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Investigating the Potentials of *Citrullus Lanatus* seed as Phytobiotic to improve growth Performance, feed efficiency and Serum biochemical Prameters in broiler chickens

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Abstract
Sixty day-old chickens were grouped into three; A, B and C. Group A, the control, received commercial diet only. Groups B and C were fed commercial diet, supplemented with 1 and 2% of *Citrullus lanatus* powder, respectively. Feed and water were offered *ad libitum* for 6 weeks. Water consumed was measured daily, birds and feed were weighed on a weekly basis. At the end of the experiment, results for feed consumption and feed conversion ratios were similar for all groups. The activity of superoxide dismutase increased statistically in group fed 2% WSP when compared to 0 and 1% WSP. From the results of this study, it may be concluded that *Citrullus lanatus* could improve the immune status of broilers and can be supplemented in feeds for broilers at 1 and 2% without interfering with the overall health and performance of the chickens.

Keywords: *Citrullus lanatus*, phytobiotics, antibiotics, antioxidant enzymes, broilers

1. Introduction
Antibiotics are naturally occurring, semi-synthetic, or synthetic compounds with antimicrobial activity [1, 2]. They are common types of growth promoters used in poultry industries. However, one of the drawbacks of excessive use of antimicrobial drugs is that they get built up in tissues and organs of treated animals as residues and eventually become part of the food pyramid [3]. Excessive usage of antibiotics in food producing animals can serve as a reservoir of antibiotic-resistant bacteria strains which may be transferred to humans [4]. Subsequently, the effectiveness of antibiotics in humans decreases, resulting in treatment failures [5, 6]. Due to the fear of creating cross resistance between animals and humans, the European Union has placed a ban on the application of antibiotics as growth enhancers since 1st January 2006, which was also followed by other parts of the world including North America [7].
Restriction in the usage of antibiotic growth promoters has however, resulted in an increased incidence of enteric disorders such as necrotic enteritis and dysbacteriosis in poultry [8]. Due to this restriction, poultry industries have now focused on the use of biological products, such as enzymes, probiotics, prebiotics, symbiotic, organic acids and plant extracts (photobiotic), as alternatives to antibiotic feed additives in diets for monogastric animals [9, 10]. Photobiotic are plant-derived natural bioactive compounds used in animal diet as alternatives to antibiotic growth enhancers that are added to feed to improve performance in animals [11, 12].
Researchers have documented beneficial effect of photobiotic on the performance of poultry [13-16]. Many photobiotic show promising results for applications in organic and conventional poultry production.
Watermelon (*Citrullus lanatus*) is a creeping plant belonging to the family of cucurbitaceous. The center of diversity and possible origin of *Citrullus* is southern Africa [18]. It is mainly propagated by seeds. It is a tropical plant that requires a lot of sunshine and high temperature of over 25 °C for optimum growth [19].
Watermelon fruit is known to be a very good source of Lycopene, a red pigment of the

carotenoid class found in few fruits and vegetables. It is a powerful oxygen radical scavenger and a highly effective antioxidant [20 21]. Watermelon seeds are known to be highly nutritional; they are rich source of protein, vitamin B, minerals (such as magnesium, potassium, phosphorous, sodium, iron, zinc, manganese and copper) and fat among others, as well as some phytochemicals [23]. This present was designed to investigate the potentials of *Citrullus lanatus* seed as phytobiotic to improve growth performance, feed efficiency and serum biochemical parameters in broiler chickens.

2. Materials and Method

Sixty day-old broiler chicks were purchased from a commercial supplier in Minna, Niger state, Nigeria and transported to the research facility of IBBU Lapai. The chicks were exposed to cross-ventilation throughout the study period. Chicks were reared in cages of dimension 1.5 m x 1.5 m x 2.0 m; length x breadth x height, which were previously cleaned and disinfected. Chicks were then allowed to acclimatize for 5 days. During the acclimation, the chicks were fed commercial starter feed (23.62% Crude Protein, 14.7% Crude fat) and water was supplied *ad libitum* under strict biosecurity control according to previously published protocol [24].

2.1 Study location: The feeding trial was carried out at the animal research facility of biochemistry department, Ibrahim Badamasi Babangida University, Lapai, Niger State.

2.2 Study design: Sixty day old chickens were randomly distributed into three treatments and fed commercial starter feed (Chikun, Olam poultry feed mill, Kaduna, Nigeria) mixed with 0, 1 and 2% WSP. Pre-weighed feed were provided to all treatments. Feed and water were offered *ad-libitum* and reared without vaccination. Cages were cleaned at the end of each week as weighing of birds took place.

2.3 Study duration: The experiment lasted for a period of 6 weeks.

2.4 Sample size: Sixty day-old broiler chicks'

2.5 Methodology

2.5.1 Proximate composition analysis of experimental feeds and watermelon seeds powder

Moisture content, crude fat, crude fiber, and crude protein (Microkjeldahl N x 6.25) were all determined following standard Methods as described by the Association of Analytical Chemists [25].

2.5.2 Calculations for feed efficiency and growth performance parameters

Various parameters were calculated by applying the appropriate formulae where necessary using the following:

Chicks survival (%) = (total chicks' survival/ total chicks stock) x 100

Weight gain% (WG%) = $[(W_f - W_i)/W_i] \times 100$

Specific growth rate (SGR%) = $[(\ln W_f - \ln W_i)/T] \times 100$

Feed intake (FI) = total feed intake/number of live chicks

Feed conversion ratio (FCR) = wet weight gain (g)/feed intake (g)

Where W_f refers to the mean final weight, W_i is the mean initial weight and T is the feeding trial period in days.

2.5.3 Determination of Serum biochemical analysis

Serum biochemical parameters such as AST, Aspertate aminotransferase; ALT, Alanin aminotransferase; ALP, Alkline phosphate, bilirubin, albumin, creatinine, proteins, potassium, chloride, sodium and urea were determined spectrophotometrically following the manufacturer's instructions of AGAPPE diagnostics, Cham, Switzerland.

2.5.4 Determination of activities of antioxidant enzymes

Antioxidant enzymes including superoxide dismutase (SOD) and catalase (CAT) were determined spectrophotometrically from the broilers' serum where lipid peroxidation was assayed according to the method described by Health and Parker [26].

2.6 Statistical analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) and the means were compared using Turkeys test. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using SPSS software Version 20.0. Data for all determinations are presented as mean ± SEM of three replicates.

3. Results

Results for proximate composition analysis of WSP and all dietary treatments are shown in Table 1. From the Table, it may be observed that WSP is a good source of fat, protein and fibre. The moisture content of WSP was lower when compared to the three treatment groups. The result also showed that incorporation of WSP into diet significantly increased the ash content. This, suggesting that higher quantity of WSP in diet would provide higher essential minerals than the control diet. Inclusion of WSP up to 2% did not significantly ($P > 0.05$) modify carbohydrate, fat and moisture content of all dietary treatments when compared to control diet.

Table 1: Proximate composition of experimental feeds and WSP

			Treatments	
Parameters	**WSP**	**0% WSP**	**1% WSP**	**2% WSP**
MC%	5.40±0.00	8.44±0.06^a	7.56±0.93^a	8.14±0.35^a
CF%	33.80±0.03	4.90±0.01^a	7.70±0.50^b	8.80±0.80^b
ASH%	3.35±0.07	4.30±0.03^a	6.40±0.07^b	7.30±0.01^c
FAT%	30.25±0.03	14.70±0.02^a	6.49±0.02^a	13.17±0.01^a
CP%	23.62±0.05	23.62±0.09^a	24.01±0.40^a	30.62±0.03^b
CHO%	3.60±0.01	44.04±0.05^a	28.87±16.01^a	31.97±13.05^a

Data are expressed as mean ± SEM (n = 20).

Mean ± SEM followed by different superscript letter within a row are significantly different (P <0.05).

MC, Moisture content; CF, Crude fibre; CP, Crude protein; CHO, Carbohydrate (Nitrogen free extract).

Mean initial and final weights of the three dietary treatment groups as shown on Table 2 were within the same range, therefore did not show a significant difference. This indicates that the three experimental groups had no hindrance to growth, although the group fed the highest percentage of WSP recorded a higher mean final weight compared to the control group and 1% WSP group. Feed intake increased progressively from week 1 to 6 for all the treatment groups as shown in Table 4.3, where the highest feed intake for the experimental groups was in week 6, whereas the lowest was at week 1. The 2% WSP treatment group recorded among the highest at weeks 4, 5, 6 and lowest at week 1.

Table 2: Feed efficiency and growth performance of broiler chickens fed graded level of *C. lanatus* seed powder for 6 weeks starter period

		Dietary treatments	
Parameters	**0% WSP**	**1% WSP**	**2% WSP**
Growth performance			
Mean initial weight (g)	70.25±1.80[a]	70.80±1.84[a]	71.70±1.74[a]
Mean final weight (g)	2084.15±52.37[a]	2064.42±58.30[a]	2171.05±42.95[a]
Specific growth rate (%)	8.07±0.24	8.03±0.13[a]	8.12±0.10[a]
Weight gain (g)	2013.9± 0.27[a]	1993.62±0.13[a]	2099.35±0.20[a]
Av. Daily weight gain (g)	47.95±0.22	45.01±0.34	49.98±0.02
Feed efficiency			
Feed intake (g)	3622.25±0.12[a]	3615.35±0.45[a]	3647.28±0.29[a]
Feed conversion ratio	1.80±0.00	1.81±0.01	1.74±0.02

Data are expressed as mean ± SEM (n = 20).
Mean ± SEM followed by different letter within a row are significantly different (P <0.05).

The results for serum metabolic parameters such as; total protein, chloride and sodium did not significantly (P > 0.05) differ across all the treatments.
Other results obtained for serum metabolic parameters such as urea concentration of 0% WSP was lower compared to those fed diet containing 1 and 2% WSP. Increase in serum urea was noticed as the amount of WSP increased. Creatinine level was highest in the group consuming the commercial diet without the addition of WSP. Creatinine concentration in the blood of 1% WSP was noted as comparable to that of 0% WSP, both of which are statistically different from 2% WSP.

Table 4: Serum metabolic parameters of broiler chickens fed graded level of *C. lanatus* seed powder for 6 weeks starter period.

		Treatments	
Parameters	**0%WSP**	**1% WSP**	**2% WSP**
Total protein (g/dl)	2.07±0.29[a]	2.07±0.13[a]	1.53±0.09[a]
Creatinine (mg/dl)	1.77±0.14[a]	1.57±0.12[a]	1.60±0.15[b]
Urea (mg/dl)	46.00±0.23[a]	48.70±0.15[b]	49.03±0.14[b]
Potassium (mmol/l)	2.23 ± 0.22 [a]	3.73 ± 00.14 [b]	3.93 ± 0.37 [b]
Chloride (meq/l)	61.33±1.01[a]	57.93±3.62[a]	56.47±2.13[a]
Sodium (meq/l)	117.03 ± 6.43 [a]	102.40 ± 23.36 [a]	139.07 ± 24.77 [a]

Data are expressed as mean ± SEM (n = 3).
Mean ± SEM followed by different letter within a row are significantly different (P <0.05).

Results for the biomarkers monitored showed no significant difference (P > 0.05) across all treatments. Values of AST, ALT and ALP obtained in broiler chicks fed WSP containing diet at different levels and the experimental group fed the commercial di*et al*one (0%) were similar, which still fell within the normal range recorded in chickens.

Table 5: Hepatic and Renal function biomarker of broiler chickens fed graded level of *C. lanatus* seed powder for 6 weeks starter period.

		Treatments	
Parameters	**0% WSP**	**1% WSP**	**2% WSP**
AST (µL)	13.90 ± 0.67 [a]	15.07 ± 0.77 [a]	14.37 ± 1.22 [a]
ALT (µL)	17.77 ± 0.33 [ab]	20.03 ± 0.47 [a]	17.10 ± 1.13[b]
ALP (µL)	28.50 ± 0.45 [a]	27.87 ± 1.67 [a]	29.87 ± 2.33 [a]
Bil-total (mg/dl)	1.90 ± 0.23 [a]	2.00 ± 0.29 [a]	2.23 ±0.78 [a]
Bil-direct (mg/dl)	1.13 ± 0.03 [a]	1.57 ± 0.14 [a]	3.63 ± 2.04 [a]

Data are expressed as mean ± SEM (n = 3).
Mean ± SEM followed by different letter within a row are significantly different (P <0.05).
AST, Aspertate aminotransferase; ALT, Alanin aminotransferase; ALP, Alkline phosphate.

The activity of superoxide dismutase of the control group and 1% WSP group were statistically similar. However, when the concentration of the additive increased to 2%, the activity of SOD also increased. This led to statistical difference in SOD values between 2% WSP and that in the other two experimental groups. Lipid peroxidase and catalase as antioxidant enzymes were insignificantly different (P > 0.05) among the treatment groups and the control group.

Table 6: Serum antioxidant enzyme activity of broiler chickens fed graded level of WSP for 6 weeks starter period

		Treatments	
Parameters	**0% WSP**	**1% WSP**	**2% WSP**
SOD (µ/l)	1.93±0.37[a]	1.57±0.26[a]	3.26±1.12[b]
CAT (µ/l)	4.49±0.23[a]	4.58±0.14[a]	4.21±0.75[a]
LPO (nmol\ml)	2.39±0.10[a]	1.97±0.99[a]	2.13±0.31[a]

Data are expressed as mean ± SEM (n = 3).
Mean ± SEM followed by different letter within a row are significantly different (P<0.05).
SOD, Superoxide dismutase; CAT, Catalase; LPO, Lipid peroxidation assay.

4. Discussion

Results for proximate composition of watermelon seed is presented on Table 1. Proteins are important component of diet required for survival of animals as well as humans, which supplies adequate amounts of essential amino acids [27]. Protein content of WSP in the present study was in line with that described by Achu [28].
Crude fibre value of WSP was found to be higher than soya bean seed of 5.44% as reported by Etiosa [29]. Fibre has important physiological effects in the gastrointestinal tract. It expands inside the walls of the colon, making passage of waste products easy, thus relieving constipation.
Ash percentage of samples indicates the total inorganic content of that sample from where the mineral content could be obtained. The results for ash content showed significant (p > 0.05) difference within the three experimental diets. It was observed in the present study that as the percentage of WSP in the feed increased, the ash content also increased. Samples with high percentages of fibre and ash contents are expected to assist peristaltic movement as well as enhance metabolic processes necessary for improvement of growth and development [30, 31].
The results obtained for proximate composition of WSP in this present study varies from results for proximate analysis of *Citrullus lanatus* obtained by Oyeleke [22]. The possible reason for the variation in the results could be attributed to varietal and regional differences. The addition of watermelon seed powder to the commercial feed increased crude fiber, and crude protein. Therefore, birds consuming feed with watermelon seed powder at the different percentages received

more fiber and protein than the control group fed only the commercial feed. The survival of chickens from all treatment is an indication that *C. lanatus* may be free from any toxic substances. No vaccines or antibiotic growth promoters were used in this present study.

There was no significant difference observed in both initial and final average weight of broiler chickens fed different percentages of *Citrullus lanatus* seed powder. Although the group fed the highest percentage of *C. lanatus* recorded a higher average weight gain compared to the control group and 1% WSP group. This could be attributed to the fact that herbal plants establishes ideal microbial population in animal's digestive tract, which in turn improves secretion of digestive enzymes, that aid digestion and absorption of nutrients in diets, leading to improved growth of chicks [32]. However, this result is not in agreement with the findings of Guo [34], Jamroz and Kamel[15] who reported that herbs and herbal products had significant positive effect on broiler body weight gain.

Generally, it has been shown that inclusion of phytogenic compounds in broiler chicken diets improves feed conversion ratio [34-36]. Although other findings contradicts this statement, a study on photobiotic suggests that inclusion of 0.2% peppermint or thyme (w/w) characterized by menthol and thymol (70 mg/kg diet) in broiler diets did not affect feed intake and feed conversion ratio [38]. This was also similar with the observations in the present study. Nonetheless, feed intake of 2% WSP was higher followed by 0% WSP and the least being 1% WSP Blood biochemical analysis is widely used to assist the diagnosis and characterization of diseases in most animal species. Moreover, it is an important tool, as some metabolic disorders are difficult to detect only by clinical signs [47]. It can also help in monitoring the health of poultry animals, the diagnosis and treatment of diseases, and to assess their health status [48].

Creatinine concentration of 1% WSP is comparable to that of 0% WSP, both of which were statistically different from 2% WSP. This suggests the safety of *C. lanatus* on renal function. Increase in serum creatinine could be an indication of poor renal function [49]. Several physiological and pathological factors have been investigated to describe change in the concentrations of blood proteins, reflecting the actual general health state and condition of the evaluated animal, including bird species [51]. The serum total protein levels of the treatment groups were unaffected by the addition of *C. Lanatus* to diets in this study. This result is consistent with that of Erdog [52] who also reported that serum total protein level was unaffected by the addition of symbiotic and phytobiotics to poultry diets.

The results for serum urea showed a significant difference between the control group and the groups receiving *C. lanatus* additive. This is due to the increase in protein content of the commercial feed brought about by the addition of *C. lanatus* seed powder. Abdourhamane [53] revealed that cake from cucurbits seeds are a good source of proteins and could be used in poultry feed. Iyayi and Tewe [54] showed that serum urea and total protein depend mainly on the quality and quantity of protein in the diet.

Alanine Amino Transferase (ALT) and Aspartate amino transferase (AST) are liver enzymes; therefore, higher serum hepatic enzymes (AST and ALT) could be indicative of improper liver function due to inefficient performance [55]. Results from this study showed that the Amounts of ALT, AST and ALP in serum of supplemented birds with different levels of *C. lanatus* were not significantly different compared to control group. This result is in line with that obtained by Tollba [56] who reported that adding aromatic herbal extract to broiler diet did not alter the ALT and AST when compared to control. Similarly, Moomivand [57] reported that Amounts of ALT, AST and ALP in birds supplemented with different levels of drinking thyme essence were not significantly different from the control group. Also, Khursheed [58] reported that supplementation of mint leaves in broiler diet did not significantly affect ALT and AST levels. Similar values of AST and ALT that were not significantly varied ($p>0.05$) in this study for the control (0% WSP) relative to treatments are indicative of normal liver and kidney functions [59] which did not worsen by increasing the percentage of WSP.

Domestic birds like broilers are believed to be particularly susceptible to oxidative stress due to genetic selection toward larger breast muscles, increased total weight, and faster growth rates [60]. Oxidative stress is defined as an imbalance between the production of free radicals and the ability of the organism to detoxify them or counteract their harmful effects through neutralization by antioxidants and is responsible for the cause of several diseases [61, 62].

Activity of superoxide dismutase of the control group and 1% WSP group were statistically the same. However, when the concentration of the additive increased to 2%, the activity of SOD simultaneously increased. This led to a statistical difference between 2% WSP and the other two experimental groups. Increase in the activity of SOD in the current study may be considered as a protective mechanism against oxidative stress [63, 64]. The addition of phytogenic additives may help to reduce fear responses in broilers when exposed to stressful situations [65]. Conversely, lipid peroxidation profile decreased numerically in treatments with WSP when compared to the control (Table 6). Lipid peroxidation is the process in which free radicals steals electrons from the lipid in cell membranes, resulting in cell damage. It was observed in the present study that although there was a decrease in the activity of lipid peroxidase, it was not statistically significant. Tülüce reported that dietary inclusion of black cumin seeds at levels of 0.5% and 1% decreased the production of lipid peroxidases in chickens [66].

4.1 Conclusion

Based on the results of this study, it can be concluded that using *Citrullus lanatus* in diet for broiler chickens up to 2% did not enhance growth performance and feed efficiency, but statistically increased the activity of superoxide dismutase. *C. lanatus* supplementation improves the immune status of broilers, thereby making them more resistant to infection without interfering with the overall health and performance of the chickens.

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