

# Condensed Tannin in Two Varieties of Sorghum (*Sorghum bicolor*): Effect on the Growth Performance and Nutrient Digestibility of Broiler Chickens

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## Abstract

Two hundred (200) 1-d old (Hubbard strain) broiler chicks were used to evaluate the effect of condensed tannin in different sorghum (*Sorghum bicolor*) varieties on the growth performance and nutrient digestibility of broiler chickens. The birds were randomly assigned to five (5) experimental diets of four (4) replicates each at ten (10) birds per replicate in a completely randomized design in a forty-two day feeding trial. Diet D1 contained 100% maize; D2 and D3 contained 50% and 100% red sorghum variety (RSV) while D4 and D5 contained 50% and 100% white sorghum variety (WSV) respectively as replacement for maize. The RSV had higher tannin content than WSV. Birds fed WSV based diets had higher ( $P \leq 0.05$ ) weight gain with better feed conversion ratio than those fed RSV based diets. The sorghum based diets had a more digestible ether extract and nitrogen free extract ( $P \leq 0.05$ ) than those fed D1. Conclusively, WSV could completely replace maize because of the lower (condensed) tannin levels while above 50% RSV replacement for maize may be deleterious.

**Keywords:** broiler, chicken, digestibility, growth.

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## 1. Introduction

The significance of sorghum grain as food for human and feedstuff for livestock in many parts of the world especially semi-arid region is well documented. Sorghum has been considered as the next alternative to maize in terms of nutritive value, availability and cost in poultry feed [1]. However, presence of anti-nutritional factors such as tannins and phytate [2] has been reported to influence feed intake and performance of different poultry species [3]. Sorghum tannins are of two types namely: hydrolysable and non-hydrolysable (condensed) tannins [4]. Hydrolysable tannins are characterized by a central carbohydrate core with

a number of phenolic carboxylic acids bound by ester linkages. Condensed tannins have no carbohydrate core, but rather they are derived from the condensation of flavonoid precursors without enzymes participation. Condensed tannins are more widely distributed in higher plant species than the hydrolysable variety and are thought to be more active in precipitating proteins [4].

The condensed groups of tannins may form stable complexes with protein, metal ions and other macro-molecules including polysaccharides [5, 6]. Stable complexes of tannins with proteins may lead to coagulation or precipitation of protein molecules [7]. However, nature of tannins and that of proteins plays an important role that can determine the strength and degree of interaction between tannins and proteins [8], although, [9] later reported that sorghum tannins may bind and precipitate at least 12 times their own weight of protein. Tannins-carbohydrate complex has also

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been observed particularly with starch molecules but with less affinity than for proteins. The quantity of sorghum used has also shown a linear effect on digestibility of starch [10]. Tannins also interact with some minerals, form precipitates and thus reduce their availability [11]. Tannins-nutrient interaction may be one of the means by which tannins affect the digestive processes resulting into reduced availability of the nutrients in the gut [7].

Tannins have also been reported to form soluble, insoluble and sometimes irreversible complexes with digestive enzymes in the digestive tract of pigs and poultry [9]. Formation of these complexes increases with molecular size of the tannins and inhibit enzymatic breakdown of protein and can increase endogenous amino acid loss. Tannins can increase the size of the parotid glands and damage the mucosal lining of the gastro intestinal tract of chickens [12].

Therefore, the aim of this feeding trial is to determine the effect of condensed tannin in sorghum on the growth performance and nutrient digestibility of broiler chickens.

## 2. Materials and methods

### *Experimental site*

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm, Ladoké Akintola University of Technology, Ogbomoso. Ogbomoso lies on longitude 4° 15' East of the Greenwich Meridian and Latitude 8° 07' North of the equator. The latitude is between 300 and 600 meters above sea level. The mean annual temperature is about 27°C while that of average rainfall is 1247mm. The vegetation of the study area is in the derived savannah zone. (This experiment was approved by the appropriate ethics committee" (Ref./LAU/FAS/IACUC/APH-AAA030069: 4<sup>th</sup> January., 2017), based on the "principles of laboratory animal care (nih publication no. 85-23. Revised 1985").

Two sorghum varieties: red and white were used for this study. The two sorghum were purchased from a reputable commercial feed mill.

### *Source of test ingredients and preparation of experimental diets*

Diet 1: Control diet contained maize as the main source of energy.

Diet 2: 50% replacement of maize with red sorghum.

Diet 3: 100% replacement of maize with red sorghum.

Diet 4: 50% replacement of maize with white sorghum.

Diet 5: 100% replacement of maize with white sorghum.

The composition of the starter (1-22d) and finisher (22-42d) diets are presented in the Tables 1 and 2 respectively.

### *Experimental birds, design and management*

Two hundred (200) 1-d old (Hubbard strain) broiler chicks were used for this study. The birds were randomly assigned to the five (5) experimental diets of four (4) replicates each and at ten (10) birds per replicate in a completely randomized design. The experimental diets and water were supplied *ad libitum* to the birds throughout the experimental period of 42 days. Standard management practices and routine vaccination were strictly observed.

### *Data collection*

#### *Growth characteristics*

Growth performance parameters measured were:

Feed intake: This was obtained by subtracting the weight of feed left at the end of the day from feed offered to the birds.

Body weight gain: Birds were weighed on weekly basis and the weight gain was calculated by subtracting the weight in the preceding week from that of the current week.

Feed conversion ratio: Calculated from the weight gain and feed intake thus,

$$\text{Feed conversion ratio} = \frac{\text{Average feed intake (g)}}{\text{Body weight gain (g)}}$$

### *Nutrient utilization*

Prior to the end of sixth week of the experiment, two birds were randomly selected per replicate, weighed, tagged and transferred to metabolic cages to determine the nutrient retention. The birds were acclimatized for three days. On the fourth day, the feed intake of each bird was measured and faecal samples collected in aluminium foil sheet for three consecutive days. Faecal sample were first air dried during the period of collection and later oven dried at 60°C to a constant moisture level and afterwards grounded

by using mortar and pestle to obtain a homogenous mixture for proximate analysis.

**Table 1.** Gross composition of the experimental diets at the starter phase (1-21d)

Parameters (%)	D1	D2	D3	D4	D5
Maize	52.40	26.20	-	26.20	-
Sorghum	-	26.20	52.40	26.20	52.40
#Fixed Ingredients	47.60	47.60	47.60	47.60	47.60
Total (kg)	100.00	100.00	100.00	100.00	100.00
Calculated nutrient composition					
Crude protein(%)	23.48	23.87	24.26	23.87	24.26
Crude fibre (%)	3.14	3.16	3.17	3.17	3.72
ME (Kcal/kg)	2928.92	2867.61	2806.30	2867.61	2806.30
Determined proximate composition (%)					
Dry Matter	92.62	91.96	92.48	92.32	91.94
Crude protein	23.31	23.86	24.23	23.84	24.26
Crude fibre	6.02	5.84	5.68	5.74	5.72
Ether extract	6.25	7.58	7.50	7.92	7.56
Ash	2.50	2.25	2.42	3.00	2.82
NFE	54.54	52.43	52.65	51.82	51.58

ME=Metabolizable Energy, NFE=Nitrogen free extract

#Fixed ingredients=30.50% Soybean meal, 6.00% Wheat offal, 6.00% Fish meal,2.00% Bone meal, 2.00% Limestone, 0.25% Lysine, 0.25% Methionine, 0.35% \*Vitamin-mineral premix, 0.25% Salt.

**Table 2.** Gross composition of the experimental diets at the finisher phase (22-42 d)

Parameters (%)	D1	D2	D3	D4	D5
Maize	58.20	29.10	-	29.10	-
Sorghum	-	29.10	58.20	29.10	58.20
#Fixed Ingredients	41.80	41.80	41.80	41.80	41.80
Total (kg)	100.00	100.00	100.00	100.00	100.00
Calculated nutrient composition					
Crude protein (%)	20.22	20.65	21.12	20.65	20.22
Crude fibre (%)	3.17	3.18	3.20	3.20	3.84
ME (Kcal/kg)	2957.59	2889.49	2831.00	2889.49	2957.59
Determined proximate composition (%)					
Dry Matter	93.88	94.09	92.68	94.15	93.89
Crude protein	20.21	20.64	20.15	20.66	21.12
Crude fibre	6.82	6.96	6.49	6.86	6.71
Ether extract	4.5	7	6.95	6.88	6.26
Ash	2.5	2.43	2.61	3.25	3
NFE	59.85	56.76	56.48	56.5	56.8

ME=Metabolizable Energy, NFE=Nitrogen free extract

#Fixed ingredients (Finisher phase)=22.00% Soybean meal, 10.00% wheat offal, 5.00% Fish meal, 2.00% Bone meal, 2.00% Limestone, 0.15% Lysine, 0.15% Methionine, 0.25% \*Vitamin-mineral premix, 0.25% Salt.

\*Broiler vitamin and mineral premix contained (/kg): 11.7 g dicalcium phosphate; 4.5 g salt; 6500 IU vitamin A; 2000 IU vitamin D<sub>3</sub>, 25 mg vitamin E; 5 mg vitamin B2 B12; 0.01 mg vitamin; 30 mg niacin 30; 0.2 mg folic acid; 0.2 mg biotin; 500 mg choline; 3 mg dicalcium phosphate

D1 (control diet), D2 (50% replacement of maize with red sorghum), D3 (100% replacement of maize with red sorghum), D4 (50% replacement of maize with white sorghum), D5 (100% replacement of maize with white sorghum)

*Determination of tannin concentration*

*Hydrolysable tannin (HT)*

2 g of grounded sample was weighed into a 250ml beaker and 30ml of 80% aqueous acetone were

added 3 times consecutively with vigorous shaking or stirring with glass rod after each addition of 80%aq. acetone each of the three times to dissolve the sample in the acetone and extract

the phenolics efficiently [13]. 0.50ml of the above extract was diluted with distilled water to 2ml in a 10ml measuring flask; 1ml of Folin-Ciocalteu phenol reagent added and shaken vigorously. 5ml portions of 20% Na<sub>2</sub>CO<sub>3</sub> was pipetted into the mixture in the 10ml flask and made up to mark with distilled water, with vigorous shaking again. This was left to stand for 20min for colour development.

Standard Gallic acid of range 0 to 10ppm were prepared from stock Gallic acid of 100ppm and treated in a similar manner as sample above. Absorbance of sample as well as standard was read on a spectronic 21D spectrophotometer at a wavelength of 735nm. %Hydrolysable Tannin was calculated using the formula:

%HT=Absorbance of sample x gradient Factor x Dilution factor/Wt. of sample x 10,000.

#### Condensed tannin (CT)

0.1ml of the phenolic extract prepared as in HT determination was pipetted into a 30ml test tubes and covered with aluminium foil. 3ml of 4% vanillin (w/v) in methanol was added and the tube was shaken vigorously with the mixture. Immediately after that 1.5ml of concentrated HCl was pipetted into the tube and was shaken again. This mixture was allowed to stand for 20min for full colour development before reading of absorbance. Standard catechin solution of 0.1mg/ml to 1.0 mg/ml were prepared from stock 1mg/ml catechin in methanol and treated similarly as sample above. The absorbance of samples as well as standards was read on a spectronic 21D spectrophotometer at a wavelength of 500nm. The % Condensed tannin as catechin

equivalent was calculated using the formula:

$$\%CT = \frac{\text{absorbance of Sample} \times \text{Gradient factor} \times \text{Dilution factor} \times 10000}{\text{Weight of sample}}$$

#### Proximate Analysis

Homogenized samples of the white and red sorghum varieties, starter and finisher diets, and faecal samples were analysed for total ash, dry matter, crude protein, crude fibre and ether extract according to the method of AOAC [14] while the nitrogen free extract was estimated.

#### Statistical Analysis

All data collected were subjected to analysis of variance (ANOVA) in a completely randomized design (CRD) using version 8.1 of SAS [15] and where significant difference were observed, Duncan multiple range test of the same package was used to separate the means at 5% probability (P) level.

### 3. Results and discussion

Result: The result of the proximate composition of the two sorghum (*S. bicolor*) varieties (Table 3) showed that both had similar proximate composition except that the white sorghum variety had higher crude fibre and ash than the red variety. The red variety of sorghum had higher tannin content (condensed tannin, 0.035 g/100 g DM and hydrolysable tannin 0.094 g/100 g DM) than the white variety (condensed tannin 0.016 g/100 g DM and hydrolysable tannin 0.062 g/100 g DM).

**Table 3:** Proximate composition and tannin concentration of the two varieties of sorghum

NUTRIENTS (%)	Red	White
Crude protein	10.50	10.50
Crude fibre	2.05	2.11
Ether extract	5.25	5.25
Ash	1.85	3.42
Nitrogen free extract	74.63	73.04
Dry matter	94.28	94.32
Hydrolysable tannin (g/100g DM)	0.094	0.035
Condensed tannin (g/100g DM)	0.062	0.016

Table 4 shows the growth performance of broilers fed varying levels of different sorghum varieties at both starter and finisher phases. Significant differences (P<0.05) were observed for the average daily gain and feed conversion ratio.

At the finisher phase, birds on white sorghum based diet had significantly higher weight gain (P<0.05) than birds fed the control or red sorghum based diets especially at 50% inclusion (D2). Birds fed D4 (50% white sorghum) had the lowest

feed conversion ratio ( $P < 0.05$ ) when compared with other diets.

The result of nutrient digestibility of broilers fed sorghum based diets at the finisher phase is show on Table 5. Significant differences ( $P < 0.05$ ) were observed across the dietary treatments. Birds fed diets D1 (72.66%) and D4 (72.11%) had higher crude protein retention unlike those fed D2, D3 and D5 (70.12%, 68.92% and 71.31% respectively): those fed the control diet (D1) had the highest crude protein digestibility while those on D3 (68.92%) had the least. The crude fiber digestibility was not significantly different

( $P > 0.05$ ) in those fed the control (D1) and the sorghum based diets (D2-D5): those on D2 (41.66%) and D5 (41.93%) were significantly different ( $P < 0.05$ ) from those fed diets D3 (36.31%) and D4 (35.62%). The ether extract retention ranges between 62.84% in birds fed D4 and 63.99% in those fed D2 while those fed D1 had the least (59.50%). Birds fed the sorghum based diets had significantly higher NFE digestibility and those on the control diet. NFE digestibility ranges from 75.98–76.55% among the sorghum based diets while the control had the lowest value (72.53%).

**Table 4.** Growth performance of the broilers fed varying levels of different sorghum varieties

Parameters	D1	D2	D3	D4	D5	SEM
Starter phase (1-21 days)						
ADG (g/b)	21.35	22.25	20.56	21.18	20.96	0.27
ADFI (g/b)	37.90	38.02	36.90	37.17	36.58	0.22
FCR	1.78	1.72	1.80	1.76	1.75	0.02
Finisher phase (22-42 days)						
ADG (g/b)	27.70 <sup>b</sup>	28.34 <sup>b</sup>	29.56 <sup>b</sup>	33.59 <sup>a</sup>	30.33 <sup>b</sup>	0.50
ADFI (g/b)	76.52	77.73	82.02	79.67	78.89	0.75
FCR	2.77 <sup>a</sup>	2.75 <sup>a</sup>	2.78 <sup>a</sup>	2.38 <sup>b</sup>	2.60 <sup>ab</sup>	0.04

a-b Mean within the row lacking common superscript differ ( $P < 0.05$ )

SEM=Standard error of means, ADG=Average daily gain, ADFI=Average daily feed intake, FCR=Feed conversion ratio

D1 (control diet), D2 (50% replacement of maize with red sorghum), D3 (100% replacement of maize with red sorghum), D4 (50% replacement of maize with white sorghum), D5 (100% replacement of maize with white sorghum)

**Table 5.** Nutrients digestibility of broiler fed sorghum based diets

Nutrients (%)	D1	D2	D3	D4	D5	SEM
Crude protein	72.66 <sup>a</sup>	70.12 <sup>b</sup>	68.92 <sup>c</sup>	72.11 <sup>a</sup>	71.31 <sup>b</sup>	0.58
Crude fibre	38.77 <sup>ab</sup>	41.66 <sup>a</sup>	36.31 <sup>b</sup>	35.62 <sup>b</sup>	41.93 <sup>a</sup>	0.96
Ether extract	59.50 <sup>b</sup>	63.99 <sup>a</sup>	63.23 <sup>a</sup>	62.84 <sup>ab</sup>	63.33 <sup>a</sup>	0.56
NFE	72.53 <sup>b</sup>	76.06 <sup>a</sup>	75.98 <sup>a</sup>	76.14 <sup>a</sup>	76.55 <sup>a</sup>	0.59

a-c Mean within the row lacking common superscript differ ( $P < 0.05$ )

SEM=Standard error of means, NFE=Nitrogen free extract

Discussion: The crude protein level obtained in this study for both sorghum varieties (10.5%) is higher than 9.00% reported by NRC, (1994) [16] for maize, and 8.80 % and 9.50% reported by Olomu (1995) [17] for maize and sorghum respectively, but was lower than values reported by Nyannor et al. (2007) [18] (10.50 vs. 12.70%) for sorghum. The crude fibre values obtained in this study was lower than that reported by Nyannor et al. (2007) [18] for sorghum (2.20%). The major difference was recorded in the amount of ash content of the two varieties (3.42% White vs 1.85% Red). This shows that white variety was

46% richer in minerals than the red variety. These values were also higher than that of maize (1.40%), [16]. Consequently, white sorghum variety contains more minerals than both red variety and maize. The red sorghum variety is high in tannin (both hydrolysable and condensed) than the white variety. Medugu et al. (2010) [19] reported a tannin content of 1.54% for low tannin sorghum and 7.44% for high tannin sorghum. Douglas et al. (1990) [20] also observed the lowest tannin concentration in white sorghum variety (0.02%) and reported that amount of tannin in seven different sorghum varieties varied with the

colour of the testa of the grains. He further explained that white testa grains (white sorghum) showed the least amount of tannin while the tannin contents increased with increase in colour of the seed coat. This shows that tannin may be variety dependent.

There are diverse reports in the literature concerning tannin influence on feed intake: some authors reported that feeding tannin-containing diet to poultry depresses both feed intake and feed efficiency [20, 21], while other studies have also reported greater feed consumption [3]. Similarly, Zombade et al. (1979) [22] reported an increase in feed intake in chicks fed Sal seed (*Shorea robusta*) which contained high levels of tannins. The mechanisms by which tannins influence feed intake are not clearly known. Researchers have suggested that tannins' effect is related to their astringent properties, an effect resulting from the combination with salivary proteins to cause dryness in the mouth [23-25]. However, because taste acuity (sharpness) in chicken is not well developed [26], it seems unlikely that taste plays a role in decreasing feed intake. Moreover, in those studies where feed intake was significantly affected by sorghum tannins, there was no clear relationship between the amount of tannin in the diet and the magnitude of reduction in feed intake [27].

The results obtained for average daily gain contradicts the previous studies of Featherston and Rogler (1975) [28] and Douglas et al (1993) [29]; the higher weight gain of birds that received diet 2 at the starter phase may be a reflection of the observed increased feed intake. Since studies reporting large negative effects on weight gain due to sorghum tannins have typically involved diets containing sub-optimal levels of protein, the adequate levels used in the current study may have been sufficient to ameliorate any tannin effect, thus allowing birds to perform adequately. Similar results obtained for feed efficiency from 1-21 d suggests that birds were able to utilize all the sorghum based diets which was similar to the control diet. However, contrary to the results from 1-21 days, birds fed with white sorghum were able to utilize the diets better than those fed the control and red sorghum from 22-42 d. This result is in conformity with the findings of Pour-Reza and Edriss (1997) [30] who stated that all the dietary maize portion of broiler diets can be replaced with low-tannin sorghum without adverse effects on

live weight gain, feed intake and feed conversion ratio.

The depressed crude protein digestibility of sorghum observed in this study may be related to the presence of tannin in sorghum especially in the red sorghum variety. This is similar to the findings of Mahmood (2014) [7]. Osuntogun et al. (1987) [31] had earlier explained that tannins inhibit the digestibility of proteins in diets containing leaf protein concentrates. The reduced digestibility could be due to tannins forming complex compounds with protein [2] and carbohydrate components of the diet, thus making them unavailable for normal digestion [32]. Tannins bind proteins to form insoluble compounds that are excreted in the faeces [31]. Tannins, like many other anti-nutritive factors, stimulate an increase secretion of endogenous gut proteins and cause erosion of the intestinal mucosa [3]. These effects are likely to have a negative influence on the utilization of protein. An effect of tannins on protein used was more clearly evident in the efficiency of retention, which was significantly less for birds fed red sorghum than those fed maize and white sorghum. Although birds retained similar amount of protein from maize and sorghum diets, those receiving sorghum had to consume more feed because of their lower protein retention efficiency. This over consumption may reflect an attempt to increase the intake of essential amino acids as Parr and Summers (1991) [33] suggested, rather than being related to dry matter *per se*.

This observation was however contrary to that of Hamaker et al., (1986) [34] who reported that sorghum is almost as digestible as maize. There is likelihood that tannin levels in sorghum may be variety dependent. In general, it is evident that the tannins present in some sorghum cultivars may have only a limited effect on their feeding value for poultry. This observation supports findings that measurable tannin content in sorghum may be a reliable indicator of expected biological response in poultry [35, 36]. Kumar et al. (2007) [37] revealed that feeding reconstituted red sorghum-based diet (tannin content of 16 g/kg) to broiler chicken did not exert any appreciable influence on nutrient utilization. Enzyme-tannin complex has been reported [38] but the higher ether extract digestibility in birds fed sorghum based diets can be explained in the words of Mahmood et al. (2014) [7] that tannins have less

affinity for lipase. This was earlier reported by Mahmood et al (2009) [39] that tannins may not exhibit any effect on ether extract digestibility in broiler cockerels.

#### 4. Conclusions

WSV could completely replace maize because of the lower (condensed) tannin levels (improved growth performance and nutrient digestibility) while above 50% RSV replacement for maize may be deleterious.

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