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The effects of cowpea (*Vigna unguiculata*) feeding on basal, exogenous cholecystokinin (CCK33) and secretin stimulated pancreatic secretions of the anaesthetized rat

K.H. Erlwanger[1] #, E. Umapathy[2], C. Musara[1], E. Kandiwa[1], D. Kruszewska[3], I. Mattsson[4] and S.G. Pierzynowski[5]

[1]University of Zimbabwe, Faculty of Veterinary Science
Box MP 167, Mt Pleasant Harare, Zimbabwe
[2]Department of Physiology, Faculty of Health Sciences, University of Transkei
P. Bag X01, Umtata, 5117 Eastern Cape Province, South Africa
[3] Department of Medical Microbiology, Dermatology Infection
Lund University, Sölvegaten 23, S-223 62 Lund, Sweden
Institute of Ecology, Polish Academy of Sciences
Dziekanów Leśny, 05-092 Łomianki, Poland
Department of Pharmaceutical Microbiology, Medical University of Warsaw
02-007 Warszawa, Oczki 3, Poland
[4]Department of Animal Physiology, Lund University
Helgonawägen 3b, SE-223 62 Lund, Sweden
[5]Department of Animal Physiology, Lund University
Helgonawägen 3b, SE-223 62 Lund, Sweden
R&D, Gramineer Int. AB, Ideon beta
SE-223 70 Lund, Sweden

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ABSTRACT

To study the effects of chronic feeding of cowpea (*Vigna unguiculata*) on the exocrine pancreatic secretions of the rat, 32 four-week old rats were randomly assigned to four dietary groups: I. *ad libitum* standard rat feed (controls), II. raw cowpea *ad libitum*, III. cooked (boiled cowpea) *ad libitum*, and IV. restricted access to standard rat feed. The pancreatic duct was catheterized for total collection of pancreatic juice. After 12 weeks of dietary treatments the rats kept on cowpea

Corresponding author

diet and restrictively fed rats exhibited reduced growth and feed consumption compared to the controls. Diet had no significant effect on the volume of pancreatic juice and total protein output under basal conditions. Trypsin activity outflow in basal and stimulated conditions showed a tendency to be higher in rats fed a restricted diet and cooked cowpea diet as compared to the rats fed a standard diet and raw cowpea diet. Intra-venous CCK33 and secretin significantly increased the volume and pancreatic juice protein output in all the groups. However, potency of the stimulation for volume and pancreatic protein outputs were higher in rats fed standard diet compared to the other groups. Dietary cowpea enhanced basal as well as stimulated pancreatic secretion (volume, protein and trypsin outputs) in a manner similar to that observed in the rats fed restricted diet.

KEY WORDS: rat, exocrine pancreas, secretion, CCK33, secretin, cowpea

INTRODUCTION

Cowpeas (*Vigna unguiculata*) like most other legumes have been shown to contain various antinutritional factors such as trypsin inhibitors, lectins and tannins (Gatehouse and Boulter, 1983; Mnembuka and Eggum, 1995) that are known to have deleterious effects on the growth performance, body composition and gastrointestinal systems (Grant et al., 1995; Makinde et al., 1996). However, the effects of most of the antinutritional factors can be minimized and even neutralized by various processing methods such as moist or dry heat treatment and fermentation (D'Mello, 1995). Given the shortage of protein sources in the arid regions, cowpea, which is a drought resistant crop, can go a long way toward meeting the demands for plant protein in the arid regions and also the search for novel legumes (Makinde et al., 1996).

Some of the deleterious effects of cowpea seen in other studies have been attributed to effects on the pancreas either directly or indirectly (Umapathy et al., 1999). The enzyme secretion of the pancreas is highly dependant on the diet composition (Pierzynowski et al., 1990), feeding regimen (Botermans et al., 1999), age (Pierzynowski et al., 1999) and even circadian rhythm (Thaela et al., 1995). On the other hand it has been postulated that growth of the animals and their dietary performance is closely correlated with pancreatic enzyme digestive efficiency (Botermans and Pierzynowski, 1999). Thus, as cowpea is recognized as a potential source of protein for animal nutrition in arid regions, there is need to study some of the chronic effects of cowpea on the pancreatic secretions as this is the site of origin of most of the important digestive enzymes, and the secretions of the pancreas are required for digestion.

The aims of this study were to investigate the effect of chronic feeding of cowpeas on the basal and stimulated pancreatic secretions of the pancreas in anaesthetised rats.

MATERIAL AND METHODS

Thirty two 4-week old rats were randomly assigned to one of four groups fed on different diets: group one were fed *ad libitum* standard rat feed (in %: yellow maize, 20; starch grits, 20; meat-and-bone meal, 3.2; cotton seed cake, 10; soyabean meal, 20; limestone flour, 1; salt, 0.5; wheat feed, 25.5; mineral/vitamin premix, 0.3) as controls, group two were fed on raw cowpea *ad libitum*, group three were fed cooked (boiled cowpea) *ad libitum*, and group four were fed on standard rat feed but with restricted access (60% of daily ration fed to group one). All groups had free access to water at all times. The rats were adapted to a 12 h light cycle. After 12 weeks, the rats were starved overnight in preparation for surgery the next morning.

Surgery

The rats were anaesthetized subcutaneously with a mixture of ketamine (Ketalar, Parke-Davies, Barcelona Spain, 0.2 ml/100 g body weight) and azaperone (Stresnil, Janssen, Beerse, Belgium, 0.05 ml/100 g body weight). A silicon tubing (Silastic, i.d. 0.51 mm, o.d. 0.94 mm, Dow Corning, Midland, MI, USA) was surgically placed into either the left or right jugular vein. The jugular vein was reached through a ventral incision in the throat region. The catheterization was made for intravenous infusions.

A ventral midline abdominal incision was made into the abdominal cavity. The bile duct was located and ligated close to the liver to ensure pure pancreatic juice inflow in the common pancreatic-biliary duct. The common pancreatic-biliary duct was then catheterized with silicon tubing (Silastic, i.d. 0.30 mm, o.d. 0.63 mm) near its entry into the duodenum for total collection of pure pancreatic juice.

After the surgery the rats were allowed 30 min to stabilise before the commencement of the experimental procedure. The rats were maintained on a heated table during the surgery and experiments and kept at a body temperature of 38°C.

Experimental protocol

Four thirty minute pancreatic juice collections were made for each rat:

I. basal secretion
II. i.v. infusion of 0.35% BSA (Bovine Serum Albumin, Sigma, St. Louis, MO, USA) at the rate of 8 ml/h/rat
III. i.v. infusion of CCK33 (50 pmol/h/rat, CCK 33; Ferring AB, Malmö, Sweden) together with secretin (50 pmol/h/rat, Ferring AB, Malmö, Sweden) at the rate of 8 ml/h/rat. Both hormones were mixed in 0.35% BSA as a carrier
IV i.v. infusion with CCK33 (250 pmol/h/rat) together with secretin (250 pmol/h/rat) also made up in 0.35% BSA.

The infusions were made with an infusion pump (Harvard infusion/withdrawal model 2270, Harvard, UK). Each half hour collection was made into an Eppendorf tube and after collection the volumes were measured by visual comparison to volume standards. The samples were then stored at -20°C for further analysis.

Analysis

The pancreatic juice was analysed for total protein using the Lowry method (Lowry et al., 1951) and modified to be performed on 96 well micro plates with bovine serum albumin (A-7638, Sigma) as a standard (Pierzynowski et al., 1990). Trypsin activity was measured using Na-benzoyl-DL-arginine-p-nitroanilide (Sigma) as a substrate on 96 well micro plates (Pierzynowski et al., 1990). The chemical composition of the diets was determined according to AOAC (1990). Trypsin inhibitor content was determined by the method described by Smith et al. (1980) except that the enzyme was added later as suggested by Liu and Markakis (1989).

Statistical analysis was performed by analysis of variance (ANOVA) with Turkey correction using the Instat 2 computer program.

RESULTS

The chemical composition and trypsin inhibitor content of the diets is shown in Table 1. The trypsin inhibitor content of the raw cowpea was four times higher than the cooked cowpea and thirty times higher than the control diet (Table 1). On commencement of the study when the rats were allocated to the four groups, the mean weight of the rats in the groups was 31±2.5 g for the control group, 30±3.2 g for the raw cowpea group, 31±2.6 g for the cooked cowpea group and 32±2.1 g for the restricted feed group. After twelve weeks the rats were 202±9 g, 110±12 g, 128 ± 15 g and 150±7 g for the four groups, respectively. Feed intake in the restricted feed group was 60%, in cooked cowpea group 90% and in raw cowpea

TABLE 1

Chemical composition and trypsin inhibitor content of the standard diet and the cowpeas, as fed basis

	Standard diet	Raw cowpea	Cooked cowpea
Crude protein, %	22.0	26.7	25.7
Fat, %	2.4	1.2	1.5
Ash, %	7.2	3.6	3.5
Gross energy, MJ/Kg	20.0	18.9	18.8
Trypsin inhibitor[1]	0.2	6.1	1.6

[1] as mg trypsin inhibited/g

group 80% of those in control group fed standard diet. The different diets caused no significant difference in the volume of pancreatic juice and total protein content secreted by the rats under basal conditions and when BSA was infused i.v. as a carrier control to hormone infusion (Tables 2 and 3). Trypsin activity in basal secretions showed a tendency to be higher in the groups fed the restricted and cooked cowpea diets (Table 4).

Intravenous CCK + secretin significantly increased the volume and protein in all the groups in a dose dependent manner, however, the stimulation of the pancreatic juice volume outflow tended to be lower and was significantly lower in the control group as compared to the group fed cowpea diet and restricted diet, respectively. Trypsin activity outflow did not increase after exogenous CCK33+secretin treatment in any of the groups. However, higher trypsin outflow was observed in rats fed cowpea diets and restricted the diet as compared to the control rats.

TABLE 2

Pancreatic juice outflow ml/h/kg in basal and stimulated conditions in anaesthetised rats fed different diets (n = 8, mean ± SD)

Treatments	Standard diet *ad libitum*	Raw cowpea	Cooked cowpea	Standard diet restricted feed
Basal	0.44 ± 0.17^{a}	0.46 ± 0.31^{a}	0.38 ± 0.25^{a}	0.57 ± 0.29^{a}
Basal + BSA[1]	0.46 ± 0.20^{a}	0.51 ± 0.37^{a}	0.41 ± 0.19^{a}	0.61 ± 0.63^{ab}
CCK[2]+SEC[3]	1.53 ± 0.99^{ab}	1.91 ± 0.87^{ab}	2.00 ± 0.73^{ab}	2.30 ± 0.66^{bc}
(CCK+SEC*) x 5	2.37 ± 1.27^{b}	2.80 ± 0.80^{bc}	2.60 ± 0.94^{bc}	3.35 ± 0.60^{c}

[1] Bovine Serum Albumin

[2] CCK + cholecystokinin

[3] SEC – secretin

data with different superscripts in the columns and rows are significantly different at $P<0.05$

TABLE 3

Pancreatic juice protein outputs mg/h/kg in basal and stimulated conditions in anaesthetised rats fed different diets (n = 8, means ± SD)

Treatments	Standard diet *ad libitum*	Raw cowpea	Cooked cowpea	Standard diet restricted feed
Basal	54 ± 31^{a}	40 ± 37^{a}	47 ± 17^{a}	68 ± 46^{a}
Basal + BSA	60 ± 24^{a}	63 ± 36^{a}	66 ± 20^{a}	82 ± 50^{a}
CCK+SEC	121 ± 77^{ab}	156 ± 75^{ab}	172 ± 71^{ab}	153 ± 52^{ab}
(CCK+SEC) x 5	160 ± 68^{b}	321 ± 211^{b}	190 ± 103^{b}	234 ± 190^{b}

BSA, CCK and SEC as in Table 2

data with different superscripts in the columns and rows are significantly different at $P<0.05$

TABLE 4

Pancreatic juice trypsin outflow U/h/kg in basal and stimulated conditions in anaesthetised rats fed different diets (n = 8, mean ± SD)

Treatments	Standard diet *ad libitum*	Raw cowpea	Cooked cowpea	Standard diet restricted feed
Basal	290 ±19^{ab}	240 ± 61^{ab}	355 ± 12^{b}	405 ± 14^{b}
Basal + BSA	275 ± 57^{ab}	259 ± 35^{ab}	356 ± 99^{b}	391 ± 95^{b}
CCK+SEC	191 ± 60^{a}	249 ± 64^{ab}	313 ± 11^{b}	415 ± 80^{b}
(CCK+SEC) x 5	184 ± 51^{a}	241 ± 58^{ab}	246 ± 98^{ab}	389 ± 83^{b}

BSA, CCK and SEC as in Table 2

data with different superscripts in the columns and rows are significantly different at $P<0.05$

DISCUSSION

A comparison of the rats growth and enzyme secretion exhibited an interesting pattern: the best growth performance with minimal enzyme secretion was observed in the rats fed conventional diet *ad libitum*. Restricted access to the feed and dietary cowpea made the pancreas more sensitive to hormonal stimulation and caused higher basal pancreatic secretion. A 40% reduction in conventional feed only inhibited the growth by 25%. Considering that at the same time pancreatic secretion was enhanced, one can speculate that high pancreatic secretion when animals are exposed to the lack of food increases utilization of the food. This however, needs to be studied more profoundly. On the other hand, feeding the rat cowpea slightly lowered their feed consumption and significantly increased their pancreatic secretion. This however, was not correlated with improved feed utilization. On the contrary, feed utilization in both cowpea groups was significantly low. Thus, we can speculate that antinutrients in cowpea inhibited pancreatic enzyme digestion capacity in the intestinal lumen and in this way *via* feedback mechanisms stimulated higher pancreatic secretion (Fushiki et al., 1999). It is not to be excluded that cowpea – other than trypsin inhibitor – antinutritional factors could also have diminished the utilization of the food e.g., *via* diminished absorption of the nutrients since trypsin inhibitor level in one of experimental feed was effectively reduced *via* heat treatment and this only slightly improved growth of the animals in that group.

The rats that were fed the cowpea (raw and cooked) did not receive any other supplements thus the effects on the exocrine pancreatic secretions can be attributed to the cowpea. Diet had no effect on volume and total protein of the basal secretion of pancreatic juice. In rats and chickens, it has been shown that feeding raw soyabean or soyabean trypsin inhibitors resulted in hypersecretion of pancre-

atic enzymes and hypertrophy of the pancreas (Corring et al., 1986; Sauer and Mosenthin, 1999). Our results are in agreement with those since trypsin activity outflow was higher in rats fed raw and cooked cowpea. Sauer and Mosenthin (1999), in a review on antinutritional factors and the exocrine pancreas in pigs, have shown that there have been contradicting results on the effects of raw and processed soyabean on the exocrine pancreas. Żebrowska et al. (1983) showed an increase in volume of pancreatic secretion and protein but no effect on the activities of trypsin, chymotrypsin, carboxypeptidases A and B and amylase whilst Schumann et al. (1983) reported an increase in volume of secretion and doubling of protein secretion. Pancreatic enzymes were also reported to have been increased by feeding soyabean trypsin inhibitors. In a study to determine the effect of trypsin inhibitors in peas on the exocrine pancreas secretion of pigs, Gabert et al. (1996) showed that there was no effect on volume, protein and enzyme activities. Dietary inclusion of faba beans (which are high in tannins) in pigs also had no effect on the pancreatic secretions (Gabert et al., 1996). In a study on the effects of cowpea feeding on exocrine pancreatic secretions in pigs, dietary cowpea significantly reduced volume of secretions and total protein but trypsin activity was increased (Umapathy et al., 1999). Even pancreatic homogenates from pigs fed cowpea did not show any differences in protein content and trypsin activity (Erlwanger et al., 1999). It would thus appear that the differences that have been noted in studies may have been based on species differences and differences in the methods of collection of pancreatic juice and whether total or specific activities of enzymes are measured. Thus the finding that there was no difference in the protein and volume of pancreatic juice between our dietary groups could be in line with findings from pigs. The major differences in our study are seen in the trypsin activity, which, in the basal state was lowest in the raw cowpea fed rats, and, highest in the restricted fed rats. This could indicate that the raw cowpea had a suppressive effect on the trypsin activity outflow and the suppressive "factor" was heat-labile. Although we did not measure the weights of the pancreas from the rats for comparison, chronic feeding of cowpea to rats has been shown to induce an extensive increase in the relative and absolute weights of the pancreas and also caused an increase in the incidence of macroscopic pancreatic nodules (Grant et al., 1995). Infusion of the secretagogues resulted in an increase in volume and protein content of the pancreatic juice as expected. The lack of the effect on trypsin activity is puzzling and needs to be explored in further experiments.

It can be concluded that raw cowpea did not affect the basal and stimulated volume and protein output of pancreatic juice in the rat and slightly stimulated basal trypsin activity outflow. However, considering the body weight gain of the animals kept on the diets containing raw and cooked cowpea we postulate that cowpea antinutritional factors reduce utilization of the food probably *via* altered digestion of the feed components. Proportionally the best performance was seen in

the group kept on the restricted diet and as it was characterized by the highest pancreatic function led us to speculate that higher pancreatic secretion can improve utilization of the diet.

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STRESZCZENIE

Wpływ skarmiania krowiego grochu (*Vigna unguiculate*) na podstawową oraz stymulowaną cholecystokininą (CCK33) i sekretyną zewnętrzną sekrecję trzustki u szczurów w narkozie

Badania nad wpływem długotrwałego skarmiania krowiego grochu na zewnętrzne wydzielanie trzustki u szczurów, przeprowadzono na 32 czterotygodniowych szczurach, podzielonych losowo do 4 grup żywieniowych: I. zwierzęta żywione do woli standardową paszą (kontrolna), II. surowy krowi groch podawany do woli, III. gotowany krowi groch podawany do woli, oraz IV. ograniczony dostęp do paszy standardowej. Do przewodu trzustkowego założony był kateter umożliwiający całkowitą kolekcję soku trzustkowego.

Po 12 tygodniach żywienia, szczury otrzymujące diety z krowiego grochu oraz ograniczoną ilość paszy standardowej pobierały mniej paszy i gorzej rosły niż zwierzęta grupy kontrolnej. Rodzaj diety nie miał istotnego wpływu na objętość i ogólną ilość białka wydzielanych w warunkach podstawowych. Wystąpiła tendencja zwiększonej aktywności trypsyny w podstawowych i stymulowanych warunkach u szczurów otrzymujących ograniczoną ilość paszy oraz gotowany krowi groch w porównaniu z dwiema pozostałymi grupami. Dożylne podawanie CCK33 i sekretyny powodowały istotny wzrost objętości i białka wydzielanego w soku u szczurów wszystkich grup. Jednakże stymulowane wydzielanie soku trzustkowego (objętości) i białka było większe u szczurów otrzymujących dietę standardową w porównaniu z pozostałymi.

Skarmianie krowiego grochu zwiększa podstawową oraz stymulowaną sekrecję trzustki (objętość, wydzielanie białka i trypsyny) w podobnym stopniu jak u szczurów, którym podawano ograniczoną ilość paszy.