



Research Article

Combined Effects of *Dichrostachys glomerata* (phytobiotic) with Graded Levels of Probiotics on the Production Performance of Japanese Quails

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ABSTRACT

Introduction: The ban on the use of antibiotics as feed additives in livestock farming has led to the development of alternative products. This study aimed to evaluate the effects of combining phytobiotic (*Dichrostachys glomerata*) with a probiotic (Thepax®) on the growth performance of Japanese quails.

Materials and methods: A total of 160 quails, 2 weeks old with an average weight of 55g, were raised in cages in a completely randomized design with five treatments, including two controls. Each treatment was replicated four times, with each replicate consisting of eight quails (four males and four females). The control treatments included a negative control (T0) without any additive and a positive control (T0+) supplemented with the antibiotic Doxycycline® at a dosage of 1g/kg feed. The experimental groups were supplemented with 0.4 ml, 0.5 ml, and 0.6 ml of probiotic per liter of drinking water and 4 g of *Dichrostachys glomerata* /kg feed. The study lasted 35 days and focused on growth performances, feed component digestibility, hematological and biochemical parameters, and gut microbial load of quails.

Results: The findings indicated that quails fed on 0.6 ml probiotic / L of drinking water had the highest weight gain and the lowest feed conversion ratio. Combining phytobiotic with probiotics at 0.4 ml and 0.5 ml improved the apparent digestibility of crude protein and organic matter. Although the treatment did not significantly affect the carcass yields, the relative weight of the liver and pancreas were lowest with 0.4 ml probiotic. Haemato-biochemical parameters increased with higher levels of probiotics combined with 4 g of phytobiotics in the feed, leading to an increase in serum content in ALT and creatinine. Combining 4 g of the phytobiotic with 0.6 ml of probiotic resulted in a decrease in pathogens (*Salmonella* and *E. coli*) count in the gastrointestinal tract of quails.

Conclusion: The combination of *Dichrostachys glomerata* (4g /kg feed) to a probiotic (0.6ml /L drinking water) improved feed conversion ratio and live weight gain while reducing the pathogenic microorganisms in the gastrointestinal tract of Japanese quails.

1. Introduction

A number of feed additives, including probiotics, symbiotics, enzymes, and phytobiotics, have been used in animal nutrition to replace antibiotics that induced

resistance in microorganisms present in animal products. Some feed additives improve feed digestibility, modulate the immune system, and regulate the intestinal flora^{1,2}. The

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fruit of mimosa small bell (*Dichrostachys glomerata*) is known for its antimicrobial, anti-inflammatory, and antioxidant properties, as well as its regulatory effect on the intestinal flora due to the presence of chemical compounds among which are flavonoids, phenols, and alkaloids². According to Kana et al.³, supplementing broilers with *Dichrostachys glomerata* improves growth performance, compared to the control diet. In the same line, Ebile et al.⁴ reported that the incorporation of this phytobiotic at a rate of 4 g/kg feed could improve weight gain and carcass yield in Japanese quails.

Hill et al.⁵ defined probiotics as live microorganisms that confer a health benefit on their host when administered in adequate amounts. They have positive effects on the gastrointestinal tract and in immunomodulation of the poultry immune system hence improving production performance^{6,7,8}. Probiotics provide the body with microorganisms that change the pH of the gut contents, stabilize its mucosa, and assist digestion. They eliminate entero-pathogens through their anti-microbial activity and contribute to maintaining intestinal health^{9,10}. Bai et al.¹¹ found that supplementing broiler rations with probiotics (*Lactobacillus fermentum* and *Saccharomyces cerevisiae*) improved growth performance.

Based on the different beneficial effects of the different additives, their combination may affect the production performance of animals. The main objective of this study was to contribute to the search for growth-promoting antibiotic substitutes in livestock farming. Specifically, it aimed to assess the combined effects of a phytobiotic (*Dichrostachys glomerata*) and a probiotic (Thepax®) on growth performance, feed components digestibility, carcass characteristics, haemato-biochemical markers, and gut microbial load of Japanese quails.

2. Materials and Methods

2.1. Ethical approval

Quails raised during this study were handled according to the main recommendations mentioned in the guidelines of the ethical standards from the 1964 Helsinki Declaration with its latterly amendments.

2.2. Study area

This study was designed at the Teaching and Research Farm of the University of Dschang, Cameroon, located at latitude 5°26' North, longitude 10°26' East at an average altitude of 1420m. Dschang is in the Western Highlands Agro-ecological zone of Cameroon, with an equatorial climate at high altitude.

2.3. Feed additives

The additives used were antibiotic (1g per kg of feed, Doxycycline® ws, Holland), the commercial probiotic

(0.5l/1000l drinking water, Thepax®, Tunisia) containing *Saccharomyces cerevisiae*, and the *Dichrostachys glomerata* dried fruits were milled to obtain a fine powder (phytobiotic). They were all bought from the local market.

2.4. Experimental birds

A total of 160 two-week-old Japanese quails (*Coturnix japonica*) with an average weight of 55 g were purchased from a local farmer. The birds were allocated to cages (45 cm x 39 cm x 40 cm) using a completely randomized design. Each treatment consisted of 32 quails, divided into four replicates, with each replicate containing four males and four females.

2.5. Experimental diets

The different feed additives were used to formulate five rations. The controls were T0 (basal diet without additive) and T0+ (T0 + 1g Doxy /kg feed) corresponding to negative and positive controls, respectively. The other diets were T1, T2, and T3, consisting of basal diet + 4g *Dichrostachys glomerata*/kg feed and supplemented with 0.4 ml, 0.5 ml, and 0.6 ml probiotic/L drinking water, respectively. The composition of the basal diet is shown in Table 1.

2.6. Data collection growth performances

At the beginning of the trial and every 7 days thereafter, the birds were weighed to assess changes in live weight.

Table 1. Composition of the basal diet in quails

Ingredients	Quantity (kg)
Maize	60
Wheat bran	4
Soybean meal	22
Groundnut meal	4
Fish meal	4
Bone meal	0.5
Oyster shell	0.5
*Premix 5%	5
Total	100
Analysed chemical composition	
Dry Matter (%)	92.54
Crude protein (%DM)	19.78
Ash (%DM)	8.28
Crude cellulose (%DM)	7.41
Fat (%DM)	2.06
Calculated chemical composition	
Metabolizable energy (kcal/kg DM)	3117.16
Lysine (%)	1.33
Méthionine (%)	0.46
Calcium (%)	1.04
Available phosphorus (%)	0.54
Sodium (%)	0.03

* Vitamin premix provided per kilogram of diet: vitamin A: 3000000 IU; vitamin D3: 600000 IU; vitamin E: 4000 mg; vitamin K: 500 mg; vitamin B1: 200 mg; vitamin B2, 1000 mg; vitamin B6: 400 mg; vitamin B12: 4 mg; Mn: 80 mg; Fe: 8000 mg; Zn: 10000 mg; Cu: 2000 mg; Methionine: 200000 mg; Lysine: 78000 mg; Se: 20 mg. DM: Dry Matter

The difference between two consecutive weekly live weights was used to calculate weight gain. The quantity of feed served and the leftovers at the end of the week were weighed, and the difference was used to determine feed consumption. Water consumption was measured using a graduated cylinder. The feed conversion ratio was calculated as the ratio of feed intake to weight gain. At the end of the 35-day experiment, 12 quails per treatment (6 males and 6 females) were randomly selected, fasted for 12 hours, then weighed, bled, plucked, and eviscerated to assess carcass yields and relative organ weights (head, legs, heart, liver, pancreas, and abdominal fat).

2.6.1. Feed component digestibility

At the end of the experiment, 24 quails of comparable average weight per treatment were selected to assess the digestibility of feed components. Digestion sheets were therefore placed under their cages to collect the feces over 3 days.

A defined quantity of feed was served; the leftovers were weighed to determine ingested feed. The feces were collected and weighed, then analyzed according to the A.O.A.C.¹² procedure for dry matter, organic matter, ash, crude protein and crude cellulose contents to calculate the apparent digestive coefficients (aDC) of feed concerning the treatment received, using the following formula¹².

$$\text{Feed component aDC} = \frac{\text{Ingested feed component (g)} - \text{Excreted feed component (g)}}{\text{Ingested feed component (g)}} \times 100$$

2.6.2. Haematological and Biochemical analysis

From quails sacrificed at 35 days to assess carcass characteristics, blood was collected in test tubes containing anticoagulant and without anticoagulant in order to assess haematological parameters (white blood cell, red blood cell, haemoglobin, hematocrit and platelets) using an automatic blood cell haematometer (Model PCE- 210N Hong Kong, China) and biochemical parameters (aspartat aminotransferase [AST], alanin aminotransferase [ALT], Creatinine, Urea, Total Protein,

Albumin, Globulin, Total Cholestérol, Triglycérides, HDL-cholesterol and LDL-cholesterol) using commercial kits (Chronolab®, Spain).

2.6.3. Analysis of microbiological parameters

After 35 days, feces samples (5 gr) were collected aseptically from the cloaca using cloacal swabs for the identification and quantification of lactic acid bacteria, *Escherichia coli*, staphylococci, and salmonella in specific culture media (MRS AGAR, Mac Conkey AGAR, MST Agar and SS AGAR, India) respectively⁵.

2.7. Data analysis

The software SPSS (version 20.0) was used to carry out the one-way analysis of variance (ANOVA) of the data collected. Where there was a significant difference between treatments, the means were separated by Duncan's test at the 5% threshold.

3. Results

3.1. Growth performances

Table 2 summarises the effects of combining *Dichrostachys glomerata* with different levels of probiotics on the growth performance of Japanese quails. The treatments had no significant effect on water intake and feed conversion ratio of Japanese quails, while the highest weight gain was recorded with the combination of 4g *Dichrostachys glomerata*/kg feed + 0.6 ml probiotic/L drinking water, compared to all the other treatments ($p < 0.05$).

3.2. Feed component digestibility

Table 3 summarizes the combining effects of *Dichrostachys glomerata* with graded levels of probiotics on the apparent digestive coefficient (aDC) of feed components. The digestibility of feed components tends to decrease with the increasing level of probiotics in the ration. However, the aDCs of crude protein and organic matter increased significantly in quails fed on 0.4 and 0.5 ml/L, compared to all other rations ($p < 0.05$).

Table 2. Effects of Combining *Dichrostachys glomerata* with graded levels of probiotics on growth performance of Japanese quails during 35 days of the experiment

Parameters	Treatments					p-value
	T0	T0+	T1	T2	T3	
Feed intake (g)	717.76±56.77 ^b	853.17±47.97 ^a	792.98±50.91 ^{ab}	743.49±39.05 ^b	792.31±39.63 ^{ab}	0.014
Water intake (ml)	1175.82±47.88	1195.61±33.63	1168.69±88.91	1198.33±88.21	1327.91±79.81	0.106
Weight gain (g)	115.23±9.34 ^{bc}	123.95±6.73 ^{ab}	113.97±5.05 ^{bc}	109.26±7.51 ^c	131.31±4.59 ^a	0.004
Feed conversion ratio	6.25±0.51	6.92±0.76	6.95±0.38	6.84±0.75	6.03±0.37	0.121

^{a, b, c}: Means with the same letter on the same line are not significantly different ($p > 0.05$). T0: Basal diet (negative control), T0+: T0 + 1g Doxycycline®/kg feed (positive control), T1: T0 + combination of 4g *Dichrostachys glomerata* /kg feed + 0.4ml probiotic / L drinking water, T2: T0 + combination of 4 g *Dichrostachys glomerata* /kg feed + 0.5ml probiotic / L drinking water, T3: T0 + combination of 4 g *Dichrostachys glomerata* /kg feed + 0.6ml probiotic / L drinking water

Table 3. Combining effects of *Dichrostachys glomerata* with graded levels of probiotics on apparent digestibility of feed components in quails during 35 days of the experiment

Parameters	Treatments					p-value
	T0	T0+	T1	T2	T3	
DM aDC	68.00±3.91 ^b	65.51±3.74 ^b	78.88±1.83 ^a	77.95±1.85 ^a	66.22±1.90 ^b	0.000
CC aDC	62.43±0.62 ^a	51.75±2.95 ^b	63.67±5.31 ^a	59.76±5.67 ^{ab}	38.36±1.69 ^c	0.000
CP aDC	73.51±3.03 ^b	71.62±5.65 ^b	80.97±3.22 ^a	81.14±1.81 ^a	73.81±1.55 ^b	0.025
OM aDC	74.80±3.60 ^b	72.78±2.51 ^b	83.26±3.00 ^a	82.25±2.95 ^a	72.55±1.81 ^b	0.001

a, b, c: Means with the same letter on the same line are not significantly different ($p > 0.05$). T0: Basal diet (negative control), T0+: T0 + 1g Doxycycline® /kg feed (positive control), T1: T0 + combination of 4g *Dichrostachys glomerata* /kg feed + 0.4ml probiotic / L drinking water, T2: T0 + combination of 4 g *Dichrostachys glomerata* /kg feed + 0.5ml probiotic / L drinking water, T3: T0 + combination of 4 g *Dichrostachys glomerata* /kg feed + 0.6ml probiotic / L drinking water, aDC: Apparent digestibility coefficient, DM: Dry matter, CC: Crude cellulose, CP: Crude protein, OM: Organic matter

3.3. Carcass characteristics

The combining effects of *Dichrostachys glomerata* with the graded levels of probiotics on carcass characteristics of Japanese quails are presented in Table 4. With the exception of carcass yields and the relative weights of the legs and heart, all other parameters were significantly influenced by the different treatments ($p < 0.05$). There was a significant increase in abdominal fat and a decrease in the relative weight of the pancreas with increasing levels of probiotics combined with phytobiotic ($p < 0.05$). Treatment with antibiotic (T0+) and 0.4 ml/L of probiotic induced a significant decrease ($p < 0.05$) in the relative weights of the head and liver compared to the other treatments.

3.3. Digestive organs characteristics

The different treatments used as presented in Table 5

Table 4. Effects of combining *Dichrostachys glomerata* with graded levels of probiotics on the carcass characteristics of Japanese quail during 35 days of experiment

Parameters (%BW)	Treatments					p-value
	T0	T0+	T1	T2	T3	
Carcass yield	66.10±4.03	65.54±5.53	66.85±2.03	67.63±4.78	67.49±3.76	0.720
Head	5.15±0.65 ^a	4.33±0.53 ^b	5.05±0.67 ^a	4.94±0.73 ^a	5.08±0.56 ^a	0.015
Legs	2.17±0.17	1.96±0.21	2.05±0.19	2.06±0.33	2.07±0.4	0.427
Heart	0.79±0.09	0.82±0.11	0.80±0.11	0.80±0.13	0.86±0.10	0.548
Liver	1.93±0.17 ^{ab}	1.71±0.20 ^c	1.98±0.28 ^a	1.47±0.20 ^d	1.81±0.17 ^{bc}	0.001
Pancreas	0.26±0.09 ^{ab}	0.22±0.05 ^b	0.30±0.08 ^a	0.22±0.07 ^b	0.21±0.07 ^b	0.034
Abdominal fat	0.46±0.13 ^c	1.21±0.32 ^a	0.61±0.10 ^{bc}	0.89±0.24 ^{ab}	1.10±0.34 ^a	0.001

a, b, c: Means with the same letter on the same line are not significantly different ($p > 0.05$). T0: Basal diet (negative control), T0+: T0 + 1g Doxycycline® /kg feed (positive control), T1: T0 + combination of 4g *Dichrostachys glomerata* /kg feed + 0.4ml probiotic / L drinking water, T2: T0 + combination of 4 g *Dichrostachys glomerata* /kg feed + 0.5ml probiotic / L drinking water, T3: T0 + combination of 4 g *Dichrostachys glomerata* /kg feed + 0.6ml probiotic / L drinking water

Table 5. Effects of combining *Dichrostachys glomerata* with graded levels of probiotics on the digestive organs of Japanese quails during 35 days of experiment

Parameters	Treatments					p-value
	T0	T0+	T1	T2	T3	
Gizzard (% BW)	1.86±0.21 ^a	1.52±0.15 ^b	1.91±0.27 ^a	1.77±0.21 ^a	1.88±0.21 ^a	0.000
Intestinal weight (g)	4.56±1.50	4.44±1.12	4.22±1.03	4.51±1.02	4.05±0.99	0.798
Intestinal length (cm)	63.42±7.47	63.08±9.22	62.75±8.61	61.30±4.52	64.42±6.26	0.910
Intestinal density	0.07±0.02	0.07±0.02	0.07±0.02	0.07±0.02	0.06±0.01	0.619

a, b: Means with the same letter on the same line are not significantly different ($p > 0.05$). T0: Basal diet (negative control), T0+: T0 + 1g Doxycycline® /kg feed (positive control), T1: T0 + combination of 4g *Dichrostachys glomerata* /kg feed + 0.4ml probiotic / L drinking water, T2: T0 + combination of 4 g *Dichrostachys glomerata* /kg feed + 0.5ml probiotic / L drinking water, T3: T0 + combination of 4 g *Dichrostachys glomerata* /kg feed + 0.6ml probiotic / L drinking water, BW: Body weight

had no significant effect on the digestive organs with the exception of the relative weight of the gizzard which decreased in quails supplemented with the antibiotic with respect to all the other treatments ($p > 0.05$).

3.4. Hematological parameters

There was a significant increase ($p < 0.05$) in red blood cells and platelets, as well as a significant decrease ($p < 0.05$) in mean corpuscular hemoglobin (MCH), regardless of the level of phytobiotic-probiotic combination used, compared to the negative control treatment (Table 6). Similarly, quails fed an antibiotic-supplemented diet recorded a significantly lower mean corpuscular volume (MCV), compared to all the other treatments. Additionally, the combination of phytobiotic and probiotics in the diet induced a significant decrease in white blood cell (WBC) count, compared to quails supplemented with antibiotics (T0+).

Table 6. Effects of combining *Dichrostachys glomerata* with different levels of probiotic on haematological parameters during 35 days of experiment

Blood Parameters	Treatments					p-value
	T0	T0+	T1	T2	T3	
WBC (10 ³ /µl)	208.60±5.28 ^{bc}	219.77±6.29 ^a	200.60±8.03 ^c	207.08±8.85 ^{bc}	210.77±7.45 ^b	0.003
RBC (10 ⁶ /µl)	2.99±0.18 ^c	3.46±0.06 ^a	2.89±0.14 ^c	3.25±0.16 ^b	3.26±0.15 ^b	0.001
Hb (g/dl)	17.20±0.45 ^b	18.17±0.88 ^a	16.40±0.94 ^b	17.78±0.92 ^a	17.83±0.48 ^a	0.004
HCT (%)	49.96±3.74	55.47±5.82	49.52±4.65	53.17±6.55	55.04±2.98	0.212
MCV (fL)	166.80±1.60 ^a	162.52±1.06 ^c	166.50±1.09 ^a	164.15±1.37 ^b	167.30±1.12 ^a	0.001
MCH (pg)	57.92±0.73 ^a	53.42±0.16 ^c	55.10±0.84 ^b	55.08±0.90 ^b	55.18±0.46 ^b	0.000
MCHC (g/dL)	34.10±0.96 ^{ab}	32.78±0.81 ^c	34.30±0.68 ^a	33.55±0.85 ^{abc}	33.22±0.78 ^{bc}	0.024
PLT (10 ³ /µl)	6.40±1.14 ^d	11.25±0.50 ^a	8.20±0.84 ^c	9.67±0.58 ^b	11.50±0.58 ^a	0.001

a, b, c, d: Means with the same letter on the same line are not significantly different (p > 0.05). T0+: T0 + 1g Doxycycline® /kg feed (positive control), T1: T0 + combination of 4g *Dichrostachys glomerata* /kg feed + 0.4ml probiotic / L drinking water, T2: T0 + combination of 4 g *Dichrostachys glomerata* /kg feed + 0.5ml probiotic / L drinking water, T3: T0 + combination of 4 g *Dichrostachys glomerata* /kg feed + 0.6ml probiotic / L drinking water. WBC: White blood cells, RBC: Red blood cells, Hb: Haemoglobin, HCT: Haematocrit, MCV: Mean corpuscular volume, MCHC: Mean corpuscular haemoglobin concentration, MCH: Mean corpuscular haemoglobin, PLT: Platelets.

3.5. Biochemical parameters

As shown in Table 7, the different treatments significantly affected the various parameters studied, except for urea, total protein, albumin, globulin, and HDL cholesterol levels (p < 0.05). There was a significant increase in total cholesterol and LDL-cholesterol levels with the *Dichrostachys glomerata*-probiotic combination compared to the negative control (p < 0.05). Additionally, there was an increase in serum levels of ALT and creatinine with increasing levels of probiotics compared to the control ration without additives (p < 0.05). Feeding quails with a combination of phyto-biotic and probiotics decreased

triglyceride content regardless of the level of incorporation (p < 0.05).

3.6. Gut microbial load

The combined effects of *Dichrostachys glomerata* with graded levels of probiotics on gut microbiota are summarized in Table 8. Supplementation of quails with 0.5 ml/L of probiotics induced a significant increase in both beneficial bacteria (*Lactobacilli*) and pathogens (*Staphylococcus*, *E. coli*, and *Salmonella*) counts compared to 0.4 and 0.6 ml/L (p < 0.05).

Table 7. Effects of combining *Dichrostachys glomerata* with graded levels of probiotics on biochemical parameters during 35 days of experiment

Biochemical parameters	Treatments					P-value
	T0	T0+	T1	T2	T3	
AST	144.38±7.21 ^{ab}	132.56±17.20 ^{bc}	153.38±13.79 ^a	143.11±7.36 ^{ab}	128.48±15.02 ^c	0.01
ALT	45.35±5.23 ^c	46.92±3.82 ^c	54.50±8.66 ^b	51.43±5.65 ^{bc}	64.38±7.81 ^a	0.01
Creatinine	0.04±0.01 ^c	0.06±0.01 ^a	0.04±0.01 ^b	0.04±0.02 ^b	0.05±0.01 ^{ab}	0.01
Urea	19.35±1.79	18.29±1.66	18.86±2.23	18.92±2.75	18.91±3.52	0.91
Total Protein	4.05±0.20	3.88±0.64	4.13±0.40	3.92±0.26	4.20±0.50	0.45
Albumin	1.79±0.12	1.72±0.16	1.70±0.20	1.71±0.11	1.72±0.21	0.79
Globulin	2.26±0.21	2.16±0.55	2.43±0.25	2.21±0.24	2.48±0.40	0.24
Total Cholesterol	155.43±30.42 ^b	162.55±28.00 ^b	180.40±16.03 ^{ab}	171.74±27.82 ^{ab}	194.16±41.02 ^a	0.04
Triglycerides	128.12±19.69 ^a	109.56±11.89 ^b	118.67±18.13 ^{ab}	105.23±11.19 ^b	107.34±8.05 ^b	0.01
HDL-Cholesterol	92.34±18.82	91.80±16.71	84.90±14.02	89.29±17.61	98.12±19.31	0.6
LDL-Cholesterol	48.68±5.05 ^d	59.09±6.64 ^c	70.24±9.80 ^b	55.33±8.03 ^{cd}	80.74±9.95 ^a	0.01

a, b, c, d: Means with the same letter on the same line are not significantly different (p > 0.05). ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, HDL: High density lipoproteins, LDL: Low density lipoproteins, T0+: T0 + 1g Doxycycline® /kg feed (positive control), T1: T0 + combination of 4g *Dichrostachys glomerata* /kg feed + 0.4ml probiotic / L drinking water, T2: T0 + combination of 4 g *Dichrostachys glomerata* /kg feed + 0.5ml probiotic / L drinking water, T3: T0 + combination of 4 g *Dichrostachys glomerata* /kg feed + 0.6ml probiotic / L drinking water.

Table 8. Variation of gut microbiota loads as affected by the combining effects of *Dichrostachys glomerata* with graded levels of probiotics during 35 days of experiment

Bacteria load (Log ₁₀ cfu)	Treatments					p-value
	T0	T0+	T1	T2	T3	
<i>Lactobacilli</i>	5.06±0.05 ^b	5.11±0.10 ^b	4.94±0.15 ^b	5.50±0.33 ^a	4.46±0.15 ^c	0.001
<i>Staphylococcus</i> spp.	5.02±0.12 ^b	5.62±0.15 ^a	4.94±0.25 ^b	5.25±0.09 ^b	5.06±0.14 ^b	0.003
<i>Salmonella</i>	5.28±0.03 ^a	5.34±0.11 ^a	4.92±0.33 ^b	5.32±0.21 ^a	4.65±0.16 ^b	0.006
<i>E. coli</i>	5.40±0.29 ^b	5.06±0.11 ^{bc}	5.31±0.12 ^b	5.79±0.13 ^a	4.94±0.23 ^c	0.002

a, b, c: Means with the same letter on the same line are not significantly different (p > 0.05). T0+: T0 + 1g Doxycycline® /kg feed (positive control), T1: T0 + combination of 4g *Dichrostachys glomerata* /kg feed + 0.4ml probiotic / L drinking water, T2: T0 + combination of 4 g *Dichrostachys glomerata* /kg feed + 0.5ml probiotic / L drinking water, T3: T0 + combination of 4 g *Dichrostachys glomerata* /kg feed + 0.6ml probiotic / L drinking water.

4. Discussion

Feeding quails with *Dichrostachys glomerata* combined with 0.5 ml probiotic/L in drinking water resulted in a significant decrease in feed intake, compared to the antibiotic-supplemented diet ($p < 0.05$). Weight gain increased significantly with 4 g of *Dichrostachys glomerata* combined with 0.6 ml of probiotic ($p < 0.05$). This growth improvement could be due to the availability of nutrients generated by the digestive enzymatic stimulating properties of the additives. This result is in line with the findings of Abou-Kassem et al.¹³ who recorded a significant increase in live weight with no significant difference in feed intake and feed conversion ratio in quails fed rations enriched with *Bacillus toyonensis* and *Bifidobacterium bifidum* as probiotics.

The present study revealed a significant improvement in dry matter, crude protein, and organic matter digestibility with *Dichrostachys glomerata* combined with 0.6 ml probiotic. This result aligns with the findings of Smolentsev et al.¹⁴ indicating an increase in the digestibility of feed components in quails fed on probiotic (probiolac). The present improvement in feed digestibility could be due to the capacity of *Dichrostachys glomerata* to stimulate the secretion of digestive enzymes, in association with the effect of *Saccharomyces cerevisiae* in digesting the feed, reflected in the drop in feed intake recorded.

Feeding quails with phytobiotics combined with probiotics has no significant effect on carcass yields ($p < 0.05$). The present result is in line with the findings of Qasemi et al.⁶ who recorded no significant effect on carcass yield in broilers fed on probiotic *Lactobacillus sp.* Similarly, Tang et al.¹⁵ found no significant effect in laying hens fed Primalac® as probiotics.

The additives used resulted in a significant decrease in RBC and hemoglobin compared to quails fed on antibiotics. This finding is in agreement with the results of Libanio et al.¹⁶ who reported that the various hematological parameters studied in guinea fowl decreased with stabilized sorghum products enriched with lactobacilli. The combination of phytobiotics with increasing levels of probiotics led to a significant increase in the hematological parameters studied in Japanese quails ($p < 0.05$). This corroborates the work of Hasan et al.¹⁷ who reported a significant increase in WBC in quails-fed parsley seeds supplemented with probiotics. However, the present results are contrary to those of Tang et al.¹⁵ who recorded no significant effect on the same hematological parameters in laying hens fed Primalac® probiotics. The various hematological parameters studied, although significantly affected by the different additives used, remain within the reference values in quails¹⁸.

The association of *Dichrostachys glomerata* with graded levels of probiotics induced a significant increase in ALT, creatinine, total and LDL cholesterol levels, and a decrease in triglyceride content. These results are in line with the findings of Nasr El Deen et al.¹⁹ who recorded a significant

increase in AST and ALT and a significant decrease in triglyceride contents with the probiotic "Gro-2-max" in quails. They are also in accordance with the results of Asmaa et al.²⁰, who reported a significant drop in serum triglyceride levels in quails with the supplementation of their ration with black pepper and turmeric. These results suggested that liver functions were not affected by the additives.

Combining *Dichrostachys glomerata* with 0.4 ml of probiotic induced a significant increase in lactobacilli counts in quails, while 0.4 ml and 0.6 ml probiotics induced a decrease in the pathogen microbial load. These results are in line with those of Khaksar et al.²¹ who recorded an increase in the ileal load of lactobacilli and a decrease in that of *E. coli* in quails fed thyme essential oil. The same results were reported by Mirza et al.²² in quails fed on a diet supplemented with probiotics.

5. Conclusion

After evaluating the effects of combining a phytobiotic (*Dichrostachys glomerata*) and a probiotic (Thepax®) on the growth performance of Japanese quails, it can be advisable to feed them with 4 g *Dichrostachys glomerata* /kg feed combined with 0.6 ml probiotics/L of drinking water as antibiotic growth promoter substitute to modulate gut microbiota, improve feed conversion ratio and live weight gain. However, more studies need to evaluate the combination of probiotics and phytobiotics on other poultry species.

Declarations

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Pimagha Moffo and Kana Jean conceived, designed the experiment, and wrote the manuscript. Dongmo Nguedia conducted data collection and analysis. Chongsi Margaret, Tchouan Deffo, and Edie Nounamo revised the manuscript. Its final version was read and approved by all authors.

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Availability of data and materials

The data from this trial could be available with the agreement of the corresponding author.

Ethical considerations

The authors confirm that all authors have reviewed and submitted the manuscript to this journal for the first time.

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