# Effects of feeding different levels of cowpea (*Vigna unguiculata*) on gut morphology and faecal composition in weanling pigs

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The effects of feeding diets containing different levels of raw cowpea on the histomorphometry of the small intestine and faecal composition were investigated in weaned piglets. A total of 100 21-day-old weaners were divided into five equal groups and were assigned to one of the five following meals; 100% soyabean ($T_1$); 25% cowpea ($T_2$); 50% cowpea ($T_3$); 75% cowpea ($T_4$) and 100% cowpea ($T_5$). There were five pens for each group and four piglets per pen. The feeding trial lasted 28 days. Anti-nutritional factor (ANF) assay of the feeds revealed a higher tannin content in the $T_4$ and $T_5$ groups. Live weight gain was lower in the $T_5$ group, as compared to other groups. Those animals which were fed diets $T_1$ to $T_4$ had longer villi, larger villus area and greater villi perimeter than pigs fed $T_5$ diet. Fresh faeces collected daily for up to seven days postweaning, were analysed for their pH and moisture content while osmolarity and the levels of electrolytes, glucose and protein were determined from their extracts. The faecal analysis revealed an increase in the moisture content and a decrease in osmolarity in the $T_1$ and $T_5$ groups. Glucose and electrolyte concentrations were highest in the faeces of $T_1$, $T_2$ and $T_3$ animals. A transient and mild diarrhoea was observed in the $T_1$ group. However, 100% raw cowpea feeding ($T_5$) results in pathophysiological changes in gut morphology leading to impaired absorption of nutrients. ANFs in the raw cowpeas could account for the observed changes in gut morphology and faecal parameters. The diminished growth therefore may be due to reduced digestive and absorptive capacities as a result of intestinal mucosal changes. It is suggested that processing of cowpeas is essential in addition to reducing the percentage composition of cowpeas in the pig ration, if they are to be recommended as a suitable alternative to the soyabeans in stockfeed.

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## Introduction

By the year 2000, the shortage of oilcake is expected to be well over a million tons in the Southern African region (Cloete, 1990), making it imperative to search for some alternative sources of proteins for animal feed. By virtue of its heat and drought tolerance, cowpeas (*Vigna unguiculata*) are considered to be a tough grain legume well adapted to the arid agronomic areas in Southern Africa (Nell, Siebrits & Hayes, 1992). However, cowpeas, like most other grain legumes, contain ANFs such as trypsin inhibitors, lectins and tannins which decrease protein digestibility (Bressani, 1985). These ANFs are known to have a variety of effects, including morphological changes and enzyme inhibition along the intestinal mucosa (Etheridge *et al.*, 1984; Erickson *et al.*, 1985; Kik, 1991). These effects ultimately result in poor digestibility, diarrhoea, poor growth performance and sometimes death (King et al., 1980; Huisman et al., 1990; Li et al., 1991).

Even though a number of ANFs are known to be inactivated by heat resulting in diminished biological activity (Pusztai, 1985), heat treatment can be expensive and might not be effective all the time (Prince *et al.*, 1988). The effects of feeding raw cowpea on growth and hematological and systemic changes in weanling pigs have been reported (Umapathy *et al.*, 1995). However, the effects of feeding different levels of raw cowpea in the diet on histomorphological changes in the gut and faecal composition in piglets needed to be evaluated to provide more information on the nutritive value as well as the optimum use of this legume.

The objectives of the present study were therefore to investigate the effects of feeding different levels of raw cowpeas on faecal characteristics and to determine quantitatively and qualitatively the changes induced by the raw cowpea on gut morphology. It was also hoped to determine the optimal level of cowpea in the meal that would be adequate for growth without causing adverse histomorphological changes, through the assessment of faecal characteristics.

## Materials and Methods

### Experimental animal/dietary treatments

A total of 100 Large White × Landrace cross weaners from eight litters were used for this study. They were all males and none was creep-fed. All piglets were weaned at 21d of age and were randomly assigned to one of the following five diets in meal form that differed only in the level of substitution of soyabean meal with cowpeas as shown in Table 1.

$T_1$ — 100% soyabean meal

$T_2$ — replacement of 25% of the soyabean meal with raw cowpea meal

$T_3$ — replacement of 50% of the soyabean meal with raw cowpea meal

$T_4$ — replacement of 75% of the soyabean meal with raw cowpea meal

$T_5$ — replacement of 100% of the soyabean meal with raw cowpea meal

Twenty weaners were allocated to each group and each group had five pens with four piglets in each pen. All piglets were fed *ad lib*. The feeding trial lasted for 28 days. All piglets were weighed on day 7 and day 14 and average daily weight gain was calculated.

**Table 1** Feed composition

| | Control | 25% | 50% | 75% | 100% |
|---|---|---|---|---|---|
| | | (Cowpea supplememted) | | | |
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ |
| Maize meal | 63.8 | 63.8 | 63.8 | 63.8 | 63.8 |
| Soyabean meal | 31.2 | 23.6 | 15.6 | 7.6 | – |
| Cowpea meal | – | 7.6 | 15.6 | 23.6 | 31.2 |
| Limestone flour | 1.6 | 1.6 | 1.6 | 1.6 | 1.6 |
| Monocalcium phosphate | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Antibiotic premix | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Vitamin and mineral premix | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Salt fine | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |

**Table 1a** Proximate analysis

| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ |
|---|---|---|---|---|---|
| Dry matter (%) | 91.64 | 91.80 | 91.13 | 91.11 | 89.12 |
| Ash (%) | 4.82 | 4.77 | 5.59 | 5.12 | 4.43 |
| Crude protein (%) | 19.11 | 19.65 | 18.93 | 18.30 | 19.13 |
| Crude fibre (%) | 4.0 | 4.48 | 4.51 | 3.91 | 3.81 |
| Gross energy (MJ/kg) | 16.68 | 16.20 | 16.81 | 18.10 | 18.26 |

**Table 1b** Anti-nutritional factor assay

| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ |
|---|---|---|---|---|---|
| Ether extract (%) | 4.80 | 5.54 | 6.43 | 6.20 | 3.95 |
| Nitrogen free extract (%) | 64.73 | 62.97 | 61.72 | 63.80 | 65.73 |
| Insoluble tannins (A 550/g NDF) | – | 1.32 | 2.46 | 11.34 | 9.09 |

Note: NDF – Neutral detergent fibre

## Faecal examination

Fresh faecal material was obtained daily from the weaners in all five groups from 0d to 7d postweaning, for the determination of moisture content and pH. Faeces (5 g) were weighed and dried at 90°C; water content was determined by lyophilisation for 24 h. A faecal water content of > 80% was considered to indicate the occurrence of clinical diarrhoea.

Faecal extracts were employed to determine the pH, electrolyte, glucose and protein concentrations and osmolarity. The faecal extracts were prepared as described by Etheridge *et al.*, (1984), by air-drying at 60°C the grab samples from all groups at each collection period followed by grinding the sample in a Wiley mill using a 1-mm screen. After the samples were mixed thoroughly, 1 g of faecal material was weighed into a 50 ml Omni mixer barrel. Fifteen millilitres of distilled water were added to this material and the samples homogenised for 3 min. The contents of the barrel and blades were rinsed three times with distilled water into a 25 ml volumetric flask, which was then inverted to remove air bubbles, after which the samples were made to same volume of 10 ml. Samples were thoroughly mixed by inverting 10 times, transferred to a 50 ml conical centrifuge tube and centrifuged at 2000 × *g* for 15 min. The supernatant was transferred to plastic containers and frozen at –15°C.

When required, faecal extracts were allowed to thaw and equilibrate to room temperature, after which they were filtered using Whatman 42 ashless filter paper. The pH of the faecal extract was determined using a pH meter (Kent, U.K). The concentration of electrolytes ($Na^+$, $K^+$, $Cl^-$) was estimated by the principle of ion selective electrodes using Starlyte II (Pharmacia Diagnostics Inc; The Netherlands) and glucose by the hexokinase method (Gemeni, ENI Company). Total protein was determined manually by the 3% sulphosalicylic acid and 7% sodium sulphate method.

Total osmotic excretion was measured by dissolving the dried faeces into a known quantity of water and measuring osmolality from the water content, by freezing point depression as previously described (Makinde, *et al.*, 1996), using osmometer (Osmomat 030, Gonotec, Berlin).

## Morphological examination

At 0 d and 7 d postweaning, three pigs/treatment group were selected, weighed and anaesthetised using ketamine at 5 mg/kg body weight intramuscularly. The abdomen was opened and the small intestine was excised immediately. A 10-cm segment from the mid-jejunum, mid-duodenum and mid-ileum were removed, rinsed with saline, and fixed in cold 10% formalin. Cross-sections of intestinal samples from formalin-preserved segments were fixed by standard paraffin embedding procedure. Serial sections were cut perpendicularly to the long axis and stained with haematoxylin and eosin for light microscopy. Twenty-five well-oriented villi and crypts per segment sample were measured by the same individual at 40× magnification with a binocular microscope using an ocular micrometer.

The villus height was taken as the distance from the crypt opening to the tip of the villus, while the crypt depth was measured from the base of the crypt to the level of the crypt opening (Kik, 1991). Villus height and crypt depth were measured on the stained sections in triplicate specimens for each pig within each group. The perimeter and area on the intestinal segments were measured on the sections by morphometric methods using modified Abercrombie's formula as applied to testicular tissues (Umapathy & Rai., 1982).

## Statistical evaluation

Body weight changes were analysed by Student's *t* test. Data obtained from these experiments 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). Preplanned contrasts were used to separate treatment means in the trials. The comparisons made were control compared to different levels of raw cowpea meal in the diets.

Differences among groups in duration of diarrhoea were compared by an 8 by 2 chi-square contingency test, using single pig days of diarrhoea as the individual event.

Faecal analysis results were subjected to least-squares analysis of variance. If the *F*-test was significant for main effects, then differences among means were evaluated by Duncan's (1955) multiple range test.

# Results

Table 1a shows no significant differences in the nutrient composition between the experimental diets and the control. However, tannin content, the only anti-nutritional factor that we could measure was found to be appreciably higher in $T_4$ and $T_5$ groups (Table 1b).

Table 2 shows the body weight changes and average daily weight gain in the different treatment groups. Both at 7 d and 14 d postweaning, the body weight of the $T_5$ group showed a decrease ($p < 0.05$) when compared to other groups.

## Gut morphology

Pigs fed 100% cowpea had shorter ($p < 0.05$) villus height than pigs fed either soyabean meal or lesser percentages of cowpea meal (Table 3). Pigs fed 100% cowpea had greater ($p < 0.05$) crypt depth than pigs fed soyabean meal, 25%, 50% or 75% cowpea. The area of the villus corresponded with the villus height; the longer the villi, the larger the area of the villi. Pigs from the $T_5$ group had smaller ($p < 0.05$) perimeter length and villus area than pigs from the $T_1$ to $T_4$ groups. There were no significant differences in villus height among pigs fed reduced percentages of cowpea meal, i.e. $T_2$, $T_3$, and $T_4$ groups.

## Faecal analysis

Normal faecal material was semi-solid with about 70% moisture content. At 2 d and 4 d postweaning, a mild diarrhoea (water content > 80%) was observed in the control group and in the 100% cowpea fed group but the duration of diarrhoea was a little longer in the former group ($T_1$) than in the latter group ($T_5$) (Table 4).

There was a trend for increased osmolarities in the $T_2$, $T_3$ and $T_4$ groups. Interestingly, these were the groups that did not show diarrhoea. The osmolarity of faecal material in $T_1$ and $T_5$ pigs were lower when compared with these ($T_2$, $T_3$ & $T_4$) groups ($p < 0.05$) (Table 4).

When analysed across treatments by day, the faecal glucose in $T_1$, $T_2$ and $T_3$ pigs was higher than in the $T_4$ and $T_5$ groups ($p < 0.05$). While there were no significant differences in potassium and chloride concentrations in the faecal extracts from all the groups, the sodium content was significantly reduced in the $T_4$ and $T_5$ groups as compared to the other groups (Table 5).

**Table 2** Effect of feeding different regimes of cowpea meal to weaners on body weight (kg ± SEM) changes

| Age post-weaning | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ |
|---|---|---|---|---|---|
| Day 0 | 4.72 ± 0.6 | 4.70 ± 0.9 | 4.72 ± 0.91 | 4.73 ± 0.35 | 4.72 ± 0.8 |
| Day 7 | 5.55 ± 0.67 | 4.93 ± 0.79 | 5.08 ± 1.33 | 5.18 ± 0.95 | 4.8 ± 0.72* |
| ADG | 0.12 | 0.03 | 0.05 | 0.06 | 0.01 |
| Day 14 | 6.38 ± 1.2 | 5.95 ± 0.6 | 5.72 ± 1.0 | 5.38 ± 0.8 | 5.02 ± 0.8* |
| ADG | 0.12 | 0.15 | 0.09 | 0.03 | 0.03 |

* $p < 0.05$ ADG – Average daily gain (kg)

**Table 3** Effect of feeding different levels of cowpeas on gut morphology at 7 d postweaning

| Criteria | ($T_1$) | $T_2$ | $T_3$ | $T_4$ | $T_5$ |
|---|---|---|---|---|---|
| Villus height, μm[a,b] | 366.2 | 316.5 | 330.4 | 308.3 | 275.0 |
| Crypt depth, μm[b] | 298.0 | 300.1 | 308.6 | 304.1 | 380.6 |
| Perimeter, μm[b] | 813.3 | 719.0 | 746.4 | 756.9 | 634.0 |
| Villus area, μm[a,b] | 27893 | 20987 | 23456 | 24687 | 17423 |
| Villus/crypt ratio | 1.23 | 0.72 | 1.01 | 1.07 | 1.05 |

[a] $T_1$ vs others ($p < 0.05$)

[b] $T_5$ vs others ($p < 0.05$)

**Table 4** Occurrence of diarrhoea (> 80% faecal water content) in pigs fed cowpea during the first seven days postweaning

| Treatment | Time of onset (days) (mean ± *SD*) | Duration (days) (mean) | *n* |
|---|---|---|---|
| Control | 2.0 ± 0.8* | 2.2 # | 10 |
| 100% cowpea (T5) | 4.2 ± 1.2* | 1.7 # | 6 |

* Significantly different by Student's *t* test, $p < 0.01$

# Significantly different by chi-square test, $p < 0.05$

*n* = No. of pigs

**Table 5** Summary of components measured in faecal extracts[a] from growing pigs fed different levels of raw cowpea during the first 7 d postweaning[b,c]

| Item | Dietary treatment $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ |
|---|---|---|---|---|---|
| Osmolarity (mmol/dm³) | 90 ± 14[d] | 145 ± 13[e] | 142 ± 12[e] | 153 ± 12[f] | 89 ± 5 |
| pH | 6 ± 0.1[d] | 6 ± 0.2 | 6.2 ± 0.2 | 7 ± 0.2[e] | 7 ± 0.3[f] |
| Glucose (mg/100 ml) | 37 ± 5[d] | 39 ± 4 | 40 ± 5 | 20 ± 2[e] | 22 ± 2[f] |
| Na (m eq./dm³) | 14 ± 1[d] | 15 ± 1.1 | 14.5 ± 1.2 | 8 ± 1[e] | 6 ± 1[f] |

[a] Extract = 1g of dry faecal material/25 ml distilled water

[b] Grab samples collected from 12 pigs

[c] Mean ± SE

[d,e,f] Means with different superscripts differ ($p < 0.05$)

## Discussion

The histomorphometric findings in the present study indicate that the jejunal mucosa of weaners in $T_5$ group was characterised by shortening of the villi and lengthening of the crypts. Similar findings have been made in the piglets fed *Phaseolus vulgaris* beans (Kik, 1991). Even though King et.al., (1980), in another study could not observe a change in villus length in the rats and piglets fed *Phaseolus vulgaris* beans, one would expect that in an animal with extensive villus atrophy, the total area available for nutrient absorption has become significantly reduced, a factor which may, in part, explain the reduced body weight gain in such animals. Although reports from previous studies have been rather inconsistent, it is now generally believed that the presence of ANFs in legumes causes a hypersensitivity response (Mowat & Ferguson, 1981), which in turn alter the mitotic rate of enterocytes (Miller *et al.*, 1984). These effects are associated with changes in either villus height and/or crypt depth (Makinde *et al.*, 1996). Nabuurs (1991) suggested that villus height and crypt depth influence the pathogenesis of postweaning diarrhoea by establishing the functional relationship between them whereby nutrients, electrolytes, and water are absorbed by villus enterocytes, whereas electrolytes and water are secreted by crypt cells. Therefore, shorter villi and deeper crypts have fewer absorptive and more secretory cells, absorption might be poorer and secretion increased. Thus the implication of ANFs as a contributory factor to growth inhibition is in line with previous reports (Grosjean *et al.*, 1986). However, additional factors like feed intake and differences in lysine content between cowpeas and soyabeans may also be taken into consideration.

Some investigators (Miller *et al.*, 1984) have attributed this enteropathology to immune responses consequent upon hypersensitivity reactions from the soyabean meal in weaning rations. It was suggested that local type III hypersensitivities may be induced in any tissue to which an antigen can gain access. In general our results are in agreement with the observations that a dietary antigen is associated with villus atrophy and crypt hyperplasia which, in turn, cause a malabsorption syndrome (Miller *et al.*, 1984). The increased moisture content of faeces in the $T_1$ and $T_5$ groups can be attributed to the increase in osmotically active particles, resulting in a decreased reabsorption of water from the lower digestive tract. The presence of glucose in the $T_4$ and $T_5$ groups indicates that the higher the percentage of raw cowpeas, the higher the accompanying bacterial fermentation of carbohydrates, as suggested by Etheridge *et al.*, (1984). It is worth noting that the soyabean meal-fed weaners had mild diarrhoea but no changes in faecal sodium. A decrease in the faecal sodium in both the $T_4$ and $T_5$ groups was seen, indicating greater absorption of sodium by these two groups. This may be attributed to a compensatory mechanism initiated by the intestines to conserve water.

However, it is interesting to note that the soyabean-meal-fed pigs had diarrhoea but did not have a fall in faecal sodium, which probably indicates that the changes in villus morphology induced by the cowpea may indeed be much more complex. This observation is further confirmed by the rise in faecal pH of the two groups of pigs fed high levels of cowpea. Blaxter & Wood (1953) while studying diarrhoea in calves found a lower faecal pH in calves with diarrhoea as compared to normal calves which had a pH of 6.8. Such observation is consistent with our study since we also found that the soyabean-meal-fed group had a lower faecal pH and had incidences of diarrhoea. But the 100% cowpea-fed group, although they had diarrhoea, did have a rise in faecal pH. We therefore suggest that there may be changes at the cellular level of the intestine of the cowpea-fed pigs which may be specifically affecting some transport mechanisms across the cell membranes, which may be worth investigating.

Reductions in growth performance and changes in the small intestinal morphology caused by feeding cowpea diet may be due to a hypersensitive response induced by ANFs in these diets (Li *et al.*, 1991). In order to derive optimal benefit from the use of this legume in pig rations, it does appear that there is need for its processing. Such may be achieved by mechanical (dry fractionation process and separation of fractions) and/or physical (heat treatments) as suggested by Van der Poel & Melcion (1995). Similar views were documented by Li *et al.*, (1991) in regard to soybeans and their concentrates.

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