sunflower (7), *Mucuna pruriens* (8), *Momordica charantia*, and *Ocimum gratissimum* (9). Recently, *Justicia carnea* leaf powder (JLP)'s anti-inflammatory, antioxidant, and anti-diabetic properties have been published (10). Additionally, rats exposed to phenylhydrazine had their anemia corrected and their lipid profiles enhanced by *Justicia carnea* extract (11). Therefore, this study attempted to ascertain how JLP supplementation affects broiler chickens' performance, hematological indices, serum biochemical indices, heat shock protein 70 (HSP 70), oxidative deoxyribonucleic acid damage biomarkers, and intestinal microbes in tropical environments.

2. Materials and Methods

2.1. Processing of *Justicia carnea* Leaf and Experimental Diets

Justicia carnea leaves were minced and sparingly scattered on clean stainless-steel platters. They were then left to air dry for 14 days in the shade. Full details on the processing of *Justicia carnea* leaves to JLP have been previously published (10). In this study, JLP was employed as a phytogenic supplement.

Baseline diets were created and compounded (Table 1) to meet the birds' nutritional needs during the two stages (starter and finisher) of broiler chicken production. The baseline diet was divided into four equal portions at each phase of growth and named diets 1 through 4. Diet 1 included no supplement (negative control), diet 2 included 200 mg/kg vitamin C (positive control), diet 3 included 2,500 mg/kg JLP, and diet 4 included 5,000 mg/kg JLP.

Table 1. Composition of the experimental basal diets

Ingredients	Starter feed	Finisher diet
Maize	52.33	59.32
Maize bran	7.02	0.00
Rice bran	0.00	6.03
Fish meal	3.00	3
Soybean meal	30	24
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	3.55	3.63
Crude fat	4.47	3.94
Crude protein	22.19	20.09
Calculated composition (%)		
Calcium	1.02	0.97
Available phosphorus	0.44	0.41
Methionine	0.68	0.65
Lysine	1.36	1.24
Metabolizable energy (Kcal/kg)	3018.93	3108.16

2.2. Experimental Site and Design

The feeding study was conducted on the Teaching and Research Farm of the Agricultural Technology Department, The Federal Polytechnic, Ado Ekiti, Nigeria, during February and March 2022. The HTC digital thermometer and hygrometer were used to calculate the average daily atmospheric temperature. During February and March 2022, the daily average temperature was 25.56±5.8ºC and 26.11±4.73ºC, respectively.

A total of 240 Cobb 500 broiler chicks were assigned randomly to the experimental diets (6 replicates per diet, 10 birds per replication). The experimental pen's temperature was maintained at 31±3ºC for seven days before it was decreased by 2 degrees each week until it reached 26±3ºC. On the first day, the lights were on for 24 h, and on days two through seven, they remained on for 23 h. Eighteen hours per day of lighting were available during the raising phase. The birds were fed constantly during the six-week experiment.

2.3. Performance Indicators, Sample Collection, and Analysis

Body weight, feed intake, and body weight gain (BWG) were all measured at intervals of seven days. The ratio of the birds' feed consumption to the increase in their body weight was used to calculate the feed conversion ratio. Twelve birds were randomly selected from each treatment group (2 birds/replica) on the 42nd day of the experiment, and they were bled using a syringe and needle through the brachia vein. Blood was collected from the chickens and put into bottles containing ethylenediaminetetraacetic acid (EDTA) and ordinary sample vials. The plain bottle samples were centrifuged, and their serum was split into another set of plain bottles and frozen at -20ºC before being used to measure serum chemical indices, HSP, and deoxyribonucleic acid damage lesions marker. The following serum parameters were measured using a Reflectron ® Plus 8C79 (Roche Diagnostic, GonbH Mannheim, Germany): total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol, creatinine, glucose, HSP 70 (12), and 8-hydroxy-2'-deoxyguanosine (8-OHdG) (13). The samples were collected into EDTA sample vials, and the packed cell volume (PCV), red blood cell (RBC), and hemoglobin concentration (Hbc) of the samples were measured (14). About 24 h before sample collection, agar plates were produced and streaked on the collection site to evaluate bacterial growth. The birds' caecal contents from each replicate were then removed for a subsequent serial dilution investigation of the bacterial populations. Lactic acid-producing bacteria (LAB) were grown on Man Rogosa agar, while *Coliforms* were grown on MacConkey agar. The nutrient agar was utilized to cultivate the total number of aerobic bacteria (15).

2.4. Statistical Evaluation of Data

The SPSS software (version 20) was used for the statistical analysis. All of the acquired data were subjected to a one-way analysis of variance using the general linear model technique. The Duncan multiple range test included in the same package was applied to determine mean differences between the treatment groups. $P<0.05$ was used to denote statistical significance.

3. Results

Table 2 shows the effects of JLP on the performance characteristics of broiler chickens. The BWG of the birds fed on diets 2 and 4 was higher than those on the control diet 1 (*P*<0.05). The PCV, RBC, and Hbc concentrations of the birds on diets 2, 3, and 4 were higher than those on diet 1 (*P*<0.05) (Table 3). On the other hand, the total protein, albumin, globulin, glucose, and creatinine were similar across the dietary treatments (*P*>0.05). The serum AST, ALT, and blood cholesterol were lower in the birds on supplemented diets, compared to those on diet 1 (*P*<0.05). The effects of JLP on anti-oxidant and oxidative deoxyribonucleic acid damage biomarkers of broiler chickens are shown in table 4. Lower (*P*<0.05) HSP 70 and 8-OHdG concentrations were recorded in the experimental birds on supplemented diets (diets 2, 3, and 4), compared to diet 1. The LAB count was significantly higher in the birds on diets 2, 3, and 4 than those on diet 1 (*P*<0.05) (Table 5). On the other hand, the *Coliform* bacteria count was reduced in the birds fed on diets 2, 3, and 4, compared to those on diet 1 (*P*<0.05). The aerobic bacteria counts were similar (*P*>0.05) across the dietary treatments.

Table 2. The effects of *Justicia carnea* leaf powder on the performance characteristics of broiler chickens

Characteristics	Diet 1	Diet 2	Diet 3	Diet 4	SEM	*P* value
IBW (g/bird)	40.50	41.23	40.90	40.32	0.20	0.43
BWG (g/bird)	2286.06^{b}	2602.49^{a}	2427.57^{ab}	2658.33^{a}	53.72	0.02
FI (g/bird)	3841.46	3729.63	3926.70	3952.00	65.02	0.68
FCR	1.68	1.43	1.62	1.49	0.04	0.16

Means in a row without a common superscript letter differ (*P*<0.05); JLP: *Justicia carnea* leaf powder; IBW: Initial body weight; BWG: Body weight gain; FI: Feed intake; FCR: Feed conversion ratio; Diet 1: Control, Diet 2: 200mg/kg vitamin C; Diet 3: 2,500mg/kg JLP; Diet 4: 5,000mg/kg JLP; SEM Standard error of the mean

Table 3. The effects of *Justicia carnea* leaf powder on erythrogram and serum biochemical indices of broiler chickens

	Diet 1	Diet 2	Diet 3	Diet 4	SEM	*P* value
Erythrogram						
Packed cell volume (%)	20.50^{b}	28.00^{a}	27.50^{a}	28.50^{a}	1.21	0.03
Red blood cell count (x10 ^{6}/L)	1.65^{c}	3.90^{a}	2.40^{b}	2.45^{b}	0.26	0.01
Haemoglobin concentration (g/dl)	6.83^{b}	9.33^{a}	9.16^{a}	9.50^{a}	0.41	0.03
Serum biochemical indices						
Total protein (g/l)	59.82	60.01	59.95	61.38	0.89	0.93
Albumin (g/l)	30.15	29.97	29.84	29.75	0.55	0.99
Globulin (g/l)	29.67	30.03	30.11	31.63	0.90	0.90
Glucose (mmol/l)	7.98	8.26	7.76	7.85	0.39	0.98
Aspartate aminotransferase (IU/L)	111.06^{a}	95.23^{b}	92.62^{b}	91.94^{b}	2.87	0.03
Alanine aminotransferase (IU/L)	27.32^{a}	20.79^{b}	21.12^{b}	20.19^{b}	1.03	0.01
Creatinine (mmol/l)	54.28	53.48	53.40	51.74	4.71	0.99
Cholesterol (μmol/l)	3.36^{a}	2.43^{b}	1.74^{bc}	1.35^{c}	0.26	0.01

Means in a row without a common superscript letter differ (*P*<0.05); JLP: *Justicia carnea* leaf powder; Diet 1: Control, Diet 2: 200mg/kg vitamin C; Diet 3: 2,500mg/kg JLP; Diet 4: 5,000mg/kg JLP; SEM Standard error of the mean

Table 4. The effects of *Justicia carnea* leaf powder on anti-oxidant and oxidative deoxyribonucleic acid damage biomarkers of broiler chickens

	Diet 1	Diet 2	Diet 3	Diet 4	SEM	*P* value
Heat shock protein 70 (ng/ml)	0.67^{a}	0.50^{b}	0.37^{c}	0.49^{b}	0.03	0.01
8-hydroxy-2' -deoxyguanosine (ng/ml)	66.69^{a}	116.74^{b}	101.42^{b}	102.85^{b}	5.95	0.01

Means in a row without a common superscript letter differ (*P*<0.05); JLP: *Justicia carnea* leaf powder; Diet 1: Control, Diet 2: 200mg/kg vitamin C; Diet 3: 2,500mg/kg JLP; Diet 4: 5,000mg/kg JLP; SEM Standard error of the mean

Table 5. The effects of *Justicia carnea* leaf powder on gut microbes (log 10CFU/g) of broiler chickens

	Diet 1	Diet 2	Diet 3	Diet 4	SEM	*P* value
Lactic acid-producing bacteria	8.07[d]	9.66[c]	10.78[b]	11.96[a]	0.45	0.01
Aerobic bacteria	10.87	10.83	11.14	11.17	0.15	0.84
Coliform bacteria	9.32[a]	6.95[b]	7.26[b]	7.16[b]	0.34	0.01

Means in a row without a common superscript letter differ (P<0.05); JLP: *Justicia carnea* leaf powder; Diet 1: Control, Diet 2: 200mg/kg vitamin C; Diet 3: 2,500mg/kg JLP; Diet 4: 5,000mg/kg JLP; SEM Standard error of the mean

4. Discussion

In warm environments, broiler chickens with low mitochondrial respiratory chain activity produce excessive reactive oxygen species, suffer from oxidative stress and damage, and perform poorly (3, 16). The antioxidant qualities of the supplements utilized in this study, including vitamin C and JLP, may have contributed to the increased body weight shown in the birds on diets 2 and 4, compared to those on the control diet. Vitamin C affects health and growth due to its biological functions as a water-soluble antioxidant and a co-factor for several enzymes, most notably hydroxylases, which are involved in collagen production (17). On the other hand, JLC has recently been reported to have antioxidant qualities (10).

Oxidative stress causes anemia by encouraging lipid peroxidation and DNA damage caused by reactive oxygen species, such as hydrogen peroxide, superoxide, and hydroxyl radicals during aerobic metabolism (18). The observed decreased PCV, RBC count, and HBc in the birds fed on the control diet may be explained accordingly. However, the increased erythrograms value in the birds on supplemented diets in this study, compared to the control birds, further demonstrates the benefits of the antioxidant activities of the supplements in reducing the drawbacks of oxidative stress by reversing the negative effects of reactive oxygen species that disrupt the process of normal blood formation (11). It has been noted that *Justicia carnea* has antioxidant and anti-anemic properties (10, 11).

The similar total protein, albumin, globulin, glucose, and creatinine across all the dietary treatments in this study suggests that the dietary supplements and the levels at which they were included were nutritionally safe (11, 19). This is supported by the fact that the birds fed on supplemented feeds in this study had lower serum levels of AST and ALT, which suggests that the supplements have nutraceutical qualities that safeguard the heart, liver, kidney, and skeletal muscles. This could be a result of the supplements' nutraceutical qualities manifested due to the activities of bioactive chemicals found in phytogenic supplements (8, 10, 11). Another health benefit of the supplements used in this study is the lower serum cholesterol concentration in the broiler chickens fed on diets 2, 3, and 4 than those on diet 1, as an abnormal rise in blood cholesterol is associated with arteriosclerosis and consequently, a sudden death syndrome in poultry (9). The lowered blood cholesterol may also be caused by saponin activities, such as intra-luminal physicochemical interactions, which would prevent the gut from absorbing cholesterol (9).

The HSP is a protein produced in reaction to stress in various ways, such as heat exposure and physical, chemical, or biological pressures (20). Lower serum HSP 70 concentrations in birds fed on supplemented diets suggests that JLP has anti-inflammatory and antioxidant benefits in birds grown in a normal tropical environment outside their thermoneutral zone, which may have been related to the down-regulation of HSP 70 (10, 21). Antioxidant food supplementation resulted in decreased HSP 70 mRNA expression in birds in hot climates (22). Since 8-OHdG is a helpful indicator of DNA damage brought on by oxidative stress, the reduced concentration of 8-OHdG in the serum of birds fed on supplemented diets in this study further demonstrates another significant nutraceutical benefit of JLP, particularly in suppressing the production of ROR that may bring about oxidative damage (10, 21, 22).

Due to the significant roles that LAB, such as *Lactobacillus* species, play in the fermentation of carbohydrates or starch in the gut, particularly in the caecum, the observed progressive increase in the LAB count with the increased JLP supplementation from 2,500 to 5,000 mg/kg in the broiler chickens is of nutritional and health value (9). In addition, the aerobic bacteria counts being stable across the dietary treatments depicts another health benefit of the JLP dietary supplementation. There have been reports of the involvement of phytogenic feed supplements in regulating the gut microbial population or inhibiting the growth of harmful microorganisms (15). This explains to an extent the reason for the *Coliform* bacteria counts that were generally lower in birds fed on diets 2, 3, and 4, compared to those on diet 1. The experimental birds' gut health and general performance may have been enhanced by the bioactive ingredients in the dietary supplement, which may have selectively suppressed the population expansion of some gut microorganisms in favor of others (15, 23).

In summary, the dietary supplementation of JLP at doses of 2,500 mg/kg and 5,000 mg/kg boosted BWG, improved erythrogram parameters, and decreased blood AST, ALT, cholesterol, HSP70, 8-OHdG, and caeca *Coliform* population but raised caeca LAB population.

Authors' Contribution

Study concept and design: O. D. O. and A. S. O.
Acquisition of data: O. D. O., A. S. O., J. A. O., O. D. A., and O. C. O.
Laboratory analysis: O. D. A., O. D. O., and J. A. O.
Analysis and interpretation of data: O. D. O., A. S. O., J. A. O., O. D. A., and O. C. O.
Drafting of the manuscript: O. D. O., A. S. O., J. A. O., O. D. A., and O. C. O.
Critical revision of the manuscript for important intellectual content: O. D. O. and J. A. O.

Ethics

The experiment's requirements and criteria for animal and animal protocol were accepted by the Research and Ethics Committee of the Agricultural Technology Department of the Federal Polytechnic, Ado Ekiti, Nigeria.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Kpomasse CC, Oke OE, Houndonougbo FM, Tona K. Broiler production challenges in the tropics: A review. Vet Med Sci. 2021;7(3):831-42.
2. Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria J Med. 2018;54(4):287-93.
3. Mishra B, Jha R. Oxidative Stress in the Poultry Gut: Potential Challenges and Interventions. Front Vet Sci. 2019;6:60.
4. Weaver WD. Poultry Housing. In: Bell DD, Weaver WD, editors. Commercial Chicken Meat and Egg Production. Boston, MA: Springer US; 2002. p. 101-11.
5. Lee MT, Lin WC, Yu B, Lee TT. Antioxidant capacity of phytochemicals and their potential effects on oxidative status in animals - A review. Asian-Australas J Anim Sci. 2017;30(3):299-308.
6. Cardozo LF, Pedruzzi LM, Stenvinkel P, Stockler-Pinto MB, Daleprane JB, Leite M, Jr., et al. Nutritional strategies to modulate inflammation and oxidative stress pathways via activation of the master antioxidant switch Nrf2. Biochimie. 2013;95(8):1525-33.
7. Adeyeye S, Oloruntola OD, Ayodele s, Falowo A, Agbede J. Wild sunflower and goat weed LMC in broiler chickens. Acta Fytotech Zootech. 2020;23:205-12.
8. Olugbenga David O, Simeon Olugbenga A, Idowu Samuel O, Samuel Adebowale A, Moyosore Joseph A. The effect of dietary supplementation of mucuna leaf meal on the growth performance, blood parameters, and carcass quality of broiler. Acta Sci Anim Sci. 2022;44(1).
9. Oloruntola OD, Ayodele s, Olowu O, Falowo A, Adeyeye S, Omoniyi I, et al. The Proximate Analysis, Phytochemical Screening, Antioxidant Activity and

Mineral Composition of Momordica charantia and Ocimum gratissimum Leaf Powder. Asian J Res Biochem. 2021:30-9.

10. Oloruntola OD, Ayodele S, Adeyeye S, Oluwagbemiga Samuel F, Osowe C, Ganİyu T. Proximate Composition, Phytochemical Profile, Antioxidant, Antidiabetic and Anti-inflammatory Properties of Justicia carnea Leaf Powder. BSJ Agri. 2022;5.
11. Onyeabo C, Achi NK, Ekeleme-Egedigwe CA, Ebere CU, Okoro CK. Haematological and biochemical studies on Justicia carnea leaves extract in phenylhydrazine induced-anemia in albino rats. Acta Sci Pol Technol Aliment. 2017;16(2):217-30.
12. Chebotareva NV, Neprintseva NV, Bobkova IN, Kozlovskaia LV. [Investigation of 70-kDa heat shock protein in the serum and urine of patients with chronic glomerulonephritis]. Ter Arkhiv. 2014;86(6):18-23.
13. Zhang C, Nestorova G, Rissman RA, Feng J. Detection and quantification of 8-hydroxy-2'-deoxyguanosine in Alzheimer's transgenic mouse urine using capillary electrophoresis. Electrophoresis. 2013;34(15):2268-74.
14. Cheesbrough M. District Laboratory Practice in Tropical Countries. 1st ed. UK: Cambridge University Press; 2000.
15. Seidavi A, Simões J. Evaluation of dietary fish oil plus green tea supplementation on the gizzard, ileum and cecum microflora in broiler chickens. Arch Zootec. 2015;64:397-402.
16. Tan GY, Yang L, Fu YQ, Feng JH, Zhang MH. Effects of different acute high ambient temperatures on function of hepatic mitochondrial respiration, antioxidative enzymes, and oxidative injury in broiler chickens. Poult Sci. 2010;89(1):115-22.
17. Traber MG, Stevens JF. Vitamins C and E: beneficial effects from a mechanistic perspective. Free Radic Biol Med. 2011;51(5):1000-13.
18. Madhikarmi NL, Murthy KR. Antioxidant enzymes and oxidative stress in the erythrocytes of iron deficiency anemic patients supplemented with vitamins. Iran Biomed J. 2014;18(2):82-7.
19. F.B A, Adu O, Chineke C, Oloruntola OD, Omoleye S, Adeyeye S, et al. The Performance, Antioxidant Status, Blood Chemistry Analysis and Tissue Histology of Broiler Birds Fed a Diet Containing Chromium Picolinate and Vitamin C. 2022:129-42.
20. Gu XH, Hao Y, Wang XL. Overexpression of heat shock protein 70 and its relationship to intestine under acute heat stress in broilers: 2. Intestinal oxidative stress. Poult Sci. 2012;91(4):790-9.
21. Anthonia OC, Ikechukwu UR, Uzoma NO, Sunday ELU. Nutritive properties of aqueous extract Justicia carnea leaves and its effects on haematological and some biochemical indices of anaemia induced male wistar albino rats. Biomed Res. 2019.
22. Jang IS, Ko YH, Moon YS, Sohn SH. Effects of Vitamin C or E on the Pro-inflammatory Cytokines, Heat Shock Protein 70 and Antioxidant Status in Broiler Chicks under Summer Conditions. Asian-Australas J Anim Sci. 2014;27(5):749-56.
23. Lillehoj H, Liu Y, Calsamiglia S, Fernandez-Miyakawa ME, Chi F, Cravens RL, et al. Phytochemicals as antibiotic alternatives to promote growth and enhance host health. Vet Res. 2018;49(1):76.