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Negro Pepper (*Xylopia ethiopica*) in Feed is Antibacterial and Can Improve Broiler Chickens Productivity

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Abstract: An experiment was conducted to determine the proximate composition of Negro pepper, its antibacterial effect and its dietary effect on growth performance of broilers. A total of 150 Ross 308 chicks were used. There were five treatments each replicated three times. Each replicate had ten birds. The experiment was arranged in completely randomized design (CRD). Starter and finisher basal (Control-T1) diets without Negro pepper were formulated. T2 was the basal diet plus 2.5g/kg Negro pepper; T3 = 5g/kg; T4 had 7.5g/kg and T5 was 10g/kg. The diets and water were offered to the birds *ad libitum* for 56 days the experiment lasted. Negro pepper reduced bacterial load from 3.6×10^4 - 1.7×10^3 cfu/g. At the starter phase, final live weight and daily gain were improved ($P<0.05$) by 2.5g/kg but negatively affected by 10g/kg. Total feed intake was increased by 7.5 and 10g/kg. Feed: gain ratio was deteriorated by 7.5 and 10g/kg. Addition of 2.5 and 5g/kg at the finisher phase improved ($P<0.05$) live weight and total feed intake. 10g/kg negatively affected final live weight, total feed intake, and feed: gain ratio. Therefore 2.5g/kg Negro pepper could be added to broiler diets for optimum productivity.

Key words: Bacterial Load · Broilers · Growth Performance · Negro Pepper · Proximate Composition

INTRODUCTION

Successful poultry production is dependent on optimal feed intake throughout the growing period. This optimal feed intake is a function of several factors such as quantity, quality of feed, type of feed, physical form of feed and proper management system [1]. According to Oluyemi and Roberts [2] the success of poultry venture depends on the birds having good health, required nutrition and good management. Broilers are good sources of quality protein and there is growing desire to obtain high productivity in broiler production in terms of quality and quantity of meat. This could be achieved by use of specific feed ingredients and additives. In facing the challenge of high cost of feed, broiler producers are making efforts to exploiting alternative feed ingredients which include feed additives.

According to Olomu [3] feed additives are materials which are added to feed with the aim of protecting the animal from diseases, improving growth rate and feed efficiency and increasing overall performance. Feed additives include antibiotics, enzymes, hormones and recently organic acids and spices [4]. According to Suriya *et al*. [5] and Ndelekwute *et al*. [6] massive use of antibiotics for disease prevention and growth promotion in animal nutrition has been implicated in the emergence of microbial drug resistance in animal products which caused public health concerns and hazards.

Owning to several criticisms on health risks of using antibiotics, El-Hussein *et al*. [7] and Dono [8] reported their ban in animal feed by European Union (EU) and United States Department of Agriculture (USDA). The ban has encouraged the use of plant-based compounds called phytogenics or phytobiotics that are incorporated into diets of farm animals to improve their productivity, performance and quality of their products [9]. Therefore, the search for natural feed additives as replacement for growth promoting antibiotics is gaining more research attention [10]. Hence, African Negro pepper (*X. aethiopica*) could be seen to have potentials to be among the league of these feed additives. Negro pepper is a tree which can be 20 meters tall. The seeds and fruits are used as spices. It has pharmaceutical and medicinal properties according to Isikwenu and Udomah [10].

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Because of its phytochemical contents such as essential oils, phenols, *flavonoids* and *carotenoids*, the spice is used in diets for nursing mothers to stabilize the gastrointestinal tract [11]. Recently experience gathered on its use in human nutrition is being applied in monogastric animals due to its anti-oxidant and anti-microbial activities. Its positive effect on human could qualify it as an alternative growth promoter to synthetic antibiotics in broiler diets, owing to the fact that human being and monogastric animals have similar digestive system [11]. It could be used to improve growth performance and reduce bacterial load of feed of broiler chickens [10]. Therefore the objective of the study was to determine the antibacterial effect of Negro pepper on feed and its dietary effect on growth of broiler chickens.

MATERIALS AND METHODS

Experimental Site: The experiment was conducted at the Poultry Unit of the Research and Teaching Farm of The University of Uyo, Uyo, Nigeria. Uyo is located at latitude 5°32°N and longitude 7°54°E with average rainfall of 1500mm.

Processing of Experimental Material: Ripe fruits of Negro pepper were harvested and dried under the sun. Thereafter they were grinded and stored in a plastic container. A portion of it was taken to the laboratory where proximate analysis was conducted according to Association of Official Analytical Chemists [12].

Experimental Design: The experiment was conducted using 150 day old Ross 308 broiler chicks. They were randomly allotted to five treatment (T) groups. Each group had 30 birds replicated three times and each replicate contained 10 birds. The experiment was arranged in completely randomized design (CRD). Starter and finisher basal diets were formulated to which ground Negro pepper was added at different levels respectively. Treatment one (T1) which was the control contained no Negro pepper, T2, T3, T4 and T5 contained 2.5, 5.0, 7.5g and 10.0g/kg respectively for both the starter and finisher diets. Feeding of the experimental diets started from the first week of age. The starter and finisher diets were offered for four weeks each giving a total of 8 weeks (56 days) which the experiment lasted.

Statistical Analysis: To evaluate the effects of dietary treatments on the parameters measured (Final live weight, weight gain, feed: gain ratio etc) data collected were analyzed by one way analysis of variance (ANOVA) using the procedure of SAS (SAS Institute Inc., Cary, NC, USA) at 5% level of significance. Significant means were separated using Duncan New Multiple Range Test (DNMRT) according to Steel and Torrie [13]. The statistical model applied is shown below.

Table 1: Ingredient and nutrient composition of experimental diets.

Ingredients	Starter	Finisher
Maize	51.0	50.0
Soybean meal	30.0	28.0
Fish meal	4.00	2.00
Palm kernel cake	10.20	15.3
Bone ash	4;00	4.00
Salt	0.25	0.25
Lysine	0.20	0.10
Methionine	0.10	0.10
*Premix	0.25	0.25
Total	100	100
Nutrient composition (%)		
Crude protein	22.10	20.05
Crude fibre	4.38	4.96
Ether extracts	4.56	3.65
Lysine	1.20	1.00
Methionine	0.47	0.47
Calcium	1.25	1.10
Phosphorus	1.00	0.85
Energy (KcalME/kg)	2 880	2 901

*premix supplied per kg starter diet: vitamin A 15,000 i.u., vitamin D_3 13000 i.u, thiamine 2mg, riboflavin 6mg, pyridoxine 4mg, niacin 40mg, cobalamine 0.05g, biotin 0.08mg, choline chloride 0.05g, manganese 0.096g, Zinc 0.06g, iron 0.024g, copper 0.006g, iodine 0.014g, selenium 0.24mg, cobalt 0.024mg and antioxidant 0.125g. *premix supplied per kg finisher diet: vitamin A 10, 000 i.u., vitamin D_3 12,000 i.u. vitamin E 20 i.u., vitamin K2.5mg, thiamine 2.0mg, riboflavin 3.0mg, pyridoxine 4.0mg, niacin 20mg, cobalamin 0.05mg, pantothenic acid 5.0mg, Folic acid 0.5mg, Biotin 0.08mg, choline chloride 0.2mg, manganese 0.006g, Zinc 0.03g, copper 0.006g, iodine 0.0014g, selenium 0.24g, cobalt 0.25g and antioxidant 0.125g.

$$Y_{ij} = \mu + Ti + eij$$

where Yij is the response variable recorded by the broiler chickens, μ is the overall mean, T_i is the treatment (Negro pepper) effect on the broilers and eij is the random or residual effect.

Management of Experimental Birds: The day old chicks were purchased from a reputable hatchery and managed under welfare best practices. Ethics regulating experimenting with live animals stated by Ward and Elsea [14] were adhered to. The research protocol was approved by the University Research and Ethics Committee. The day old chicks were randomly allotted to five treatment groups in an enclosed house. Kerosene stoves were used to raise the temperature (30-35°C) of the house to supply warmth to the chicks for 21days. At the end of 21 days,

the birds were transferred to an open-sided experimental rearing house providing 0.15m^2/bird for adequate space. At day one glucose was added to their drinking water. From the second day vitamin, mineral and antibiotics commercial preparations were added to their drinking water for seven days. Maize-soybean meal based formulated diets containing nutrients levels as required by broiler chickens in the humid tropical environment (Table 1) were fed to the birds *ad. libitum.* Newcastle disease and infectious bursa disease (Gumboro) vaccines were administered by a veterinary Officer. Antibiotics were later given only at the advice of a veterinary officer.

Data Collection and Antibacterial Determination: Data on live weight and feed intake were collected weekly using a 20kg capacity Avery scale and used to calculate the feed: gain ratio. The effect of Negro pepper on total bacterial load of the experimental diet was determined by mixing 0.0, 2.5, 5, 7.5g and 10g Negro pepper of respectively to 1.0kg of the basal diet and homogenized. Then 5.0g of each diet was incubated at 37°C for 24 hours in agar medium in glass plates. Serial dilution method according to Ochi and Kochatkar [15] was used to determine the bacterial load.

RESULTS

Proximate Composition of Negro Pepper: The proximate analysis of the Negro pepper is shown in Table 2. The moisture and nitrogen free extracts were low. The crude fibre content was high while it had 32.96% and 8.33% of ether extract and protein respectively.

Effect of Negro pepper on Bacterial Load of experimental Diet: Table 3 shows the antibacterial action of Negro pepper in the diet. It was observed that the spice had dose dependent action on the bacterial load. As the level of Negro pepper was increased the number of bacterial load was significantly reduced ($p<0.05$) and hence the bacterial load was reduced from 3.6×10^4 to 1.7×10^3 cfu/g

Growth Performance of Starter Broilers: The result of effect of Negro pepper on growth performance of starter broiler chicks (1 - 4th weeks) is shown in Table 4. There were no significant differences ($P>0.05$) in initial weight and daily feed intake. Negro pepper significantly ($P<0.05$) influenced the final live weight, total feed intake and feed: gain ratio. The highest live weight was observed in group that consumed 2.5g/kg Negro pepper while those

Table 2: Proximate composition of test Negro pepper (% dry matter).

Composition	(%)
Moisture	8.50
Crude protein	8.33
Ether extract	32.96
Crude fibre	9.23
Ash	6.05
Nitrogen free extract	38.93

Table 3: Effect of dietary Negro pepper on bacterial load of experimental feed.

Xylopia Levels (g/kg diet)	Bacterial load Load ($1x10^4$ cfu/g)
0.00	3.6
2.50	3.5
5.00	3.0
7.50	2.5
10.0	1.7

Table 4: Effect of dietary Negro pepper (g/kg diet) on growth performance of starter Broilers (1 - 4 weeks)

Parameters Treatments	Initial live weight (g)	Final live weight (g)	Daily weight gain (g)	Total feed intake (g)	Daily feed intake (g)	Feed: gain ratio
T1(0.00)	44.12	743^b	24.96^b	1 289^b	46.04	1.84^b
T2(2.5)	43.72	800^a	27.01^a	1 389^{ab}	49.61	1.84^b
T3(5.0)	43.56	750^b	25.23^b	1 370^{ab}	48.93	1.94^b
T4(7.5)	43.68	714^b	23.94^b	1 405^a	50.18	2.10^a
T5(10.0)	45.20	664^c	22.10^c	1 418^a	50.64	2.29^a
SEM values	4.01	48.72	1.35	114	4.56	0.16

abc means within the same column with different superscripts are significantly ($P<0.05$) different

Table 5: Effect of dietary Negro pepper (g/kg diet) on the performance of finisher broilers (5-8 weeks)

Parameters Treatments	Initial live weight (g)	Final live weight (g)	Daily weight gain (g)	Total feed intake (g)	Daily feed intake (g)	Feed: gain ratio
T1(0.00)	743[b]	2 205[b]	52.21[b]	4 595[b]	164.11	3.14[b]
T2(2.5)	800[a]	2 336[a]	54.86[a]	4 711[a]	168.25	3.07[b]
T3(5.0)	750[b]	2 332[a]	56.50[a]	4 735[a]	169.11	2.99[b]
T4(7.5)	714[b]	2 125[b]	50.39[b]	4 559[c]	162.82	3.23[b]
T5(10.0)	664[c]	1 935[c]	45.39[c]	4 549[c]	162.46	3.58[a]
SEM values	48.72	78.50	4.34	152	22.09	0.25

abc means within the same column with different superscripts are significantly (P<0.05) different

that consumed 10g/kg produced the least live weight. There was no difference between the live weight of birds on control diet, those on 5g/kg and 7.5g/kg. Total feed intake was improved by diets containing 7.5g/kg and 10g/kg compared to control diet. There was no difference (P>0.05) in total feed intake within the groups that consumed Negro pepper. It was also observed that the total feed intake was similar in birds on control diet, 2.5 and 5g/kg Negro pepper diets. The feed: gain ratio was reduced by 7.5g/kg and 10g/kg Negro pepper diets. Feed: gain ratio produced by 2.5g/kg and 5g/kg was similar to that of the control.

Growth Performance of Finisher Broilers: The performance of finisher broilers (5-8th week) fed Negro pepper is shown in Table 5. Final live weight, daily weight gain, total feed intake and feed: gain ratio were significantly (P<0.05) influenced by dietary treatment. Feeding of 2.5g/kg and 5.0g/kg Negro pepper diet gave higher live weight while 10g/kg produced the least. The daily gain followed similar pattern as the final live weight. Total feed intake was significantly (P<0.05) improved by 2.5g/kg and 5g/kg diet, while 7.5g/kg and 10g/kg negatively affected it. The daily feed intake was not influenced significantly (P>0.05). Poorest feed: gain ratio was recorded by 10g/kg and there was no significant difference in feed: gain ratio between the control, 2.5, 5.0 and 7.5g/kg.

DISCUSSION

Proximate composition is one of the parameters used to determine the nutritive value of feeds and feed ingredients. It shows the levels of crude protein, crude fibre, ether extract, ash and nitrogen free extract in a given feed or feedstuff. The result of the proximate analysis indicated that Negro pepper contained appreciable level of ether extract and protein which were comparable to other spices such as ginger, garlic, onion, black pepper and selim pepper [16, 17]. The ether extract and crude protein were higher and lower respectively compared to the report of Barminas *et al.* [18]. The ether extract was higher compared to other spices such as black pepper (20.30%) and *tetrappluera tetraptera* (5.60%), though the ether extract was high it was less than 53.40% reported for *Moringa myristica* [19]. The level of the ether extract is an indication that the Negro pepper could be a source of oil in diets for broilers. Spice oils such as garlic and rosemary oils have been incorporated into diets for broilers which resulted to positive effect [20].

Reduction in bacterial load of the diet was an indication that the Negro pepper was anti-bacterial. Antibacterial action is one of the characteristics of spices and phytogenics in general which makes them a potential replacement for pharmaceutical antibiotics as growth promoters [4, 21]. Usman *et al.* [22] reported that Negro pepper contained essential oils which contained bioactive compounds such as Eugenol which showed various degree of inhibitory effect against gram negative bacteria. This confirmed earlier assertion that Negro pepper had antibacterial properties which could be used to improve the productivity of broilers [23].

The result of growth performance recorded at the starter phase is an indication that Negro pepper is a substance that could be used to improve the growth of starter broiler chicks. Young chicks lack the full capacity to utilize feed effectively because their gastro intestinal tract is not well developed and endogenous secretions such as gastric juice components (e.g. hydrochloric acid) and mucus are inadequate [24]. The gastro intestinal tract needs a booster to enhance the capacity to secrete vital substances for effective utilization of nutrients. The Negro pepper could have assisted in this regard as spices have reported to aid endogenous secretions [25]. The result which indicated that as the level of the spice was increased above 5g/kg, live weight and feed: gain ratio were negatively affected clearly showed that the Negro pepper had inclusion limit which when exceeded could be detrimental to broiler birds. It also suggests that the spice could have contained some substances that are anti-nutrient. This was shown in the result of feed: gain ratio which was negatively affected. The poor feed: gain ratio

recorded by 7.5 and 10g/kg despite their highest total feed intake is an indication that the poor performance in final live weight recorded at the two levels was not due to their feed intake. It could be due to some anti-nutritional factors contained in Negro pepper such as tannins, cyanogenic glucosides and saponins [25]. According to Windisch *et al*. [9] contained tannins which is known to bind protein [3]. According to Lee *et al*. [26] essential oils contained in spices reduced growth of broilers at a certain level of inclusion. For instance feeding 2.5g/kg diet of black pepper, Ndelekwute *et al*. [17] reported improved live weight and feed intake but both were reduced when the level of black pepper was increased up to 10g/kg. However, Al-Kassie *et al*. [27] reported that black pepper improved both feed intake and growth at 5, 7.5 and 10g/kg. Natural feed additives such as herbs, spices and prebiotics are known to positively and affect feed intake at certain level [4, 28, 29] and also improve their health status [30]. The result is in agreement with Isikwenu and Udomah [10] who reported improvements in live body weight and feed intake of broilers fed Negro pepper but contradicted the feed: gain ratio of this result.

CONCLUSION

Current effort has been to search for natural products with antibacterial and growth promoting effects to replace antibiotics. The result of our study indicated that Negro pepper could be used to improve growth performance of broilers. It is therefore concluded that 2.5g/kg Negro pepper could be added to broiler diets.

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