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## The Effects of Dietary Raw and Heat-Treated Cowpea (*Vigna unguiculata*) on Growth and Intestinal Histomorphometry of Pigs

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**Abstract:** To investigate the effect of raw and heat treated cowpea on the GIT and growth performance of pigs, 48 weanling piglets were divided into 4 groups and fed diets containing either 30% soymeal (T<sub>1</sub>) as controls, or 30% heat-treated cowpea (T<sub>2</sub>), or 15% heat-treated and 15% raw cowpea (T<sub>3</sub>), or 30% raw cowpea (T<sub>4</sub>). Phase 1 of the feeding trial started immediately after weaning and lasted for 18 weeks. From the 19th week, T<sub>4</sub> group was subdivided into 4 groups and they were fed either T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> or T<sub>4</sub> for 16 week. Average Daily Gain (ADG) was lowest in T<sub>4</sub> group both in phase 1 and phase 2 (p<0.01). Marked hypertrophy of the villi and crypt hyperplasia were seen in the jejunum and duodenum (p<0.01) of T<sub>4</sub> group. The diminished growth and marked enteropathology in the T<sub>4</sub> group was attributed to the heat sensitive ANFs in the raw cowpea. However, in phase 2 of the trial, when substitution feeding was implemented in the T<sub>4</sub> group, they showed marked improvement in feed consumption and weight gain. The catch-up growth seen after the introduction of substitution feeding, indicates that the antinutritional effects induced by the raw cowpeas are reversible. Heat treated cowpeas could make a valuable contribution in substituting soybeans in animal feed.

**Key words:** Cowpeas, feed intake, growth, gastrointestinal histomorphometry

### INTRODUCTION

Cowpeas (*Vigna unguiculata*) are heat and drought tolerant and are considered to be tough grain legumes well adapted to diverse environmental and agronomic regions including areas where soybeans are unable to thrive (Olivera *et al.*, 2003). However cowpeas like most other grain legumes, contain antinutritional factors (ANFs) such as trypsin inhibitors, lectins and tannins (Oluwatosin, 1999) which have a growth inhibiting effect. However, when the raw cowpeas are processed many of these undesirable effects are abolished (D'Mello, 1995; Olivera *et al.*, 2003).

The growth inhibition in pigs fed raw cowpea has been partly attributed to intestinal impairment (Makinde *et al.*, 1996a) and/or due to impaired pancreatic exocrine secretion in pigs (Umamathy *et al.*, 1999). Dietary manipulations during the prenatal suckling or weaning periods have long lasting and apparently irreversible effects on some transport mechanisms (Pacha, 2000). Previous studies have focussed on the adverse effects of cowpea when used in animal feeds, however, it is not clear

whether some of the growth inhibiting effects induced by dietary raw cowpea are reversible resulting in a catch-up growth that can occur when conditions of malnutrition or endocrinological disturbance causing early growth retardation are reversed (Boersma and Wit, 1997; Gafni *et al.*, 2001).

The objectives of the present study were therefore to investigate the effects of feeding raw cowpeas, heat-treated cowpeas and mixed diet (soybean meal and raw cowpea) on growth pattern, average daily gain, feed consumption and on the intestinal histomorphometry for a period of time and then to institute substitution feeding in only the raw cowpea fed group in order to investigate whether the growth changes induced by raw cowpea feeding are reversible and whether there is a subsequent catch-up growth.

### MATERIALS AND METHODS

The study was approved by the Zimbabwe Animal Experiments Licenseate and was undertaken at the Pig Industry Board in Harare Zimbabwe in 2001.

**Experimental animals and dietary treatments:**

Forty eight male Large White×Landrace weaners 21 days old, were used in the study. The pigs had not been creep-fed. They were randomly assigned to one of four diets in meal form: (T<sub>1</sub>) 30% extruded soyabean meal (control) (T<sub>2</sub>) replacement of SBM with heat-treated cowpea (T<sub>3</sub>) replacement of SBM with 15% heat-treated cowpea and 15% raw cowpea and (T<sub>4</sub>) replacement of SBM with raw cowpea (Table 1). Heat-treatment of the cowpeas was by autoclaving the raw cowpeas under pressure, 1 atm, at 120°C, for 15 min.

There were 12 weaners in each dietary group and each group had 3 pens with 4 pigs each. The pigs had *ad libitum* access to feeds and drinking water. The duration of the phase 1 of the study was from weaning to 18 week postweaning. In phase 2 of the trial, only the pigs from T<sub>4</sub> group are randomly divided into 4 sub-groups (consisting of 3 pigs per group) and were fed either T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> or T<sub>4</sub> diet. The duration of phase 2 was from the 19th week to the 35 weeks weaning. All pigs were weighed weekly. Feed intake was measured by weighing the food offered and subtracting that left over once a day.

The diets were analysed at the Institute of Animal Production in Tropics and Subtropics, University of Hohenheim; Nutrient analysis: Nitrogen (crude protein = N×6.25), lipid and ash by AOAC (1980) and the gross energy (GE, MJ kg<sup>-1</sup> DM) using a bomb calorimeter. Proximate analysis data are shown in Table 2.

**Histomorphology of the small intestine:** In the 21st week, 2 pigs/treatment group were randomly selected and sedated with ketamine (5 mg kg<sup>-1</sup> body mass, intramuscularly). The pigs were then euthanased by an overdose of pentobarbital (Eutha-naze, Centaur labs, Johannesburg, South Africa). Segments of the small intestine were removed and the lumen rinsed in Ringer’s bicarbonate solution and thereafter with fixative (4% buffered formalin) before being immersion-fixed in the same fixative for 48 h. The tissue was stored in 70% ethanol and after dehydration with estisol (buthyldecanate) to substitute xylene, embedded in paraffin, sectioned (5-7 µm) and stained with haematoxylin and eosin, mounted and examined. The villus perimeter of the intestinal segments were measured by morphometric methods using modified Abercrombie’s formula (Umapathy and Rai, 1982).

**Antinutritional factors assays:** Tannins and condensed tannins were determined by colorimetric methods

Table 1: Composition (%) of experimental diets used in feeding trial

Components	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
Maize meal	56.3	56.3	56.3	56.3
Soya	30.0	-	-	-
Heated cowpea	-	30.0	15.0	-
Raw cowpea	-	-	15.0	30.0
Meat and bone meal	10.0	10.0	10.0	10.0
Blood meal	3.0	3.0	3.0	3.0
Antibiotic premix	0.2	0.2	0.2	0.2
Vitamin and mineral premix	0.5	0.5	0.5	0.5

Table 2: Proximate analysis of raw, heat-treated cowpeas and experimental diets. Data are expressed on a dry matter basis

Treatments	Crude protein (%)	Lipids (%)	Ash (%)	GE (MJ kg <sup>-1</sup> )
Raw cowpeas	26.7	1.2	3.6	18.9
Heated cowpeas	25.7	1.5	3.5	18.8
T <sub>1</sub>	19.5	2.7	5.6	18.2
T <sub>2</sub>	17.6	4.3	5.1	18.7
T <sub>3</sub>	17.0	3.8	4.9	18.4
T <sub>4</sub>	16.5	4.2	6.7	18.3

(Makkar *et al.*, 1993). Total phenols were quantified by Folin-Ciocalteu reagent and tannins as the difference of phenolics before and after tannin removal from the extract using insoluble polyvinylpyrrolidone. Condensed tannins were measured by butanol-HCl-Fe<sup>3+</sup> reagent (Porter *et al.*, 1986). Trypsin inhibitor activity was determined according to Smith *et al.* (1980) except that the enzyme was added later as suggested by Liu and Markakis (1989). The haemagglutination assay (Gordon and Marquardt, 1974), was used to measure the lectin activity. Saponins were determined according to Hiai *et al.* (1976). Phytate content was determined by the colorimetric procedure of Vaintraub and Lapteva (1988).

**Statistical analysis:** Body weight gain, ADG and feed intake were analysed by Student’s t-test. Data on morphometric parameters were analysed using the GLM procedure of SAS (1982). Mean values and differences between treatments were determined by the procedure of Ott (1988).

**RESULTS**

**Body mass, Average Daily Gain (ADG) and feed intake:**

Growth pattern in phase 1 of the trial was significantly reduced for the T<sub>4</sub> group, while the other groups had almost similar growth pattern (Fig. 1). However, in the phase 2 of the trial when the T<sub>4</sub> diet was replaced with either T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> or maintained on T<sub>4</sub>, the group that was substitution fed T<sub>1</sub> showed the highest catch-up growth (5) while the group that was fed T<sub>2</sub> had a moderate catch-up growth (7) and the group that was

fed T<sub>3</sub> had the least catch-up growth. The group that was continuously fed T<sub>4</sub> diet had a typical growth inhibition curve throughout the duration of feeding. The body weight of this group (4) was significantly lower (p<0.001) compared to the other groups that were on substitution diet (5, 6 and 7).

In phase 1, the live body weight (p<0.001), ADG and feed intake (p<0.05) were lowest in the T<sub>4</sub> group. In the second phase of the trial, when the T<sub>4</sub> group was switched to the other feeds, there was considerable weight gain and increase in the feed intake, indicating considerable catch-up growth (Table 3).

**Antinutritional factors:** Trypsin Inhibitor Activity (TIA) and lectins were highest in the T<sub>4</sub> diet. Total phenols, tannins, phytates and saponins were almost similar in T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> diet. Saponins were prevalent in all the

diets and the heat treatment did not affect their levels. Glucosinolate, cyanogens and amylase inhibitors were not detected in any of the diets (Table 4).

**Intestinal morphology:** Pigs from T<sub>4</sub> group that were maintained on T<sub>4</sub> diet for both phases of the trial had shorter (p<0.01) villus height than pigs fed either T<sub>1</sub>, T<sub>2</sub> or T<sub>3</sub> diets (Table 5). The same group of pigs also had greater (p<0.01) crypt depth than pigs fed the other diets. These changes were observed in both the duodenum and the jejunum, while the ileum was not affected. Pigs fed T<sub>4</sub> diets had smaller (p<0.01) perimeter length at 21 days as compared to pigs fed T<sub>1</sub>, T<sub>2</sub> or T<sub>3</sub> diets. The villus crypt ratio was lower in T<sub>4</sub> group as compared to other groups (p<0.01) (Table 5).

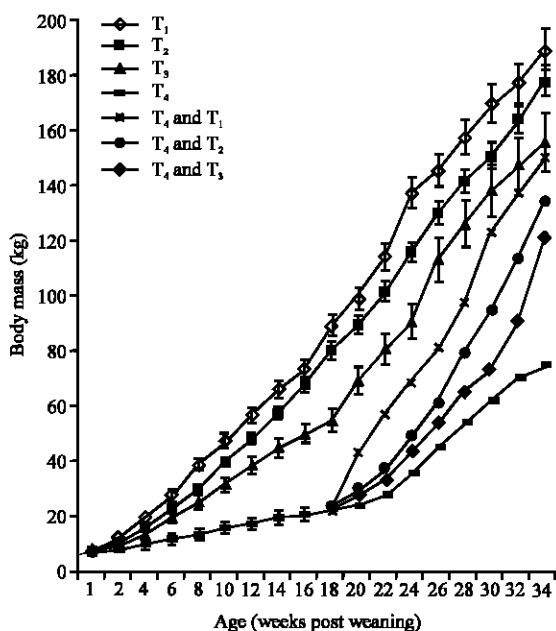


Fig. 1: Effect of diet and substitution feeding on growth rates of pigs

Table 3: Live body weight (kg), Average Daily Gain (ADG) and feed intake (kg week<sup>-1</sup>) in phase 1 and 2 of pigs fed experimental diets

Phase 1	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
Body weight	88.00±10 <sup>a</sup>	54.00±7 <sup>a</sup>	79.00±9 <sup>a</sup>	22.00±3 <sup>c</sup>
ADG	0.63±0.07 <sup>a</sup>	0.49±0.13 <sup>a</sup>	0.57±0.1 <sup>a</sup>	0.13±0.08 <sup>b</sup>
Feed intake	31.20±11.8 <sup>a</sup>	29.80±13.7 <sup>a</sup>	32.10±11.0 <sup>a</sup>	12.70±6.2 <sup>b</sup>
Phase 2	T <sub>4</sub> to T <sub>1</sub>	T <sub>4</sub> to T <sub>2</sub>	T <sub>4</sub> to T <sub>3</sub>	Fed on T <sub>4</sub> only
Body weight	154.00	124.00	132.00	73.00
ADG	0.75	0.81	0.83	0.34
Feed intake	40.50	44.50	47.20	16.80

Data in same row with different superscript differ: <sup>a, b</sup>: p<0.05; <sup>c</sup>: p<0.001, Values are Mean±SD. (Due to the small sample size for phase 2 only mean is shown)

Table 4: Anti-nutritional factors in raw and cooked cowpea and in the experimental diets

Diets	Trypsin inhibitor <sup>1</sup>	Lectins (unit) <sup>2</sup>	Total phenols (% <sup>3</sup> )	Total tannins (% <sup>3</sup> )	Phytate (% <sup>4</sup> )	Saponin (% <sup>5</sup> )
Raw cowpea	6.1	25.6	1.7	1.40	0.7	4.4
Heated cowpea	1.6	6.4	1.3	1.00	0.8	4.0
T <sub>1</sub>	0.6	13.0	0.3	0.04	0.8	2.8
T <sub>2</sub>	0.4	2.0	0.4	0.10	0.7	2.9
T <sub>3</sub>	0.9	3.2	0.4	0.10	0.8	2.7
T <sub>4</sub>	1.3	6.4	0.4	0.20	0.8	2.3

<sup>1</sup>: As mg trypsin inhibited/g; <sup>2</sup>: One unit = Inverse of minimum amount (mg) in the assay which produced haemagglutination, <sup>3</sup>: Tannic acid equivalent; <sup>4</sup>: leucocyanidin equivalent; <sup>5</sup>: Diosgenin equivalent

Table 5: Intestinal morphometric changes induced by different diets in growing pigs at 21 days postweaning

Treatments	T <sub>1</sub>		T <sub>2</sub>		T <sub>3</sub>		T <sub>4</sub>	
	VH	CD	VH	CD	VH	CD	VH	CD
Duodenum	344±9	418±63	351±26	466±52	430±45	519±63	166±46*	556±86*
Jejunum	343±4	324±38	319±58	322±50	273±43	383±52	162±28*	412±89*
Villus perimeter (µm)	467±48		543±45		642±35		331±54*	
Villus/crypt ratio	1.13		1.08		1.3		0.7*	

\*: T<sub>4</sub> versus other groups p<0.01, Values are Mean±SD (minimum of 10 different fields/segment), VH = Villi Height (µm) CD = Crypt Depth (µm)

## DISCUSSION

In the present study, growth of pigs fed on heat-treated cowpea diet was comparable to soyabean meal-fed pigs, confirming the reports that antinutritional factors could be reduced by heating (Nestares *et al.*, 1996).

The raw cowpea-fed pigs showed considerable growth depression even with 30% substitution as reported earlier by our group (Makinde *et al.*, 1996b). These antinutritional effects are attributed to the trypsin inhibitors, resulting in reduced digestibility and an excessive loss of endogenous amino acids leading to growth depression. This is supported by the high TIA levels in raw cowpea diets although the TIA for the cowpea variety in the present study was generally higher than those reported for other cowpea varieties (Ologhobo and Fetuga, 1984). The possible mechanisms involving the pancreatic exocrine secretions and the TIA in raw cowpea fed pigs (Umapathy *et al.*, 1999) and rats (Erlwanger *et al.*, 2001) have previously been documented.

Studies on intestinal transport and pancreatic enzymes in pigs fed raw and heat-treated cowpeas, strongly support the hypothesis that reduced feed intake was a major contributory factor to growth depression seen in raw cowpea-fed pigs (Erlwanger *et al.*, 1999). The present study supports the above hypothesis as the feed intake and ADG were significantly lower in the raw cowpea-fed pigs. The reduced food intake may have been due to tannins in the raw cowpea diet, as tannins have been suggested to have a bitter taste that makes the raw cowpea diet less palatable. Dietary tannins impair protein metabolism and nutrient utilisation *in vivo* (Olivera *et al.*, 2003). The capacity for tannins to depress animal growth as a consequence of poor digestibility, particularly of proteins (Trugo *et al.*, 1993) may be considered, although the malabsorption of glucose and sodium due to raw cowpeas may be rejected (Erlwanger *et al.*, 1999).

Phytate, generally regarded as an ANF, interferes with the bio-availability of minerals from plant sources (Trugo *et al.*, 1993) and inhibits digestive enzymes such as pepsin and pancreatic  $\alpha$ -amylase. The phytate levels were similar in the different diets used in the present study. Phytate values observed in the present study for cowpea were much higher than those reported by Ologhobo and Fetuga (1984) and heat treatment had no effect on phytate levels in the cowpeas. However, the extent to which the inhibition of enzyme activity by phytate contributes to its overall antinutritional effect remains uncertain.

Saponins have been implicated in decreased weight gain in different animals (Thompson, 1993) however, the level of saponins we found was not markedly different in the various diets so, the role of saponins in the process of growth depression may have been negligible.

A variety of nutritional factors have been shown to regulate gut growth and amongst these factors, dietary lectins found in high amounts in leguminous plants are known to resist degradation in the digestive tract where they may then interact with the gut epithelium; by binding to glycosyl side chains of receptors in the intestine, lectins have been shown to induce growth of the GI tract and pancreas in adult rats (Linderoth *et al.*, 2000). Lectins are implicated in growth depression (Liener, 1994). Lectins impair transport of nutrients across the intestinal wall accompanied by an increased rate of synthesis of mucosal protein and inhibition of brush border hydrolases (Liener, 1994). In the present study, the raw cowpeas had a substantial amount of lectins, which was considerably reduced by heat treatment. However, the lectins in soybean meal diet (T<sub>1</sub>) was found to be more than in cowpea meal and mixed diets (T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>). Similar findings have been reported for other legumes compared to soya bean (Leontowicz *et al.*, 2001). Yet, neither adverse histomorphometric changes in the intestinal segments nor growth depression were evident in pigs fed T<sub>1</sub> diet. This is probably attributed to the fact that depending on their sugar specificity, lectins react with different functional compartments of the intestine. Also as a result of different binding sites, the effects of different lectins may vary, as soybean lectins, have been shown to neither bind to nor damage cells of the lower parts of the villi or the crypts (Liener, 1994). Further, among plant species there are considerable differences in their severity of effect and mode of action (Kik *et al.*, 1989). It is therefore assumed that the cowpea lectins have a higher affinity towards intestinal membranes than the soyabean lectins.

ANFs in legumes, by inducing hypersensitive responses (Lalles *et al.*, 1996), alter the mitotic rate of enterocytes. These effects are associated with changes in villus height and/or crypt depth and these morphometric changes are the etiologic factors in the pathogenesis of post weaning diarrhoea (Makinde *et al.*, 1996a). Thus the antigenicity of ANFs are implicated in rapid weight loss, negative net protein utilization, neurotoxicity and low digestibility (Grant *et al.*, 1995), by virtue of their antinutritional proteins. It is presumed that these antinutritional proteins in cowpeas could be implicated as a dietary antigen associated with villus atrophy and crypt hyperplasia which leads to low digestibility. The extreme changes in the raw cowpea group could be due to immune

responses induced by the ANFs in the raw cowpeas as it is known that a dietary antigen is associated with villus atrophy and crypt hyperplasia (Hampson, 1986). As these degenerative changes were not evident in the pigs fed heat-treated cowpea, this enteropathology could be attributable to hypersensitive reactions induced by the heat sensitive ANFs in the raw cowpea diet. The analysis of cowpea antigens, therefore becomes an important tool in interpreting the actual mechanism underlying poor digestion and diarrhoea as reported for soybeans (Lalles *et al.*, 1996). The antigenicity of soya could be reduced by heat treatment (Toullec *et al.*, 1994) which further supports the possibility of cowpea antigens playing a significant role in growth inhibition although they may not be directly implicated in reduced feed intake.

Although the pigs fed raw cowpea showed considerable growth depression, they recovered significantly once substitution feeding was introduced. Thus two different phases of growth could be observed in the raw cowpea-fed pigs, one before the substitution feeding and the other after 18 weeks when catch-up growth was evident. Similar growth depressive effects have been reported when pigs were fed pigeon peas or white-flowered peas (Visitpanich *et al.*, 1985; Le Guen *et al.*, 1995). Most of these effects are attributed to the trypsin inhibitors, as they are known to inhibit trypsin activity resulting in reduced digestibility and an excessive loss of endogenous amino acids leading to growth depression. Perhaps the cowpea lectins have a higher affinity towards intestinal membranes than the soyabean lectins. In mammals, release from growth inhibiting conditions results in catch-up growth and Gafni *et al.* (2001) have proposed that this may be due to effects on the growth plates. There is also evidence supporting a neuroendocrine mechanism for catch-up growth (Gafni and Baron, 2000).

The finding of the reversibility of the effects of raw cowpea in the diet when replaced with soybean meal opens up the possibility of replacement feeding strategies where farmers in arid areas could utilise cowpea during periods when it is difficult to grow soybeans and then finish the pigs with other traditional high protein sources.

The findings of changes in intestinal morphometry due to feeding of cowpea also opens up the possibility of further investigation into the use of cowpea and specifically, the phytohaemagglutinins in inducing precocious maturation of the gut. Pacha (2000) reports that dietary manipulations during the prenatal suckling or weaning periods have long lasting and apparently irreversible effects on some transport mechanisms the demonstration of such developmental windows (critical periods) for programming of irreversible switching on or off once during the individual lifetime in response to

suitable environmental conditions has thus a great significance. The extent to which dietary cowpea may evoke such changes also needs further investigation. The mechanisms by which legume globulins modulate GIT or systemic metabolism are unclear, however some trigger excessive synthesis and secretion of mucus into the GIT leading to high endogenous N and dry matter in faeces which may distort nutrient needs and impair utilization of nutrients (Olivera *et al.*, 2003).

It is recommended that a cost benefit analysis be done on the possible role of substitution feeding with cowpea in areas where it can be grown cheaply relative to soybean. There is also need to investigate the effect of dietary cowpea on carcass quality and hormonal profile specifically those related to growth and appetite such as leptin, ghrelin and insulin like growth factors.

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