RESEARCH ARTICLE

Performance and serum biochemistry of broiler chickens fed diet supplemented with *Annona senegalensis-Garlic* Cocktail

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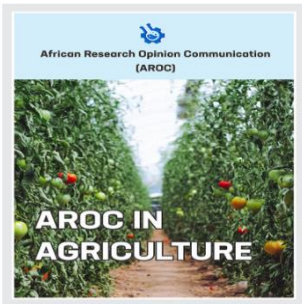

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ABSTRACT

Background: Animals eat to acquire the energy and building materials that they need to live and grow the nutrient required to achieve such, abound in phyto-materials. The synergistic, additive, or complementary immuno-modulatory effects of phytocomponents from *A. senegalensis* and *Alium cepa* were evaluated in this work. **Methods:** Seventy indigenously developed varieties of day-old broilers were divided in a completely randomized design(CRD) into 5 (n=14) treatment groups (T1-T5) treatments with 2 replicates per treatment. At the finisher level, 0.0, 0.2, 0.3, 0.4, and 0.5 kg of the *A. senegalensis*-garlic (ASG) cocktail was added to the normal finisher diet. **Results:** Feed consumption tends to increase significantly ($p \leq 0.05$) with the increased level of ASG Cocktail. values recorded 1405±17.9 g, 1450±21.5 g, 1310±20.6 g, 1450±17.3 g, 1340±15.3g for T1, T2, T3, T4, and T5 respectively were recorded. There is a significant difference ($p \leq 0.05$) in the total protein (TP) at different inclusion levels with 0.5% having the highest value of 57.95±4.07 g/dl. The triglycerides and cholesterol levels decrease as the ASG inclusion level increases which shows the anticholesteremic potency of the blend. There are significant increases ($p \leq 0.05$) in the albumin and globulin concentrations as the level of ASG inclusion increased. However, while there is a significant increase ($p \leq 0.05$) in the aspartate transaminase along the concentration gradient, the reverse is the case with regards to alanine transaminase. A significant decrease ($p \leq 0.05$) in bilirubin concentration along the inclusion gradient (except in T5). There was a significant increase ($p \leq 0.05$) in body weight across the inclusion gradient except in T3. The Feed Conversion Ratio (FCR) follows a regular pattern with a range of 0.51-0.53 except for the birds fed with 0.3% inclusion (T3) that recorded a higher (poor) value of 0.89. **Conclusion:** Supplementation with ASG cocktail improves the performance and the immune-competence of the bird at the finisher stage.

Keywords: Broiler finisher; *Anona senegalensis; Alium cepa;* Supplementations; Biochemical indices

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1.0 Introduction

Animals eat to acquire the energy and building materials that they need to live and grow. Energy is used by animals to perform normal body functions such as breathing, walking, eating, digesting, and maintaining body temperature. Energy and material needed for the development of bone, flesh, feathers, and eggs are derived from nutrients. [1] indicated that vitamin premix (VP) withdrawal at 42 day of age did not impair feed intake or weight gain, but significantly affected feed conversion ratio of broiler chickens. In contrast, [2] reported that removing vitamin and trace mineral premixes from broiler diets from 28 to 49 days of age had little impact on growth performance. However, as reported by Majid, *et al.* [3], there are some reasons which may justify removing or reducing the usage of vitamin supplementation (an expensive essential nutrient in poultry's diets) in finisher period diets in floor system. His reason being that, the amount of vitamin supplements usually exceeds two or three times the recommended broiler chicken requirements in poultry diets; fat-soluble vitamins

may be stored by a bird in its liver and fatty tissue in sufficient quantities to meet requirements for up to 15 day or even longer; there are some vitamins in diet ingredients such as wheat, barley and soybean meal that are not considered during formulation diets and finally, floor-raised broilers can access their faeces to reach some vitamins which are produced in the intestine.

Cost of production of broiler chicks particularly in Nigeria and some sub Saharan Africa and Asian countries could substantially be minimised by substituting some of the feed supplements with the available natural products and other agricultural waste products [4] This could be achieved through series of substitution trials. This will have a ripple effect of both enhancing the immuno-competency of the birds and making proteins source both available and affordable to the poor populace in the society [5].

Natural products, especially those derived from plants, have been used to help mankind sustain its health since the dawn of medicine. Over the past century, the phytochemicals in plants have been a pivotal pipeline for pharmaceutical discovery [6, 7]. The importance of the active ingredients of plants in agriculture and medicine has stimulated significant scientific interest in the biological activities of these substances [8].

One such plant with abundance of phytochemicals is *Annona senegalensis* Persoon (Annonaceae), commonly known as wild custard Apple and Wild Soursop. *A. senegalensis* is a multipurpose plant with a high traditional and medicinal uses for the maintenance of free health life. Traditionally the plant is used as stimulant, pain reliever etc. Several uses of the plant species is reported for example anti-oxidant, antimicrobial, antidiarrheal, antiinflammatory, antiparasitic, anticonvulsant, antimalarial, antitrypanosonal, anti-snake venom and antinociceptive properties and many other biomedical properties of pharmaceutical relevance. These properties of the plant possess is due to its important phytochemical constituents like triterpenes, anthocyanes, glucids, coumarins, flavonoids and alkaloids etc. [9]. It is found growing throughout Nigeria. It is very common in Northern Nigeria, primarily in Nasarawa, Kaduna, Kano, Plateau, and Niger States and in the Federal Capital Territory, Abuja and usually known as Gwándàn dààjìì (Hausa) or dukuu-hi (Fulani) [10].

Allium sativum, commonly known as garlic, is a species in the onion genus, Allium. Its close relatives include the onion, shallot, leek, chive, [11]. With a history of human use of over 7,000 years, garlic is native to central Asia [12] and has long been a staple in the Mediterranean region, as well as a frequent seasoning in Asia, Africa, and Europe. A member of the Liliaceae family, garlic (Allium sativum) is a cultivated food highly regarded throughout the world. Originally from Central Asia, garlic is one of the earliest of cultivated plants [13]. In 1997, garlic was the most widely used natural supplement in US households. Garlic was also shown to be used more than twice as much as any other natural supplement [14].

The main thrust of this study is to assess the potency of employing a blend of pulverised leaf of *Annona senegalensis* and garlic (to form a cocktail) from day 28 (4 weeks) and the effects of its inclusion on the feed intake, weight gain, feed conversion ratio and serum biochemical parameters in experimental broiler chicks.

2.0 Materials and Methods

2.1 Experimental site:

The research was conducted at both the teaching/research farm and the Central Laboratory of the Federal College of Wildlife Management, New Bussa. The experimental station (New Bussa) sits at 9^0 53'N, 9^0 83'N and 4^0 31'E, 4^0 51'E [15]. The research work was carried between the Months of May to July (early part of rainy season).

2.2 Experimental birds

Seventy-four (4) weeks old indigenously developed varieties of Anak broilers of average weight range of 964 – 1121 g, were procured from the CHI Farms along Ibadan-Lagos express way, Nigeria. The birds were divided into 5 (T1-T5: n=14) treatments with 2 replicates per treatment and 7 birds in each replicate in a completely randomised design. Fifteen pens were made and the replicates were randomly allotted using lottery method under deep litter system and reared for 7 weeks. All animals handling and experimentations were in accordance with the principles and guidelines for the care and use of agricultural animals in research (The Federation of Animal Science Societies [16] (FASS), 1999), which were consistent with the recent recommendations issued by the International Life Sciences Institute (ILSI) [17] as applicable.

2.3 Collection and identification of plant materials:

The plant (*Annona senegalensis*) was sourced from the surroundings of Federal College of Wildlife Management, New Bussa plantation and the surrounding villages where it is in abundance. Its identity was confirmed in the Department of Forestry, Plant Nursery Unit, F.C.W.M, while the *Alium sativum* (Garlic bulbs) was purchased from the Monday market in New Bussa metropolis, Niger State, Nigeria

2.4 Preparation of the plant sample

Young leaves from the plant (*Annona senegalensis*) were harvested and sliced and chopped into smaller pieces to hasten drying at room temperature. It was then pulverized using Hammer miller machine. Powdered sample was then transferred into a sterile universal bottle and stored at 4^{o}C until required for use. The garlic cloves were also chopped into smaller pieces and oven dried before being pulverised into powdered form.

2.5 Diets Formulations

Diets were formulated based on the individual nutrient analyses of each soybean meal lot. Dietary protein was provided by the dehulled soybean meal and corn. Diets were formulated to meet or exceed National Research Council (NRC), [18] values for broiler chickens. A coccidiostat, salinomycin (Sacox, Intervet Inc., Millsboro, DE), was included in all diets at a level of 50 g/ton. Broilers were fed a starter diet containing approximately 33% wt/wt soybean meal from day 0 to 21. For the rest of the study (day 21 to 42), broilers were fed a grower-finisher diet containing approximately 30% wt/wt soybean meal.

Table 1: Percentage of composition of experimenter diet at finisher phase

Ingredients	T1(Kg)	T2 (Kg)	T3 (Kg)	T4 (Kg)	T5 (Kg)
Maize	50.2	50.2	50.2	50.2	50.2
Soya beans	20	20	20	20	20
Fish meal	10	10	10	10	10
GNC	6.0	6.0	6.0	6.0	6.0
Wheat offal	9.7	9.7	9.7	9.7	9.7
Bone meal	3.0	3.0	3.0	3.0	3.0
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Vitamins premix	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Powder coccidiostat	0.05	0.05	0.05	0.05	0.05
Growth enhancer	0.05	0.05	0.05	0.05	0.05
Total (%)	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
A. senegslensis /garlic blend	0.00	0.2	0.3	0.4	0.5

2.6 Experimental design

Day old broiler chicks were divided into five groups (T1-T5; n = 14). The formulation of the *A. senegalensis*/garlic blend was done in the ratio 7:3

Treatment 1: Standard control supplemented with 0% (0.0 g/100kg) ASG at the finisher stage

Treatment 2: Test group supplemented with 0.2% (200 g/100kg) ASG.

Treatment 3: Test group supplemented with 0.3% (300 g/100kg) ASG.

Treatment 4: Test group supplemented with 0.4%(400 g/100kg) A*ASG*.

Treatment 5: Test group supplemented with 0.5% (500 g/100kg) ASG

Feed and water was supplied *ad-libitum* for about 3 weeks before the commencement of the experiment. Mortality in respective group was recorded at occurrence in starter and finisher period.

2.7 Measurement of the body weight

Individual body weights were recorded at the beginning of the experiment and further body weight increment were recorded at the end of each week to monitor the pattern of body weight changes. Group wise average weights under

different treatments were arrived at. The weighing of the birds was done in the early hours of the day before feeding.

2.8 Feed consumption and conversion ratio

The daily amount of the concentrated diet was weighed and offered to the 7 bird replicate group. The feed consumption in each replicate was recorded weekly by subtracting the weight of residual fed from the total quantity of feed supplied during the respective week. The feed conversion ratio (FCR) was determined through the relationship between amount of feed consumed (FC) to the body weight gain (BWG) under each group of birds i.e.

$$FCR = \frac{FC}{BWG(g)}$$

2.9 Blood biochemical analysis

Blood samples were also collected through the wing vein into tubes without anticoagulant and the serum analysis on blood glucose, alkaline phosphatase (ALP), serum albumin, total protein, cholesterol and blood urea was carried out. The under listed biochemical parameters as described by each procedure were determined using biochemical kits (Randox Laboratories, UK): Briefly, the activities of ALP were determined according to the method described by Tietz *et al.* [19].

The activities of transaminases (AST and ALT) were determined as described by Reitman and Frankel [20]. The level of serum total proteins was estimated using biuret in accordance with the method described by Burtis and Ashwood 1999 [21] and Deneke and Riiffersdorf, [22]. The level of serum urea was also assayed as described by Veniamin and Vakirtzi-Lemonia[23] and Deneke and Riiffersdorf [22]

2.10 Statistical analysis

Data pertaining to various parameters obtained during the experiment was analysed as Completely Randomized Block Design according to the methods described by Snedecor and Cochran [24] significance level was set at $p \leq 0.05$.

3.1 Result

3.1 Performance of finisher broiler chickens fed basal feed supplemented with *Annona senegalensis*-garlic cocktail.

There is significant difference (($p \leq 0.05$)) in all the parameters measure in initial weight. The final weight and weight gain significantly increased with increasing level of ASG cocktail across the treatments. The weight gains of the birds were within the range of 1479g (T3) to 2783g (T4). The final weight at the finisher level shows that there is no significant (($p \leq 0.05$)) between the treatments T1 to T5. Also feed consumed tends to increase significantly (($p \leq 0.05$)) with the increment level of ASG Cocktail. The value recorded were 1405.00, 1450.00, 1310.00, 1450.00, 1340.00 for T1, T2, T3, T4, and T5. The FCR follows an irregular pattern with T2 having the best value of 0.51 and birds on diet ASG cocktail 0.2% inclusion recording a poor value of 0.89 (Table 2).

Table 2. Performance of finisher broiler chickens fed basal feed supplemented with *Annona senegalensis*-garlic cocktail.

Parameters	T1 (0% ASG)	T2 (0.2% ASG)	T3 (0.3% ASG)	T4 (0.4% ASG)	T5 (0.2% ASG)
Average Initial Weight(g/birds)	1071±21.2	1106±19.0	1121±23.4	1092±18.5	964±20.6
Average Final Weight(g/birds)	3525±32.4	3925±29.3	2600±16.3	3875±42.3	3514±32.1
Average Weight Gain(g/birds)	2454±28.2	2819±20.3	1479±19.4	2783±22.8	2536±31.0
Average Feed Consumed(g)	1405±17.9	1450±21.5	1310±20.6	1450±17.3	1340±15.3
Feed Conversion Ratio (FCR)	0.57	0.51	0.89	0.52	0.53

ASG = *Annona senegalensis*-garlic cocktail.

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3.2 Serum biochemical parameters of finisher broilers fed basal feed supplemented with *Annona senegalensis*-garlic cocktail

There is significant difference (p≤0.05) in the total protein (TP) at different inclusion level with 0.5% having the highest value of 57.95±4.07 g/dl. The triglycerides and cholesterol level decreases as the ASG inclusion level increases which shows the anticholestraemic potency of the blend (Table 3). There is significant increase (p≤0.05) *in the* albumin and globulin concentrations as the level of ASG inclusion increases. While there is significant increase (p≤0.05) in the aspartate transaminase along the concentration gradient, reverse is the case in with regards to alanine transaminase. Significant decrease (p≤0.05) in bilirubin concentration along the inclusion gradient (except in T5) is an attestation of haemo-stability induced by the ASG blend.

Table 3. Serum biochemical parameters of finisher broilers fed basal feed supplemented with *annona senegalensis*-garlic cocktail

Parameters	T1 (0% ASG)	T2 (0.2% ASG)	T3 (0.3% ASG)	T4 (0.4% ASG)	T5 (0.2% ASG)
Total Protein	27.55±1.65^{a}	38.95±2.74^{bc}	41.80±10.97^{bc}	40.85±4.94^{bc}	57.95±4.07^{c}
Albumin	2.14±0.33a	5.35±0.62bc	4.07±0.87ab	4.40±0.46b	6.57±0.83c
Globulin	25.41±1.98a	33.60±3.36b	37.73±10.11b	36.45±4.48b	51.38±12.89c
Aspartate Transaminase	27.13±3.01a	56.85±2.51bc	41.40±13.51ab	28.80±8.31a	68.40±2.08c
Alanine Transaminase	24.44±10.85a	13.79±3.81a	7.52±0.60a	6.58±1.63a	7.94±9.23b
Bilirubin	1.08±0.25a	0.97±0.31a	1.51±0.06ab	1,57±0.41ab	2.21±0.03b
Triglycerides	132.96±22.51ab	113.78±29.86a	175.86±14.93b	82.76±5.97a	73.71±3.73a
Total Cholesterol	155.77±36.64a	132.69±36.64a	248.07±3.33b	144.23±23.31a	162.68±15.19a

ASG = *Annona senegalensis*-garlic cocktail

4.0 Discussion

There are no significant differences in FCR, body weight, and feed intake between the control treatment, T1, T2 and T4 with the exception of T3 that differs significantly (P≤ 0.05) with the rest of the treatments. Additionally; the diet in the treatment 3 with 0,3% ASG cocktail, shows the least body weight gain and FCR tended to be higher while the diet with 0.4% displayed an amazing feature of lowest feed consumed but with body weight gain comparable to T2 and T5. However, despite the fact that T2, T4 and T5 statistically differ (P≤ 0.05) in their respective weight gains (Table 2), it is pertinent to state that the values remain numerically non-significant in terms of profitability envisaged from raising such bird [25].

Annona senegalensis has been reported to contain low level of tannins, catechin but high in fiber [26] and it has been indicated that the growth of broiler chicks had a negative relationship with dietary tannin contents of green tea [27]. This results therefore confirms this assertion in the sense that, the low tannin level in both the *Annona* and the garlic reflect in the good performance observed in this study.

The reduction of cholesterol content in blood in T2, T4, and T5 compared with the control (Table 3) can be explained by effect of catechin and fibre/tannin contents of garlic and *Annona senegalensis* respectively. *A. senegalensis* containing tannin in low quantity, and catechin is one kind of tannin which according to [28] may have an inhibitory effect on intestinal absorption of lipid. This may prevent an excessive accumulation of lipid in the liver and other tissues as observed in T2, T4 and T5 except in T3 (Table 3).

The reduction in tissue cholesterol may also be explained by a negative effect of Garlic catechin and the *A. senegalensis* fiber on formation of micelles that mediates reabsorption of bile acid [29]. The other possible explanation on *A. senegalensis* fibre is based on the great deal of evidences [30] that dietary fibre could reduce the level of cholesterol in animals through adsorbing bile acids and various lipids on it. In addition, the phenolic compound in tannic acid of these plants resources may play an important role in the catabolism of liver cholesterol [31].

Conversion of cholesterol to bile acids occurs exclusively in the liver and represents the major pathway for the elimination of cholesterol from the body. This may also explain the reduced cholesterol and Triglyceride levels in T2, T4 and T5 (Table 3) compared with the values in birds on control basal diet. Appreciable increase (but still within the normal range) in total serum protein, albumin and globulin compared with the control (Table 3), points

to the increase feed utilisation by the bird in all the treatments.

An interesting aspect of this study is the observed low mortality rate (2%) recorded in the course and within the time frame the work was conducted on the experimental birds fed the supplement when compared with the control group that a higher mortality was recorded. The probable reason could be attributed to the presence of flavanols, (the flavan-4-ols) in both the garlic and as a phyto-component of the *Annona senegalensis* which have some immunologic, therapeutic and antitumor activity [32-34].

Flavan-4-ols revealed strong host mediated antitumor activity, which is due to the enhancement of immune response of the host animals through the actions on tumor cells and some immunocytes [35, 36], while on one hand the green, dry "folds" in the center of the garlic clove are especially pungent. The sulfur compound allicin, produced by crushing or chewing fresh garlic, produces other sulfur compounds: ajoene, allyl polysulfides (Diallylsulphides, Diallyldisulphides and Triallyltrisulphides) and vinyldithiins. the presence of S-allylcysteine has also been observed in garlic.

Garlic has been used for thousands of years as a remedy for many different ailments, including intestinal disorders, flatulence, worms, respiratory infections, skin diseases, wounds, and symptoms of aging. Modern research indicates that garlic may help improve heart health in a number of different ways. It is a blood thinner that helps to lower both high blood pressure and blood triglycerides. The presence of diallyl sulphide and thiacremonone has been found to confer on it anti-arthritic property [37]. Garlic also has anti-inflammatory properties. Several population studies also show an association between an increased intake of garlic and a reduced risk of certain cancers, including colon, stomach, esophagus, pancreas, and breast cancer. [38].

One special area of interest is the fact that meat produced from these experimental birds will besafe for human consumption particularly the women at pre-menstrual. This is true because it has been established that meat cooked at high temperatures (400°F/204°C or higher) forms heterocyclic amines (HCAs) that are cancer-related substances. One such HCA is called PhIP (2-amino-1-methyl-6-phenylimidazopyridine). PhIP is thought to be one reason for the increased incidence of breast cancer among women who eat large quantities of meat because it is rapidly transformed into DNA damaging compounds. A phyto-component in garlic called Diallyl sulfide (DAS), one of the many sulfur-containing compounds in garlic, has been shown to inhibit the transformation of PhIP into carcinogens [39, 40]. DAS blocks this transformation by decreasing the production of the liver enzymes (the Phase I enzymes CYP1A1, CYP1A2 and CYP1B1) that transform PhIP into activated DNA-damaging moiety. Additionally, garlic is a triple threat against infections, offering antibacterial, antiviral, and antifungal properties. Also, the positive immunomodulatory effect observed as a result of sustained feeding of the experimental animals with *A. senegalensis*–garlic cocktail might be a major attribute to the ability of the experimental animals to resist the infection by parasites and other pathogens.

5.0 Conclusion

Supplementation with *A. senegalensis-Alilium sativum* cocktail proved quite effective in improving the growth performance and immune-competency of the experimental birds. However, the sudden increase in the serum levels of AST in all the treatments compared with control is a worrisome indication of possible inflammation of the hepatocytes and appeared to be concentration dependent except in T4. Despite the observed set back supplementation with this cocktail still hold great potency as growth promoter if further study is undertaken to identify and isolate the component with the seeming hepato-toxic effect.

Authors' contributions:

This work was carried out as a collaboration between all authors. Authors MHG, ATA and KMA designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors LJB, NS and SMD managed the analyses of the study, performed the statistical analysis and managed the literature searches. All authors read and approved the final manuscript.

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Ethical approval

Authors hereby declare that "principles of Laboratory animal care" (nih publication no. 85- 23, revised 1985) were followed, as well as Specific national laws where applicable. All Experiments

have been examined and approved by the appropriate ethics committee.

Competing interests

Authors have declared that no competing interests exist

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