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Growth Promoting and Antimicrobial Activities of *Justicia Secunda* (Vahl) Leaf Meal Supplemented Diets in an Experimental Broiler Chickens

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Abstract: The ban of antibiotics as growth promoter in animal production in many countries due to their challenges necessitates seeking for alternatives in which medicinal plants were identified. In this study, 96 day-old broilers were experimented for 6 weeks. They were brooded for two weeks before introducing the experimental diets. This entails a random allotment of 8 broilers into four dietary treatments 0, 5, 10 and 15 g/kg (diets 1, 2, 3 and 4 respectively) of air-dried leaf meal of *Justicia secunda* in a completely randomized design (CRD). The broilers were fed with the experimental diets and water (ad libitum) for 28 days after which 3 (three) birds per replicate were sacrificed for performance evaluation. Faecal samples were collected for four weeks from each of the replicates for microbial analysis. After the analysis of the data, the treatments significantly ($p < 0.05$) influenced the broilers' feed intake, weight gain, primal cuts, feed conversion ratio, duodenal and ileal length; with best results obtained from the birds on diet 3. The weight of their selected organs and feathers did not differ significantly ($p > 0.05$). Faecal bacteria load count (cfu/mL) was significantly ($p < 0.05$) influenced by the treatments in the order; diet 1> diet 2> diet 3>diet 4. Evidently, birds on 10 g/kg of *Justicia secunda* leaf meal (JSLM) recorded the highest weight gain, suggesting its growth promoting effect. Overall, it was concluded that JSLM had growth promoting and antibacterial effects with 10 g/kg inclusion level recommended in broiler diets.

Keywords: Medicinal Plants, Antibiotics, Growth, Antibacterial, *Justicia secunda.*

Introduction

Weight gain, feed conversion ratio, and low to zero mortality rates among others are unarguably some of the most important desired production parameters in broiler farming. This is so, because broilers are fast growing specie. Therefore, their uncompromised adequate and standard daily weight gain is highly priced. This underscores the use of synthetic growth promoting factors like hormones and antibiotics for the sustainability of their rapid growth and sound health.

However, uncontrolled administration of antibiotics in poultry has resulted to antimicrobial resistance [1,2] Antibiotic resistance (AR) has been recognized to be one of the threats to the health of the public worldwide [3]. The need for alternatives, especially in the light of banning antibiotics use as growth promoter in animal production has now become very pertinent and imperative [4]. As part of the way out from this pressing challenge, probiotics, synbiotics and diet-acidifiers were recommended as potential alternatives to synthetic growth promoters [5-7]. Furthermore, plants have been identified as good supply of safe drugs with growth promoting activity, increased nutrient bioavailability and without residual effects [8-10]. Medicinal plants like dried sweet potato [11], *Moringa oleifera* [12], *Tithonia diversifolia* [13], Neem [14] etc. have been explored as potential leaf meal in broiler diets with no deleterious effects. They rather improved the physiological, immunological, growth and health status of broilers [15-17].

Justicia secunda M. Vahl (Acanthaceae family) is one of the studied medicinal plants whose extracts and leaf meal have demonstrated growth promoting activity in broilers [18] and weanling pigs [19] respectively. The plant has been characterized to be abundant in different volatile as well as numerous bioactive compounds of high industrial and pharmacological value of which phenols and flavonoids top the list [12,20].

These bioactive compounds might be responsible for its antibacterial efficacy in the ethanol-water extract of the plant against *E. coli*, *K. pneumonia*, *B. subtilis* and *S. aureus* in the study of [21]. This fact underscores the need for sustainable and pragmatic harnessing of the growth and health-promoting properties of *J. secunda* [22] especially in broiler farming. Several reports by researchers on the pharmacological usefulness of *J. secunda* in humans and animals are abundant and accessible in the literature. Contrariwise, none on the nutraceutical evaluation of the 'leaf meal' of this plant as prospective growth promoter and antibacterial in broiler diets was found. The foregoing has engendered a sort of novel research lacuna deserving a prompt attention. Our study thus examines the effects of *J. secunda* 'leaf meal' incorporated into broiler diets on the progressive growth, organs weight, carcass characteristics, feather production, gut morphometric and faecal bacteria load of broiler chickens.

Materials and Methods

Experimental Site

This study was carried out at the Teaching and Research Unit of the Department of Animal Production and Health. The poultry pen was properly cleaned and disinfected prior to the arrival of a day old broiler chicks from a reputable hatchery in Ibadan, Oyo State, Nigeria.

Plant Collection and Preparation of Leaf Meal

J. secunda leaves were collected within Akure metropolis, properly rinsed in distilled water and drained before chopping into pieces to fast track drying. The chopped leaves were arranged in trays and subjected to air drying under shade for 3 weeks. When the level of its crispiness became obvious the sample was pulverized using conventional laboratory hammer mill [23]. The meal thus formed was tagged *Justicia secunda* leaf meal (JSLM). This was stored in an airtight bag at room temperature until needed for the experiment.

Experimental Design and Feeding Trial

In this study, ninety six (96) day-old Arbor Acres broiler chicks were used. The birds were brooded for two weeks and fed commercial broiler starter. After the two weeks, they were weighed and randomly assigned to four dietary treatments in a completely randomized design (CRD) with eight (8) birds per treatment and replicated thrice. At this stage, JSLM was incorporated into commercial broiler finisher at 0 %, 5 %, 10 % and 15 % to form treatments 1, 2, 3 and 4 respectively. These diets and water were fed to the broilers for four weeks *ad libitum*. Necessary vaccinations were appropriately administered alongside with strict bio-security measures. The birds were weighed weekly in order to ascertain their respective weight gain *vis a vis* each of the treatments. Feed given was equally weighed as well as the leftover for the purpose of calculating their feed intake.

Slaughtering, Carcass and Organ Measurement

The birds were fasted overnight on the eve of their 6 weeks old attainment. In each of the replicates, three birds were selected randomly, weighed by using Digital Compact Scale A-123® and thereafter sacrificed by slaughtering for the pre-determined evaluations. They were plucked using hot water at about 75°C. They were eviscerated and cut into parts to obtain the respective weight of the neck, head, chest, right wing, left wing, right thigh, left thigh, right drumstick, left drumstick, right shank, left shank, and the back. The dressing percentage was calculated thereafter. The feathers were kept per replicates, sun-dried and weighed. Selected organs viz; pancreas, proventriculus, gizzard, heart, liver, spleen, kidney, and lung were removed and weighed accordingly with the aid of digital electronic scale. The gut morphometric parameters include the duodenum, jejunum, ileum, caeca and colon. These were identified, removed, stretched out on a clean table and measured using graduated fibre Tape.

Bacterial Load Evaluation

Faecal samples for microbial analysis were picked up from the litters in each of the replicates consecutively for four weeks. In the laboratory, pour plate method was adopted for this purpose. Briefly, 1g each of the faecal samples was weighed into 9 mL sterilized water in bijou bottles which was serial diluted to 10-5.1 mL was drawn using sterile needle and syringe into a petri dish which was later thoroughly mixed with sterilized nutrient agar. This procedure was also repeated using *Salmonella Shigella agar*. The hot sterilized agar in the petri dishes was allowed to cool down and gel before arranging them in the laboratory incubator at 37°C for 24 hours [24]. At the expiration of the incubation period, the plates were brought out from the incubator. The grown colonies on the gel were carefully counted and recorded as the total bacterial load count per plate.

Statistical analysis

All the generated data from this study were collated and analyzed using Statistical Package for the Social Sciences [25] via analysis of variance (ANOVA). Duncan's multiple range test of the same package was used for means separation. The p-value of less than 0.05 was considered statistically significant.

Results and Discussion

The weight of the birds as recorded weekly is as shown in Table 1. There was no observed significant difference in weight at week one, two and four among the treatments. However, at week three and week five, their body weight witnessed notable significant difference ($p < 0.05$). The birds whose diets contained *J. secunda* leaf meal (T2, T3 and T4) had higher weight than the control (T1). At the end of the experiment, those birds on 10 g (T3) of JSLM / kg feed had the highest body weight while the lowest body weight was recorded in the control group.

Table 1: Average weekly weight of broiler chickens fed *J. secunda* supplemented diets.

Treatments	Week 1	Week 2	Week 3	Week 4	Week 5
T1	333.80±3.92	614.48±1.22	882.59±1.34^{b}	1126.62±0.26	1305.87±7.85^{c}
T2	334.87 ±7.43	630.65±8.74	934.46±6.06^{a}	1173.86±23.27	1561.64±47.54^{a}
T3	332.47 ±0.96	626.51±4.00	928.70±13.40^{ab}	1164.01±21.54	1573.87±12.96^{a}
T4	333.44±3.77	588.68±19.83	884.95±48.28^{ab}	1121.44±53.07	1455.64±80.12^{b}

$^{a, ab, b, c}$ Means (± Standard deviation) along the same column with different superscripts are significantly different ($p<0.05$).

Feed intake is very important in broiler production because it reveals the degree of feed palatability which is directly proportional to the rate of consumption. In this experiment, there were significant differences in feed intake (Table 2). Highest feed intake was recorded in treatment three followed by treatment four (though no significant difference between T3 and T4), two and lastly treatment one.

Table 2: Average weekly feed intake of broiler chickens fed *J. secunda* supplemented diets.

Treatments	Week 1	Week 2	Week 3	Week 4
T1	483.33±5.77	645.66±0.61	692.76±3.65	771.22±13.15^{b}
T2	503.81±17.25	706.03±8.64	704.95±23.30	838.38±62.21^{ab}
T3	497.56±2.75	714.18±14.88	676.91±12.06	876.64±12.93^{a}
T4	478.78±20.05	699.29±34.77	699.00±28.910	844.90±29.73^{a}

$^{a, ab, b}$ Means (± Standard deviation) along the same column with different superscripts are significantly different ($p<0.05$).

Table 3 shows the average weekly weight gain by the experimental broilers. Significant differences were observed at week 1, week 2 and week four of the feed trial. This showed that the diets actually influenced the weight of the broilers positively. The birds in treatment three showed the highest weight gain followed by T4, T2 and lastly T1. The tested plant thus demonstrates growth promoting effect on the broilers. According to [26], leaf meals promote broilers growth. Sage powder at 8 and 12 g/kg increased body weight and also improved growth rate as well as feed intake [27].

Table 3: Average weekly weight gain of broiler chickens fed *J. secunda* supplemented diets.

Treatments	Week 1	Week 2	Week 3	Week 4
T1	280.68±2.75^{a}	268.11±2.24^{ab}	244.03±1.57	179.25±7.61^{c}
T2	295.78±3.02^{a}	303.81±3.30^{a}	239.40±28.10	387.78±66.42^{ab}
T3	294.04±2.88^{a}	302.19±9.42^{a}	235.31±30.95	409.86±11.64^{a}
T4	255.24±21.55^{b}	296.27±30.34^{ab}	236.49±5.83	334.20±35.99^{b}

$^{a, ab, b, c}$ Means (± Standard deviation) along the same column with different superscripts are significantly different ($p<0.05$).

Feed conversion ratio (FCR) shows the ability of animal to convert the feed consumed into flesh. Table 4 presents the weekly FCR of the broilers in this study. Significant differences ($p < 0.05$) were observed in the first and last week of the experiment among the treatments. The best FCR was observed in treatment three both at week one as well as at week four indicating that birds on this diet definitely performed better in weight gain than those in the remaining experimental diets. This result agrees with [28,29] who reported improved FCR and performance in broilers fed 0.5%, 1.0% and 1.5% inclusion of basil and sage leaf powder at 8 and 12 g/kg [27] in broilers diets.

Table 4: Weekly feed conversion ratio (FCR) of the experimental birds.

Treatments	Week 1	Week 2	Week 3	Week 4
T1	1.72±0.04^{b}	2.41±0.05	2.84±0.01	4.31±0.13^{a}
T2	1.70 ±0.04^{b}	2.32±0.02	2.97±0.28	2.19±0.25^{c}
T3	1.69 ±0.09^{b}	2.37±0.10	2.91±0.35	2.14±0.03^{c}
T4	1.88±0.09^{a}	2.37±0.21	2.96±0.11	2.54±0.17^{b}

$^{a, b, c}$ Means (± Standard deviation) along the same column with different superscripts are significantly different ($p<0.05$).

As shown in Table 5, the carcass characteristics of the experimental chickens present varying pattern of differentials in weight across the treatments. Significant differences ($p <0.05$) were observed among the treatments in the weight of breast muscle (chest), wings and thighs with the highest values got from birds whose diet was supplemented with 10g/kg of JSLM. The lowest values of these primal cuts were seen in those birds without JSLM in their diet (TI). The back weight of the broiler chickens also followed the same pattern as above, showing that this part was also positively influenced by the addition of JSLM in their diets. Several researchers have opined that inclusion of plants' leaf meal in livestock diets improved performance parameters [30]. The inclusion levels of JSLM (0, 5, 10, and 15 g/kg) in our study did not affect the experimental broilers negatively. Our observation aligns with [31] who reported 0, 2.5, 5, 10 and 20 g/kg of oregano leaf meal in the diets of broiler chickens though with different plants. However, the observation of [32] contradicts the above as the inclusion levels of the leaf meal of *Gliricidia sepium* at 0, 50, 100 and 150 g/kg in broiler diets depressed feed intake, body weight gain and feed conversion ratio. The deleterious effects might be attributable to too high levels of the tested leaf meal. The findings of [33] emphasize the growth promoting effect of dietary leaf meal and also describe the variability in performance subject to the plant species and feed inclusion levels.

Table 5: Carcass characteristics of broiler chickens fed *J. secunda* supplemented diets.

Parameters (g)	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Live weight	1305.37±243.35	1547.42±64.38	1547.93±50.26	1441.94±102.47
Dressed	1270.17±226.58	1483.77±57.27	1490.27±41.82	1389.16±100.42
Plucked	1217.80±221.37	1418.26±50.89	1423.59±46.40	1321.22±86.42
Eviscerated	977.87±162.41	1167.47±56.97	1189.60±38.53	1082.51±95.55
Neck	45.87±13.76	59.17±6.32	63.77±3.55	51.68±5.08
Head	38.47±7.81	42.33±0.93	40.59±2.33	40.93±3.512
Chest	273.77±38.78^{b}	325.33±16.52^{a}	334.17±15.23^{a}	296.67±25.95^{b}
Right wing	50.27±6.52^{b}	59.07±2.74^{ab}	59.70±2.94^{a}	55.59±5.27^{ab}
Left wing	52.83±8.21^{b}	59.56±2.86^{ab}	63.54±2.25^{a}	56.97±4.20^{ab}
Right thigh	59.57±10.25^{b}	80.93±4.92^{a}	84.90±3.69^{a}	73.63±11.55^{ab}
Left thigh	56.33±8.49^{b}	73.80±3.44^{a}	74.82±0.63^{a}	65.76±9.23^{ab}
Right drum	66.30±11.35	79.82±5.89	76.29±4.67	70.33±3.69
Left drum	63.90±13.16	78.36±5.53	76.30±3.39	71.73±4.07
Right shank	30.33±6.27	35.32±2.17	34.47±2.28	32.91±0.81
Left shank	29.50±5.35	34.72±2.12	34.23±33.61	33.61±1.50
Back	155.33±29.48^{b}	206.81±5.61^{a}	211.18±10.79^{a}	188.98±19.06^{ab}
Dressing %	67.59±1.89	68.17±0.56	69.79±0.81	67.55±1.80

$^{a, ab, b}$ Means (± Standard deviation) along the same column with different superscripts are significantly different ($p<0.05$).

The selected and evaluated organs include the pancreas, proventriculus, gizzard, heart, liver, kidney, spleen and lung (Table 6). There was no significant difference ($p > 0.05$) in the weight of these organs across all the treatments. This also corroborates the findings of [34] in the incorporation of basil leaf meal into broilers diets even to the level of 9%. This probably shows the non-toxicity effect of JSLM on these vital organs.

Table 6: The weight of selected organs from the experimental broiler chickens.

Treatment	Pancreas	Proven-triculus	Gizzard	Heart	Liver	Spleen	Kidney	Lung
T1	4.63±1.856	6.53±1.42	56.07±11.94	5.30±1.80	31.67±3.19	1.83±0.65	8.10±1.91	8.87±3.30
T2	5.28±0.26	7.79±0.68	51.09±2.36	6.61±0.78	36.60±3.30	2.34±0.40	8.78±1.08	10.50±0.76
T3	4.98±0.54	7.91±0.62	48.22±4.95	6.86±0.57	36.09±1.29	2.09±0.62	9.82±0.62	10.81±0.24
T4	4.52±0.66	7.54±0.20	43.80±3.55	6.87±1.06	33.84±1.24	1.88±0.29	8.81±1.42	10.63±0.82

The feathers (g) produced by the broiler chickens (Table 7) did not differ significantly ($p > 0.05$) across the treatments. This may suggest that the feed consumed by the broilers were not utilized for the production of more than necessary feathers. Also, it may be presumably asserted that JSLM in broiler diets contributed to flesh building rather than superfluous feather production.

Table 7: Average weight of feathers produced by the experimental broilers.

Treatments	Feathers (g)
T1	84.13 ± 6.55
T2	93.00 ± 13.42
T3	87.80 ± 11.00
T4	90.40 ± 5.36

The role of the gastrointestinal tracts (GIT) in the process of digestion and nutrients assimilation necessitated the measurement of selected parts viz; duodenum, jejunum, ileum, caeca and colon as presented in Table 8. The length of the duodenum and ileum were significantly ($p < 0.05$) influenced by the diets. The development of the gut is very relevant for nutrient absorption and general sound health of broilers [35]. The lowest length of these two essential parts of the gut was recorded among those birds in the control group. This observation concurs with [26] in the addition of 5g/kg of basil leaf supplementation in broilers diets.

Table 8: Morphometrics of selected parts of the gastro-intestinal tract of the experimental broiler chickens.

Treatments	Duodenum	Jejunum	Ileum	Caecum 1	Caecum 2	Colon
T1	$30.28±0.25^{b}$	84.00±2.85	$85.44±1.07^{b}$	20.37±0.75	20.29±1.31	9.11±0.84
T2	$33.11±0.51^{a}$	89.39±7.09	$101.17±10.02^{a}$	21.44±1.71	21.33±0.88	9.39±1.46
T3	$31.67±0.58^{ab}$	93.28±1.83	$95.22±2.69^{ab}$	22.39±1.44	22.11±0.77	10.06±0.82
T4	$32.78±1.71^{a}$	90.61±7.06	$97.39±5.64^{a}$	20.89±0.19	21.11±1.23	10.17±0.73

$^{a, ab, b}$ Means (± Standard deviation) along the same column with different superscripts are significantly different ($p<0.05$).

It is quite obvious from Table 9 and 10 that inclusion of JSLM in the diets of the broilers in this study demonstrated antibacterial activity. The result as shown in Table 9 indicates a significant antibacterial activity of JSLM. Throughout the four week of the feeding trial, the highest number of bacterial colonies (cfu/mL) was observed in the faecal samples of birds that did not consume JSLM. The number of bacterial colonies also found to be reducing *vis a vis* the increase in the level of JSFM in the diets. This pattern of antibacterial efficacy of JSLM was also observed at week three and four of the experiment using *Salmonella-Shigella* agar as presented in Table 10. The present result is at par with bacterial load reducing capability of phytogenics especially *E. coli*, *S. aureus*, *C. perfringens* as reported by a host of researchers [36,37]. This underscores the importance of phytogenics as alternative to antibiotics for growth promotion and general wellness of the livestock [19]. Phytogenics (phytobiotics) were considered essential in poultry production because of their ability to stimulate appetite, enhance the activities of endogenous digestive secretions and also improve immunological their response [38]. Interestingly, the abundant phytogenic compounds in *J. secunda* like saponins, flavan-3-ol, tannins, proanthocyanin, phenols, kaempferol, alkaloids, naringenin, cardiac glycosides, sapogenin etc. have been credited to its strikingly antibacterial potential [9,18,39].

Table 9: Faecal bacteria count from experimental broiler chickens on Nutrient Agar (NA).

Treatments	Weeks			
	1	2	3	4
T1	62.00±0.58^{a}	17.00±0.58^{a}	76.00±0.58^{a}	67.00±0.58^{a}
T2	17.00±0.58^{b}	8.00±0.58^{b}	40.00±0.58^{b}	55.00±0.58^{b}
T3	15.00±0.58^{c}	6.00±0.58^{c}	30.00±0.58^{bc}	30.00±0.58^{c}
T4	12.00±0.58^{d}	4.00±0.58^{d}	25.00±0.58^{bc}	25.00±0.58^{d}

$^{a, b, bc, d}$ Means (± Standard deviation) along the same column with different superscripts are significantly different ($p<0.05$).

Table 10: Faecal bacteria count from broiler chickens on Salmonella-Shigella Agar (SSA).

Treatments	Weeks			
	1	2	3	4
T1	10.00±0.58^{a}	0.00±0.00	14.00±0.58^{a}	25.00±0.58^{a}
T2	0.00±0.00^{b}	0.00±0.00	3.00±0.58^{b}	14.00±0.58^{b}
T3	0.00±0.00^{b}	0.00±0.00	0.83±0.29^{c}	4.00±0.58^{c}
T4	0.00±0.00^{b}	0.00±0.00	0.00±0.00^{d}	0.00±0.00^{d}

$^{a, b, c, d}$ Means (± Standard deviation) along the same column with different superscripts are significantly different ($p<0.05$).

The observed markedly reduction in the faecal bacteria count (Table 10) of Salmonella and *Shigella spp* in this study go along with the findings of [40] who demonstrated the antibacterial potency of *Justicia secunda* leaf extract against some pathogenic Gram positive and Gram negative bacteria including *S. typhimurium*, *E. coli*, *S. dysenteriae*, *E. faecalis*, *P. mirabilis*, *S. aureus*, *P. aeruginosa* and *S. saprophyticus* among others. Following the outcome of this study as well as the reported antibacterial activity of *J. secunda*, it is thus becoming clearer and understandable the reasons behind its regular traditionally use to cure bacterial infection. Medicinal plants have been reported to produce antibacterial effect on many bacteria that were once found to be resistant to synthetic antibiotics [41]. Apart from this fact, the sustainability, safety and accessibility of natural antibacterial agents appear to be more realistic than the synthetic.

Conclusion

This study reported the significant growth promoting and antimicrobial activities of JSLM in broiler chickens compared to the control. The faecal bacteria load count significantly decreases following the corresponding dietary increase in the levels of JSLM. Inclusion rate of JSLM at 10 g/kg consistently outperformed others in terms of the final weight gain and FCR and therefore recommended in broiler production. As far as we are concerned, this is the first report on the use of JSLM in broiler diets and as such more research on this pertinent topic is suggested in order to further validate the purported results from this study.

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