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Influence of diets containing raw or heat processed cowpea on the performance and gut health of broiler chickens

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Abstract

An experiment was conducted to determine the influence of diets containing raw or heat processed cowpeas on the performance and gut health of broiler chickens. Forty eight day-old male broiler chicks were used in this study. The birds were randomly assigned to six mobile battery cages with eight birds per cage. The cages were assigned to three isonitrogenous and isocaloric diets in duplicate so that each treatment group had sixteen birds. The birds were fed for nine days on the following diets: T1 (control diet - corn-soy); T2 (20% of raw cowpea) and T3 (20% of roasted cowpea). The results showed no effect of either cowpea treatment versus control diet on weight gain, feed gain or cumulative period performance at day 5. However, by day 9, significantly higher weight gain was observed in chicks fed raw cowpeas compared to either roasted cowpeas or the control diet ($P<0.05$). Feed conversion and cumulative performance indices did not differ between groups fed the control diet or raw cowpeas, but chicks fed roasted cowpeas performed significantly poorer by day 9 ($P<0.05$). Although the raw cowpeas supported optimal growth in the chicks, histopathology indicated that the raw cowpeas were associated with inflammation of the duodenum. Gut morphology indicated that on day 5 duodenal crypt depth was significantly greater in birds fed on roasted cowpeas compared with the other two treatments. The differences between treatments in villus length, width, crypt depth, or muscle appearance were not apparent by day 9. Gastrointestinal viscosity was higher in the group fed T3 compared with T1 and T2. Although these results are contrary to expectations, there was no indication of over processing/ overcooking of the cowpeas that may have decreased protein quality. Data suggest that 20% of raw Mozambican cowpeas can be incorporated into broiler starter diets with no negative effects on growth or feed conversion.

Key words: Cowpea, gut inflammation, heat processing

Introduction

The cowpea (*Vigna unguiculata* L.Walp) is an annual legume produced in Mozambique, commonly called "Nhemba" in the country. It can be used at all stages of growth as a vegetable crop. The tender green leaves are prepared as a pot herb, akin to spinach. Immature snapped pods are used in the same way as snap beans, often mixed with other foods. Green cowpea seeds are boiled as fresh vegetables, and the dry mature seeds are also suitable for boiling. Currently, the majority of the population in Mozambique cannot afford eggs and poultry meat because of high costs of feeds. Thus in addition to providing protein

for humans, cowpeas should also be a cheap source of protein in poultry rations.

Nutrient composition of cowpeas is equivalent to other plant protein sources such as lupins and field peas, but lower in most nutrients compared with soybeans and canola (Tshovhote *et al.*, 2003). Tropical grain legumes are cheaper protein sources than fish meal and/or meat and bone meal (Brand *et al.*, 2004) that is traditionally incorporated in animal feed. However, a number of them including cowpeas contain anti- nutritional factors (ANF) (Erlwanger *et al.*, 1999); which cause health problems and may be fatal for both human and animals (Mikiæ *et al.*, 2009) if consumed in high amounts. Among the main ANF present in legumes are trypsin inhibitor (TI), tannins, phytic acid, and lectins (Mikiæ *et al.*, 2009).

The ANF can be reduced or destroyed by a number of processing techniques (soaking, autoclaving, pelleting, dry roasting, dehulling, germination, fermentation), that could be used to minimize or even eliminate the negative effects, and to improve the digestibility of individual feed components (Amaefule and Nwagbara, 2004; Amaefule *et al.*, 2006; Babiker *et al*., 2006; Saeed and Khadiga, 2007; Udensi *et al*., 2007). The purpose of this study was to compare the performance and gut health of 10-day old chickens fed 20% of diets as raw cowpeas or roasted cowpeas.

Materials and methods

Grain legumes

Three cultivars of *V. unguiculata* (INIA 36, IT 82-78, It- 16) were mixed and evaluated in this study. The seeds were provided by the National Research Institute of Agriculture, Mozambique (IIAM), and subjected to one of two processing methods:

1) Raw Mozambican cowpeas (1.5 kg) were milled into flour using a coffee grinder to obtain a particle size of about 1.5 mm mesh.
2) Prior to heat treatment, 1700 grams of raw cowpeas were soaked in a 2 g/kg sodium bisulphate solution in water for 12 hours at room temperature. After soaking, seeds were roasted using a hotbox oven at 120° C for 60 minutes. The seeds were removed from the oven, cooled, and milled into flour as above.

Beans were analyzed in duplicate for urease index (AOCS, 1980) in order to assess the reduction of trypsin inhibitor by heating.

Animals and housing

Forty eight day-old Ross male broiler chicks were used in this study. The birds were randomly assigned to 6 mobile battery cages with 8 birds per cage. They were fed starter diet formulations (industry standard; Agristat 2009) for four days. Lighting comprised 24 hours of light (approximately 20 lux) from days 1 to 4, followed by 20 hours of light (approximately 20 lux) and 4 hours of darkness (off at 2200 hrs; on 0200 hrs) from days 5 to 9. The environmental conditions, ambient temperatures and humidity were recorded twice daily during the week and once daily on weekends.

Diets

The diets were offered in mash form, and both the feed and water were available *ad libitum*. Birds were fed the dietary treatments from day 1 through 9.

Treatments included: T1) Control corn - soybean diet (SBM); T2) 20% of Mixed Raw Cowpea (MRC); T3) 20% of Mixed Roasted Cowpea (MROC); formulations are found in Table 1.

Measurements

The birds were weighed as groups in each pen at days 5 and 9 and feed consumption was determined as the difference between offers and refusals. Mortality was recorded twice daily and weights of dead birds measured and used to adjust for feed conversion; livability (number of birds alive per group) was estimated at times of data recording. Feed Conversion Ratio (FCR) = feed consumed per unit of weight gain. Period Gain = (body weight at a weigh day) – (body weight at the previous weigh day); Period Performance Index = (period livability*gain*100/period FCR), Cumulative Performance Index ((cumulative livability*((body weight*1000) /day of study)*10)/

Table 1. Characteristics and composition of the diets

Ingredients	T1%	T2%	T3%
Corn	58.97	42.80	42.80
SBM, 48% CP	35.47	28.89	28.89
Cowpea - raw	0.00	20.00	0.00
Cowpea - toasted	0.00	0.00	20.00
Soybean oil	1.21	3.87	3.87
Dicalcium phosphate	1.76	1.80	1.80
Calcium carbonate	1.10	1.09	1.09
Salt	0.36	0.35	0.35
Sodium bicarbonate	0.26	0.26	0.26
ALIMET®	0.24	0.29	0.29
Organic trace mineral mix	0.20	0.20	0.20
L-Lysine HCl 78%	0.16	0.12	0.12
Theronine	0.10	0.11	0.11
Choline Cl-60%	0.06	0.11	0.11
Mold guard	0.05	0.05	0.05
Vitamin premix	0.05	0.05	0.05
Santoquin-Mix 6	0.01	0.01	0.01
Total	100.00	100.00	100.00
Calculated nutrient analysis			
ME (kcal/kg)	3000	3000	3000
Crude protein (%)	22.25	22.25	22.25
Available P (%)	0.45	0.45	0.45
Ca (%)	0.92	0.92	0.92
Na (%)	0.22	0.22	0.22
Choline (ppm)	1600	1600	1600
Digestible Lys (%)	1.19	1.19	1.19
Digestible TSAA (%)	0.89	0.89	0.89
Digestible Thr (%)	0.80	0.80	0.80
Digestible Trp (%)	0.248	0.227	0.227

(cumulative feed to gain corrected for dead bird weight)), were calculated.

Tissue analysis

At day 8, 2 birds per pen were injected with bromodeoxyuridine (BRDU) (10mg/kg) as a marker for gut epithelial growth. Two days later, the birds were weighted, killed and eviscerated. Duodenum was collected, rinsed with Notox (Scientific Device Laboratory, Des Plaines, IL, USA) and preserved in tubes with Notox.

Clostridium counts and viscosity measurement

On day 10, ileal digesta samples were collected from another set of two birds per pen for *Clostridium perfringens* count by culture method. The jejunal and ileal digesta from both birds were recovered and pooled by pen for viscosity measurement on day 10 using viscometer DV-1 PRIME (Brookfield Digital Viscometer, Boston). The duodenum, jejunum, and ileum sections were collected for examination by optical microscopy. The sections were fixed in Notox fixative and stained with H & E, a monoclonal antibody to BRDU, or anti-immunoglobulin A.

Statistical analyses

Data were analyzed by ANOVA procedures appropriate for randomized complete block design using the General Linear Models procedure of SAS® (SAS Institute, Cary NC, USA). When ANOVA was significant, means were separated by Fisher's protected LSD method.

Results

Anti nutritional factors

The proximate analyses and anti-nutritional factors of raw and roasted cowpea are shown in Table 2. In general, the proximate analysis did not change under different processing treatments. Roasting was effective at reducing trypsin inhibitor, urease activity and also phytic acid content (Table 2).

Performance

Table 3 shows the performance of the chicks. The body weight gain at day 5 was higher in birds fed diet T2 (raw cowpeas)

Table 2. Proximate analyses and anti nutritional factors of raw and roasted cowpeas

	Raw cowpea	Roasted cowpea (120° C - 60 minutes)
Moisture	11.35	6.0%
Gross energy /kcal/100g	347.4	357.7
Crude protein %	22.75	24.94
Crude fat %	1.8	1.7
Fiber %	4.73	3.75
Ash %	3.42	3.42
Anti nutritional factors		
Trypsin inhibitor (TIU/g)	3000-10400	< 2000
Urease assay	0.03	-0.245
Tannins %	0.86	1.2
Phytic acid %	1.2	0.86

Table 3. Chick performance criteria when fed 20% inclusion Mozambican cowpea (*Vigna unguiculata)*

Day	Parameters	T1 (Corn-soy)	T2 (Raw cowpea)	T3 (Roasted cowpea)	P-value
5	Body weight gain, kg	0.059	0.064	0.055	0.1506
	Feed conversion ratio, kg: kg	1.271	1.076	1.266	0.5442
	Cumulative period performance index	164.53	196.065	158.60	0.4433
9					
	Body weight gain, kg	0.087^{b}	0.098^{a}	0.054^{c}	0.0007
	Feed conversion ratio, kg:kg	1.780^{b}	1.645^{b}	2.657^{a}	0.0273
	Cumulative period performance index	140.56^{a}	166.98^{a}	98.71^{b}	0.0284

^{abc} means in the same row with different letters are significantly different (P< 0.05)

and lower in the birds fed T3 (roasted cowpeas) compared with control. However there was no significant difference among the three diets (P> 0.05). At day 9, a higher body weight in chicks fed diet T2 than those fed T1 and T3 was observed (P<0.05). At day 5 feed conversion ratio (FCR) corrected for dead birds was higher in chicks fed T1 and lowest in the chicks fed diet T2, but the differences were not significant (P>0.05). At day 9, chicks fed diet T3 had the highest FCR compared with T1 or T2 and the differences were significant (P< 0.05). Although chicks fed T1 had higher feed gain compared with T2, the difference was not significant (P> 0.05; Figure 2).

In the Cumulative Period Performance Index, no significant effect (P>0.05) was observed at day 5. However, birds fed T3 showed lower indices than T2 or T3. The cumulative period performance index at day 9 was significantly different among the three diets (P< 0.05). Comparing treatment T1 and T2, no significant difference was observed (P>0.05).

Gut viscosity and microbiology

Microbiology and digesta viscosity data showed that *Clostridium perfringens* counts were higher in birds fed control diet (corn-soy) compared with raw and roasted cowpeas, but differences were not significant (P>0.05) (Figure 4). The viscosity of the digesta was higher in the chicks fed T3 -roasted cowpea (see Figure 3); however, the differences among treatments were not significant (P> 0.05).

Histopathology and gut morphology

Despite performance results indicating that the raw cowpeas supported optimal growth, the histopathology results indicated that the raw cowpeas were associated with gut inflammation.

As shown in Figures 5 and 6, raw cowpeas were associated with an inflammatory infiltrate and an increased level of IgA production in the duodenum.

The gut morphology parameters indicated that on day 5, the duodenal crypt depth was significantly greater in birds fed roasted cowpea than those fed raw

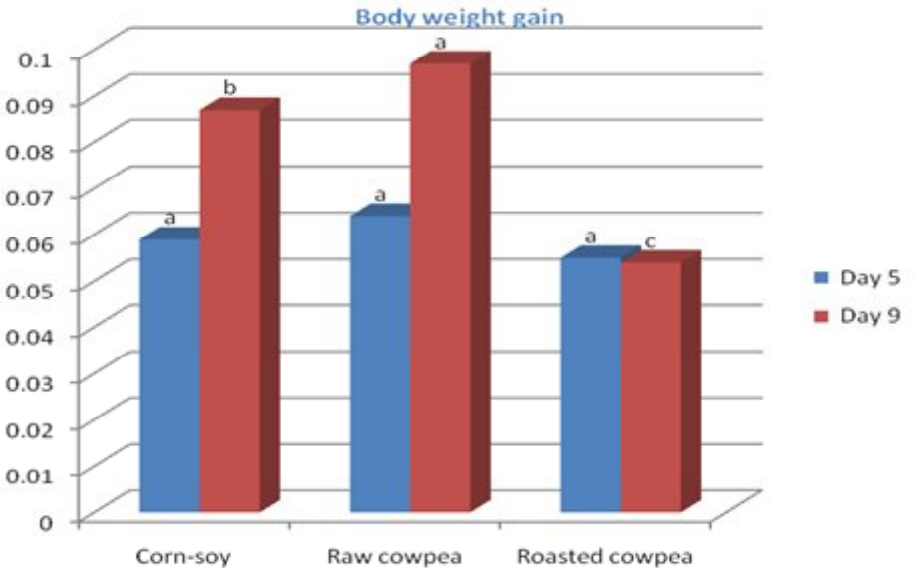

Figure 1. Body weight gain.

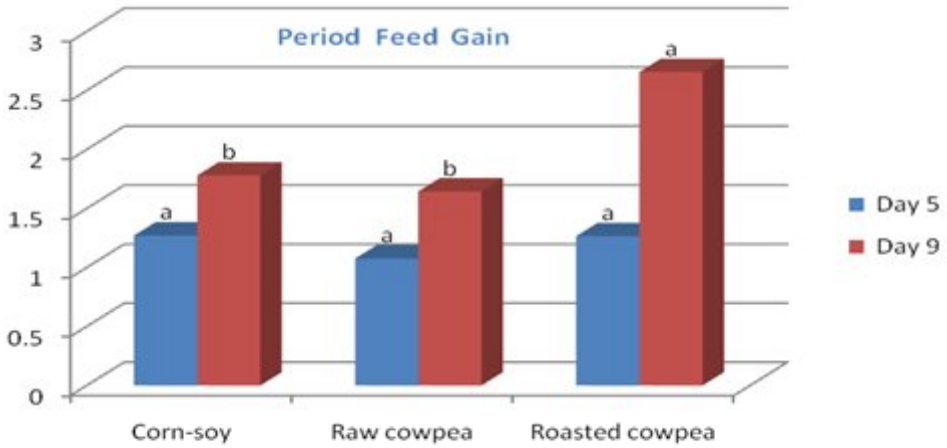

Figure 2. Period feed conversion ratio.

cowpeas or the corn soy diet. By day 9, there were no significant differences among treatments for duodenal villus length, villus width, crypt depth, or muscle thickness.

Discussion

Chemical analysis of raw and roasted cowpea confirmed that they can be a good source of dietary protein (22.8 and 24.9% respectively). This is in agreement with values previously reported by El-Jasser (2011) and Defang *et al.* (2008) of 22.9 and 24.6%, respectively, for crude protein in cowpea beans. Butt and Batool (2010) reported higher values of crude protein (27, 9%) compared to the values in the present study.

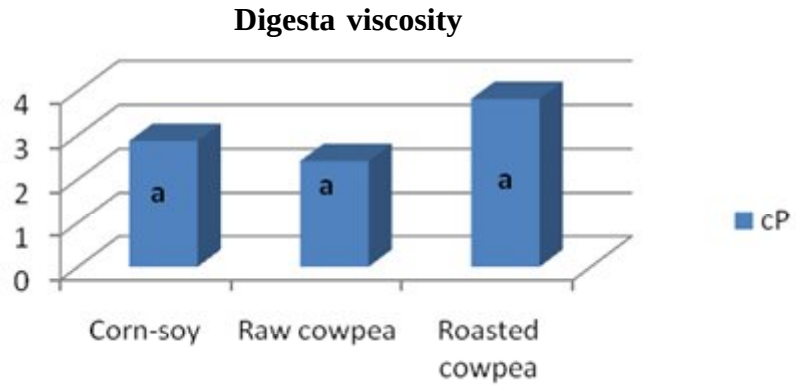

Figure 3. Digesta viscosity from gut sections of chicks fed diets containing 20% cowpea inclusion.

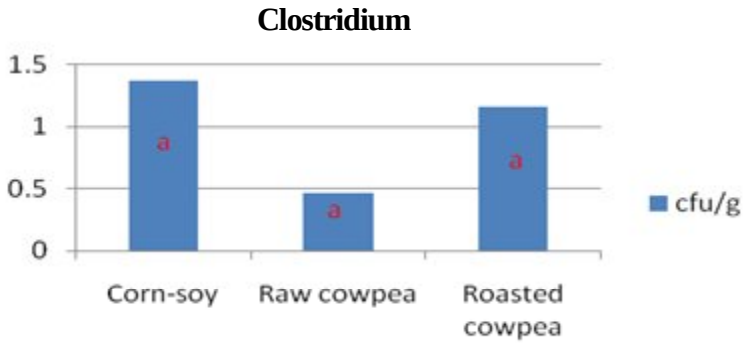

Figure 4. *Clostridium perfringens* growth (log cfu/g) from gut sections of chicks fed diets containing 20% cowpea inclusion.

The fat content of the cowpeas in our study was lower (1.8%) than reported by Hussain and Basahy (3.4%) in Saudi Arabia in 1998. The crude fiber was lower (4.73%) than presented by Butt and Batool (2010) which was 9.5%, and similar to the values observed by El- Jasser (2011). This difference could be due to the specific varieties, soil or climate growth conditions.

The data in Table 2 showed that cowpeas contain ANF such as trypsin inhibitor, phytic acid and tannins, which may limit their use as a feed ingredient for chickens. Some studies (Udensi *et al.*, 2007, Umapathy and Erwanger, 2008, Ayssiwed *et al.*, 2011 and Belal *et al.*, 2011) reported the presence of trypsin inhibitor, lectins, phenols, tannins, phytate and phytic acid, haemagglutinin, hydrogen cyanide, stachyose, raffinose and saponin in cowpeas. These authors also reported that heat treatments could reduce the ANF of the seed. Trypsin inhibitor (TI) was indeed reduced in cowpea by the heat treatment used, from an average of 6700 TIU/g (with the highest value of 10400 TIU/g for one of the varieties to levels below 2000 TIU/g). Similar results were reported by Umapathy and Erwanger (2008).

It has been recognized that the TI impairs the activity of pancreatic enzymes such as trypsin and chymotrypsin

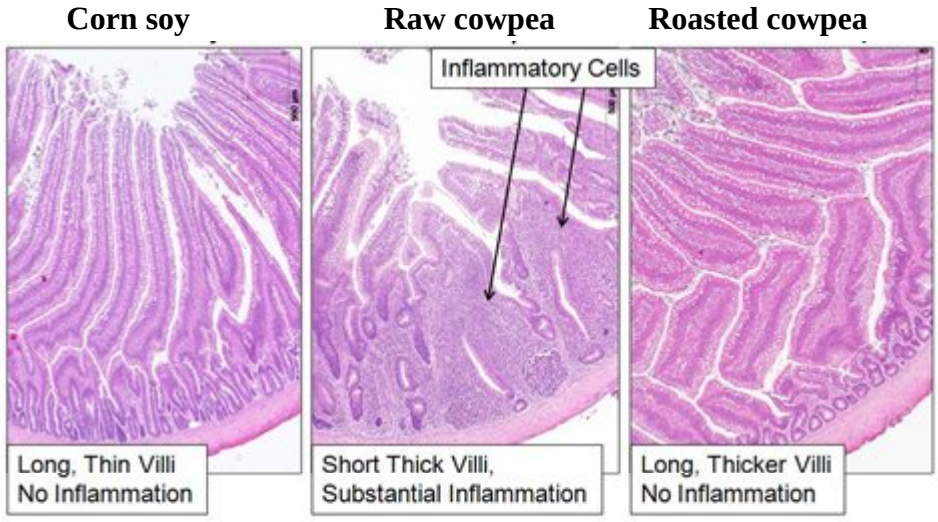

Figure 5. Morphology of duodenum from corn soy, raw cowpea and roasted cowpea fed birds: 010015 (H&E, 40x).

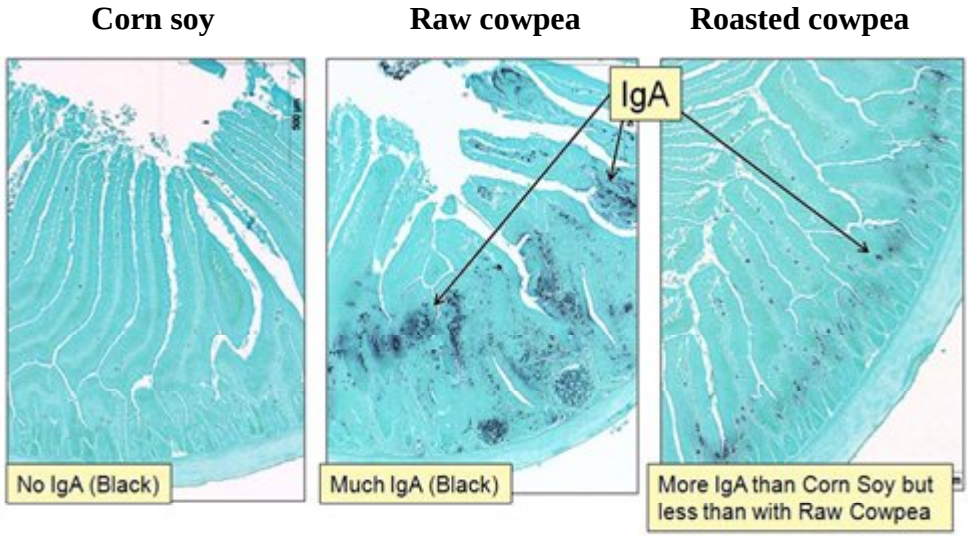

Figure 6. Morphology of duodenum from corn soy, raw cowpea and roasted cowpea fed birds:(010015 IgA, 40x).

(Tshovhote *et al.*, 2003) and inhibits growth by interfering with the digestion of protein in the intestine of animals, thereby, causing hypertrophy of the pancreas. Phytic acid present in soybean seed and soy products at levels of 1-1.5% DM chelates mineral elements, such as zinc, magnesium, iron, calcium and potassium, limiting their intestinal absorption (Mikiæ *et al.*, 2009). The most recent studies have shown that phytic acid not only limits phosphorus (P) availability from plant-derived feedstuffs for monogastric animals, but also calcium availability, metabolizable energy, and the digestibility of amino acids for poultry

(Selle and Ravindra, 2007 cited by Mingbin *et al.*, 2009). The tannins are a complex mixture of higher plant, water-soluble polyphenolic compounds of varying molecular masses that have the ability to react with proteins, polysaccharides and other macromolecules. Dietary tannins are thought to reduce the digestibility and metabolizable energy of feeds through direct interaction with proteins and carbohydrates from both exogenous and endogenous sources (Mansoori and Acamovic, 2007). Any or all of these ANFs may impact utilization of cowpeas by chickens.

Roasting cowpea at 120° C for 60 minutes decreased the growth performance of the birds during the entire experimental period. The results were unexpected since roasting cowpeas resulted in lower TI. Perhaps the reduction in performance was related to lower digestibility of nutrients due to excess heating. Defang *et al.* (2008), using 10% boiled cowpea for starter broiler diets, found low feed intake and weight gain, suggesting that ANF in the test diet was not completely eliminated through boiling. In our study, the Urease Assay indicated that trypsin inhibitor was reduced by the temperature however, the experiment demonstrated poor performance. Our results were also in contrast with those of Emiola *et al.* (2007) in feeding roasted kidney beans. The different results could be due to overcooking since we heated for 60 minutes compared to (120°C, 25-30 minutes) used by Emiola *et al.*. (2007). Emiola *et al.* (2007) also reported that roasting at 120° C for 25-30 minutes was less effective in the detoxification of tannins, phytates and oxalate compared to other measures. However, the performance of their chicks was significantly reduced in birds fed diets containing raw or dehulled kidney bean, compared with those fed aqueous and dry heated kidney bean meal diet.

According to Leeson and Summers (2001), the extent to which trypsin inhibitor is destroyed depends upon the heat temperature, time, particle size and moisture. Gut morphology data in this study was not consistent with performance results. The lack of gut inflammation in the roasted beans, but observed in the raw beans, suggests that the treatment was effective at removing some of the ANF. The histopathology results indicated that despite some inflammation associated with the raw cowpea, the animals were able to compensate and grow very well with 20% raw cowpeas in the diet. Inadequate heat fails to destroy the anti-nutritional factors, which may have a detrimental impact on animal performance. Excessive heating reduces the availability of lysine (via the Maillard reaction) and possibly, to a lesser extent, other amino acids. In this study, the temperature might have reduced nutrient availability such as protein digestibility; that might be the cause of the lower gains.

The study indicated that 20% raw cowpea could be included in the diet of young broilers without compromising growth performance despite some signs of inflammation at the gut level. Further, the roasting conditions may need to be modified to inactivate anti-nutritional factors without affecting nutritional values of cowpeas to broilers.

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