Online Journal of Animal and Feed Research

Volume 2, Issue 1: 17-22 (2012)

ISSN 2228-7701

ANTI-NUTRIENT FACTORS, PERFORMANCE AND SERUM BIOCHEMISTRY OF BROILER CHICKS FED RAW AND FERMENTED *ALCHORNEA CORDIFOLIA* SEEDS

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ORIGINAL ARTICLE

ABSTRACT: *This study was carried out to determine some anti-nutrient factors in differently processed Christmas bush (Alchornea cordifolia) seeds and the effect of the processed seed meals on the performance and blood chemistry of broiler chicks fed from 1 to 35 day of age. Ground and fermented, and dehulled Christmas bush (CB) seed meals were analyzed for their anti-nutrient contents whereas ground and sieved (GS), ground-sieved and fermented (GSF) and non-sieved and fermented (NSF) seed meals were incorporated into starter broiler diets to replace 10% of maize, respectively. Fermented and dehulled CB seed meals contain 574.4 and 21.3mg/100g phytic acid, respectively. Cyanide was not identified in any of the meals. Dehulling eliminated the anthraquinone and tannin contents whereas fermentation only eliminated the tannin content. None of the methods completely eliminated the saponin, cardiac glycoside, flavonoid and alkaloid contents of the seed meals. With GS seed meal, broilers had lower average daily weight gain (P<0.05) than the control group. Feed intake decreased (P<0.05) but feed conversion ratio was not different when compared with control. Inclusion of GSF seed meal improved growth and feed intake when compared with the NSF seed meal and by day 35, growth and feed intake were comparable to those of the control birds. Blood plasma levels of alanine aminotransferase, alkaline phosphates and aspartate aminotransferase increased with GS CB seed meal diet, while serum calcium decreased. Neither raw nor fermented seed meals altered other measures of the blood chemistry. It is concluded that CB seeds contain toxic anti-nutrient compounds and that sieving out the hulls in the ground raw seed meal before fermentation improved the feeding value of the seeds for broilers at 10% replacement for maize.*

Key words: *Alchornea seed; Anti-nutrients; Broilers; Fermentation; Performance; Serum chemistry*

INTRODUCTION

Christmas bush (*Alchornea cordifolia*) is a tropical browse plant that forms a good proportion of the ever green vegetation of the humid tropical zone of southern, Nigeria. It is locally known as 'Ububo' by the Igbo speaking tribe of south- eastern, Nigeria (Udedibie and Opara, 1998; Okoli et al., 2002). The plant produces prolific quantities of foliage and seeds in many countries including Nigeria (Iwu, 1993), Equatorial Guinea (Sebater, 1977), Republic of Guinea (Sugiyama and Koman, 1992), and Japan (Huffman, 2002) among others. The foliage including the seeds is frequently consumed in the field by Gorillas and Chimpanzee (Sebater 1977; Sugiyama and Koman, 1992) and is commonly used in zero-grazing by local villagers for the feeding of small ruminants (Udedibie and Opara, 1998; Okoli et al., 2002). It is also used by local inhabitants for the cure of many diseases of man (Lamikara et al., 1990; Iwu, 1993). However, the seeds are little known and used in the diets of humans and other monogastric animals such as chickens and pigs because there is little or no information on their production, chemical compositions, processing, marketing or use.

Published data indicates that the raw seeds contain 13% crude protein and 14Mj/KgDM gross energy and are toxic when fed to broilers, causing reductions in growth, feed intake and increased mortality (Emenalom et al., 2009). The authors also reported that the raw seeds with hulls contain 973.8mg/100g phytic acid and also show the presence of saponins, anthraquinone, cardiac glycosides, steroids, flavonoids, tannins and alkaloids. One report indicated that toxic and anti- nutritional factors in seeds are concentrated in some part of the seeds and may be reduced by pretreatments such as dehulling (Rao and Deosthale, 1982) or fermentation (Ibrahim et al., *2002*). . The

To cite this paper: Emenalom O.O, Obiora A.B, Okehie U.N. 2012. Anti-nutrient factors, performance and serum biochemistry of broiler chicks fed raw and fermented *Alchornea Cordifolia* seeds. *Online J. Anim. Feed Res.*, 2(1): 17-22.

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advantages of adding such processed non conventional feedstuffs to poultry diets have been demonstrated using jack bean (Udedibie et al., 1998), pigeon pea (Efule and Obioha, 1999), velvet bean (Emenalom et al., 2006) and Christmas bush (Emenalom et al*.,* 2011) among others. Meanwhile Christmas bush seeds have not been subjected to any form of pretreatment to determine its anti-nutritional factors or feeding value.

This study was therefore designed to determine the presence or otherwise of some anti-nutritional factors in fermented and dehulled CB seed meals and also the performance and blood chemistry of broiler chicks fed 10% ground and sieved, ground-sieved and fermented, and non- sieved and fermented seed meals as replacement for maize.

MATERIALS AND METHODS

Source of seeds

Christmas bush seeds used for this experiment were harvested from the wild in bushes around the Teaching and Research Farm of the Federal University of Technology, Owerri, Nigeria where the experiment was carried out. Owerri is located in the rainforest zone of Nigeria (5^{o} 29' N and 7^{o} 02' E) and an elevation of 90.91m. The environment has an annual rainfall of 2641mm, temperatures of $27.4^{o}C$ and relative humidity of 86.6%. The seeds were harvested alongside with the stalk.

Seed processing

The seeds with stalks were sun dried for 4 -6 days (depending on the intensity of the sun) on concrete slabs. The dried seeds with hulls were then detached from the stalks and divided into two batches. The first batch was dehulled and ground into meal while the second batch was ground with the hulls using a hammer mill and further subdivided into three parts. The first part was sieved using a 2mm sieve to remove coarse hulls. The second part was sieved, soaked in water for 48 hours at room temperature, drained into a jute bag, pressed and allowed to stand for 12 hours under pressure. The third part was soaked in water without previous sieving and treated as the second part above. The second and third parts were both sun dried on concrete slabs until they were crispy at touch.

Anti-nutrient analysis

Samples of the fermented seed meal with hulls and dehulled seed meal were screened for the presence of cyanide, phytic acid, saponnin, anthraquinone, cardiac glycoside, steroids, flavonoids, tannins and alkaloids at the Department of Biochemistry and Applied Molecular biology, National Veterinary Institute VOM, Jos, Nigeria.

Experimental diets

Four experimental broiler starter diets were formulated such that the control diet contains no CB seed meal. Diets 2, 3 and 4 contain 5.2% (10% replacement of maize) of raw ground and sieved (RGS), sieved and fermented (SF) and non sieved and fermented (NSF) CB seed meals, respectively. The ingredient composition of the diets is shown in table 1.

Experimental birds and design

One hundred and twenty (120) day old Anak broilers procured from Amazing Grace Farm, Owerri, Nigeria were used for the experiment. The birds were divided into four groups of 30 birds each. Each group was further divided into three replicates of ten birds each. The birds were then randomly assigned to the four experimental diets in a completely randomly design and housed in a 2 x 1m pens covered with wood shavings as the litter material. The pens were cover with black polythene for the first two weeks to control heat and wind and provided with lantern and stoves as sources of light and heat, respectively. Feed and water were given *ad libitum*. The experimental lasted for 35 days.

Data collection

The birds were weighed at the beginning of the experiment and weekly thereafter. Feed intakes were taken daily. At 35 day of age, three birds per treatment were randomly selected, and blood samples were collected from their brachial wing vein with syringes and needles between 8:00 am and 9:00 am. Some blood biochemical parameters including AST (aspartate amino transferase), ALP (alkaline phosphatase) ALT (alanine amino transferase), total protein, calcium, phosphorus and uric acid were evaluated. The Federal Medical Center, Owerri, performed the blood analyses.

Data analysis

The data generated in the experiment were subjected to one way analysis of variance (ANOVA). The standard error of means (Steel and Torrie, 1980) was used in the separation of means

RESULTS

Fish growth performance

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The effect of lemon balm (*Melissa officinalis*) and Aloe (*Aloe vera*) supplementation on body weight, feed conversation ratio (FCR), specific growth rate (SGR) and hepatosomatic index (HSI) are presented in Table 2. No differences (P>0.05) were observed among treatments in growth parameters.

Fermented CB seed meals with hulls, and dehulled seed meal contain 574.41 and 21.29mg /100g phytic acids, respectively and show the presence of tannins, saponins, cardiac glycosides, steroids and flavonoids (Table 2). Cyanide was not identified in any of the meals. Dehulling eliminated the anthraquinone and tannin contents whereas fermentation only eliminated the tannin content. None of the methods completely eliminated the saponin, cardiac glycoside, flavonoid and alkaloid contents of the seed meals.

Table 1 - Ingredient Composition of experimental diets (0-5 weeks)

Ingredients (%)	Control 0%	RGS 10%	GSF 10%	NSF 10%
Maize	52.00	46.80	46.80	46.80
Soybean meal	30.00	30.00	30.00	30.00
Alchornea seed meal	0.00	5.20	5.20	5.20
Fish meal	5.00	5.00	5.00	5.00
Brewers dried gain	5.00	5.00	5.00	5.00
Palm kernel cake	3.00	3.00	3.00	3.00
Bone meal	3.00	3.00	3.00	3.00
Wheat offal	2.00	2.00	2.00	2.00
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Vitamin premix[1]	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Total *Calculated analysi*	**100.00**	**100.00**	**100.00**	**100.00**
Phosphorus	0.92	0.93	0.93	0.94
Calcium	1.31	1.31	1.31	1.31
Ether extract	3.75	3.79	3.60	3.64
Crude fibre	4.57	4.74	4.60	4.64
Crude protein	22.83	22.87	22.80	22.78
ME (Keal/kgDM)	2853.33	2851.87	2838.09	2830.90

[1]*Guarantied chemical analysis per kg of feed: Vit.D_3. 400g, Vit.E.8g,* Vit. K. 30.4g, Vit.B_1.0.32g, Vit.B_2. 0.56g, Vit. B_6. 4g, Calpathonerate 1.6g, Folic acid 0.16g, Biotin 8mg, Chlorine 18g, Zinc 7.2g, Copper 0.32g, BHT 32g, Iodine 0.25g, Cobalt 36mg, Selenium 16mg, Oxytetracycline 100mg and Vit.C.25g. *RGS: Raw ground and sieved seed meal. GSF: Ground sieved and fermented seed meal. NSF: Non- sieve and fermented seed meal*

Table 2 - Effect of fermentation and dehulling on the anti- nutrient contents of *Alchornea* seeds

Components	Ground raw (GR)	Ground and fermented (GF)	Dehulled (D)
Phytic acid (mg/100g)	973.80	574.41	21.29
Cyanide	-ve	-ve	-ve
Saponins	+ve	+ve	+ve
Anthraquinone	+ve	-ve	-ve
Cardiac glycoside	+ve	+ve	+ve
Steroids	+ve	+ve	+ve
Flavonoid	+ve	+ve	+ve
Tannin	+ve	+ve	-ve
Alkaloid	+ve	+ve	+ve
Where			

-ve = negative; +ve = positive

Birds fed SF Christmas bush seed meal diet had a higher (P<0.05) feed intake than those fed RGS and NSF diets (Table 3) whereas those fed RGS Christmas bush seed diet had lower (P<0.05) feed intake than the other birds. Body weight gain of birds fed the control diet was highest but compared statistically (p>0.05) with those of birds fed SF Christmas bush seed meal diet, but the value however, differed (P<0.05) from those of birds fed RGS and NSF seed meal diets. Birds fed RGS seed meal diet recorded the lowest weight gain and gained 75.2 and 73.6% of the control and SF seed meal diets, respectively. There were no significant differences in feed conversion ratio among birds fed the control and fermented Christmas bush seed meal diets. Mortality value was relatively high in RGS seed meal diet group when compared with other dietary groups.

Of the blood analyses done, increased plasma levels of AST, ALP and serum calcium were observed in birds fed CB seed meal diets (Table 4). ALT values were lower (p<0.05) in birds fed fermented seed meal diets than those fed the control and RGS seed meal diets.

Birds fed RGS seed meal diets had a statistically lower (P<0.05) AST and ALP than the control. Values of total protein, phosphorus and uric acid were similar among the dietary groups.

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Table 3 - Performance of starter broilers fed differently processed *Alchornea* seed meal (0- 5 weeks)					
Parameter	**Control 0%**	**RGS 10%**	**GSF 10%**	**NSF 10%**	**SEM**
Initial body weight (g)	33.3	36.0	36.0	36.0	-
Final body weight (g)	726.7^{a}	536.7^{c}	716.7^{a}	643.3^{b}	23.85
Body weight gain (g)	693.7^{a}	500.7^{c}	680.7^{ab}	607.3^{b}	27.30
Feed intake (g)	1519.8^{ab}	999.5^{c}	1618.7^{a}	1317.0^{b}	38.64
Feed conversion ratio	2.2^{ab}	2.0^{b}	2.4^{a}	2.3^{ab}	0.08
Mortality rate[1]	23 (5)	39 (7)	22 (4)	22 (4)	-

^{a,b,c}: means within a row with different letter superscript differ significantly ($P<0.05$). RGS, GSF and NSF are as in table1; SEM: Standard error of means. [1]Number of birds is presented in parenthesis.

Table 4 - Serum chemistry of broiler fed *Alchornea* seed diet (0 – 5 weeks)					
Parameters	**Control 0%**	**RGS 10%**	**GSF 10%**	**NSF 10%**	**SEM**
Alanine aminotransferase (ALT)(iu/l)	24.0^{a}	26.0^{a}	16.5^{b}	16.5^{b}	2.14
Aspartate aminotransferase (AST)(iu/l)	34.0^{b}	54.0^{a}	53.5^{a}	42.5^{b}	3.33
Alkaline phosphatase (ALP) (iu/l)	302.0^{b}	624.5^{a}	317.5^{b}	500.5^{ab}	97.50
Serum total protein (g/dl)	4.70	4.00	3.00	3.45	0.67
Serum calcium (ca^{++}) (mg/dl)	7.90^{c}	8.90^{bc}	11.60^{a}	9.40^{b}	0.48
Phosphorous (mg/dl)	3.65	3.10	3.05	3.25	0.21
Uric acid (gm/dl)	1.80	2.05	1.70	1.50	0.25

^{a,b,c}: means within a row with different letter superscript differ significantly ($P<0.05$). RGS, GSF and NSF are as in table1; SEM: Standard error of means

DISCUSSION

The result of the anti- nutrient screening showed that dehulling reduced the phytic acid level eliminated the tannin content in CB seeds (Table 2). Emenalom et al. (2009) reported that raw CB seeds with hulls contain 973.8mg/100g phytic acid and showed the presence of anthraquinone and tannin among others. It therefore follows from the present result that more than 97% of the whole seed phytic acid is located in the hulls hence the very low level of phytic acid and absence of tannin recorded in the dehulled seed meal. Similar reduction in tannin has been reported in dhal pulses (Rao and Deosthale, 1982) thus indicating that under this treatment condition, tannin and phytic acid in CB seed can be reduced or eliminated. The reduction in the phytic acid level by fermentation agrees with similar report in literature (Ibrahim et al., 2002) but dehulling was more efficient than fermentation as indicated in the present result.

Little systematic research has been done on the nutritional quality of CB seed for any monogastric species. Raw CB seed with hulls are deleterious to starter broiler at 5% dietary inclusion level (Emenalom et al., 2009). Fermenting the seed meal with hulls only partially improved the nutritional quality of the seeds for finisher broiler at 10% dietary inclusion level (Emenalom et al., 2011). However, the method of processing that will produce optimal result has not been established.

In the present study, it was found that inclusion of RGS CB seed meal in diets for starter broilers had major effects on body weight gain and serum chemistry. Body weight and feed intake were substantially depressed when RGS seed meal replaced 10% of maize in the ration consumed by starter broiler chicks fed to five weeks of age. The growth depressing factors present in the RGS seed meal are unknown, but part of the adverse negative effect may be explained by lack of knowledge about availability, especially amino acids in the seeds, although the diets were nutritionally balanced based on the data available. On the other hand, a large part of the negative effect of the raw seeds on growth is undoubtedly explained by one or more of the toxic factors present in the seed (Table 2). Moreover, the presence of possibly, other unidentified anti-nutrient factors in the seeds may have also contributed to the poor performance of the birds.

Differences were observed however, between raw and fermented CB seeds on growth and feed intake. Consumption of SF seed meal diet gave a much better body weight gain and feed intake than NSF seed meal diet, thus indicating the negative effects of RGS and NSF seed meals in the inhibition of metabolic processes leading to growth, apart from an equally depressed effect on appetite. Thus the marked growth depression from feeding the RGS and NSF seed meal diets cannot be entirely explained by decreased feed intake considering the fact that chicks on NSF seed meal diet had a statistically similar feed intake with the control. This suggests that the effects of CB seed on metabolic processes should be examined further.

Feed conversion was not markedly affected by both raw and fermented CB seed meal diets. Contrary to the improved feed conversion ratio observed in the present study, Emenalom et al. (2011) reported depressed feed conversion ratio for starter and finisher broilers fed raw and fermented seed meals with hulls, respectively. This show that sieving out some of the coarse hulls from the raw seed meal or sieving before fermentation, both encouraged better feed conversion ratio than non-sieved and fermented seed meal. The 39% mortality recorded in birds fed RGS seed meal diet may not be blamed totally on toxic or anti-nutritional factors since the control diet group had similar mortalities.

Dietary raw ground and sieved CB seed meal diets caused several changes in blood chemistry. Plasma ALT decreased significantly with fermented CB seed meal diets while ALP and AST increased. The enzyme ALT is a cytoplasmic enzyme whose increase in blood plasma frequently signals either liver or muscle damage (Lumeji, 1997). Therefore a factor in raw CB seeds apparently causes either hepatic or muscular damage. On the other hand, alkaline phosphates (ALP), another indicator of liver damage (Kramer and Hoffman, 1997) also increased in broiler fed RGS seed meal diet. This observation coupled with the increase in ALT and AST may argue more strongly for the occurrence of muscle or liver damage in chicks fed raw, but not fermented CB seed meal diets. All these are metabolically related to the detoxification processes carried out by the liver and they strongly indicate the presence of toxic compounds in the blood of the chicks whose livers could not properly eliminate them.

The total protein, phosphorous and uric acid contents were not markedly affected by both raw and fermented CB seed meal diets but varied among the treatments. The serum calcium level increased significantly in chicks fed SF seed meal diet. The reason(s) for the varied level of serum calcium in the diets is not known but could be attributed to varied metabolic activities in the chicks fed the different diets. However, there appears a reciprocal relationship between serum calcium and phosphorous in chicks fed the different diets. Increase in inorganic phosphorous are known to be associated with a decrease in serum calcium which is in line with the findings of this research.

Generally, birds fed sieved and fermented CB meal diet grew as well as the control. This suggest that sieving off the coarse hulls or dehulling before fermentation reduced some feed inhibiting and growth depressing factor(s) found in the non-sieved and fermented seed meal and allowed starter broiler chicks to tolerate the 10% replacement of maize in the diet. Thus it appears that when properly sieved and fermented CB seed could be possibly used in commercial broiler rations. It was also evident by comparing the feed intake and growth of broilers fed non-sieved and fermented CB seed meal diet that the sieved and fermented seed meal diet has less drastic effect on growth. Other measures such as feed intake and feed conversion ratio were also better in broilers fed sieved and fermented seeds compared to non-sieved and fermented. Since fermented CB seeds have been studied very little in any species including chickens, it is not known what factor(s) depressed growth or which were destroyed by fermentation. However, we analyzed CB seeds for some phytochemical compounds (Table 2). Dehulling and fermentation of undehulled seed meals only reduced the phytic acid levels and eliminated anthraquinone content of the raw seed with hulls, while dehulling alone eliminated the tannin content. Therefore, the improvement in broiler performance can be partially explained by a reduction in the anti-nutritional factors, although the exert quantities removed were not determined.

CONCLUSION

Christmas bush seed meal contains some anti-nutrient compounds that were not completely destroyed by dehulling or fermentation. The partial replacement of maize by 10% raw ground and sieved CB seed meal produced a highly significant detrimental effect on starter broiler chicks' performance as was shown by extreme low rate of growth and feed intake, high ALT, AST and ALP values. Of all the treatments applied to CB seeds, sieving before fermentation was the best and superior to other processing methods in term of broiler chicks' performance and blood chemistry. The effect of higher dietary inclusion levels of the sieved and fermented CB seed meal in broiler diets should be investigated while exploring other processing methods.

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