Physiological response in rats consuming crude cowpea (*Vigna unguiculata*) grains as substitute for commercial soybean cake. Morphometry and histological analysis of the digestive organs

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Thirty-six Sprague-Dawley rats of 100 ± 5 g liveweight were used according to a random block design with three replications. Six rats per treatment were used to study the effect of the intake of crude grains of *Vigna unguiculata* (var INIFAT 93) as substitute for commercial soybean cake on the morphometry and histology of the main organs related to protein metabolism. Five levels of substitution (20, 40, 60, 80 and 100 %) were studied from a basal diet of soybean-maize. There were no changes due to the treatments in the stomach, small intestine, large intestine and pancreas. Only a small decrease in absolute weight of liver and kidneys was found not in weight relative to liveweight or metabolic weight, which was explained by a decline in animal weight. Histologically, no differences were observed between treatments in the cuts from the duodenum, the jejunum, the ileum and the kidneys. Some anomalies were reported in the cuts from the liver and the kidneys in the animals consuming more than 80 % cowpea (congestion, hemorrhage and turbid tumefaction in the former and vacuolar degeneration and degenerative processes in the renal tubes). It is concluded that it is physiologically possible to substitute up to 60 % the commercial soybean cake by meal of crude cowpea grains, without affecting the main organs related to protein metabolism in rats.

Key words: *morphometry, digestive organs, histology, rats, cowpea, soybean*.

Cowpea (*Vigna unguiculata*) var INIFAT-93 is a promising legume for the feeding of farm animals. This is due to its good agricultural, foliage and grain yields, adequate bromatological composition and low content of anti-nutritional factors similar to the species of this family (Díaz and Padilla 1997 and Aguirre *et al.* 2002).

When introducing a new feed source in animal diets, the injuries it can cause to the organs of the digestive tract and those related to protein metabolism must be taken in mind. Several authors have described the causes of such injuries (Ly and Díaz 1979, Ibañez and González 1981 and Savón *et al.* 1995). The main ones are related to diet composition: fiber content and quality, passage rate and anti-nutritional factors (ANF) content. The bromatological study of the legume grains suggests that ANF are generally the potential cause of injuries in the digestive organs: protease inhibitors, lectins and non-digestible carbohydrates, among others (Etheridge *et al.* 1984 and Cheeke and Kelly 1989).

For the study of these injuries, the weighing of the different sections of the digestive tract and the microscopic analysis of cuts of their respective membranes is a common practice. This analysis demonstrates short and long term effects of the unbalance on protein supply and the negative interactions between the diet components with anti-nutritional properties and the digestive organs (main and accessory). Also, it provides a microscopic explanation for the decrease in the productive indicators. Despite being cowpea a species with high ANF, preliminary studies (Aguirre *et al.* 1998) and those in the literature (Makinde *et al.* 1996) suggest that the average protein quality of this legume could be due to these substances. Therefore, the objective of this experiment was to study the effect of the

substitution of the commercial soybean cake by crude cowpea grains meal on the physiology of rats, as model for monogastric animals, in the morphometric and hystological traits in the different sections of the gastrointestinal tract and the main accessory organs of the digestive function.

Materials and Methods

Thirty-six male albine Sprague- Dawley (conventional) rats of 100 ± 5 kg of liveweight were selected. They were provided by the National Center for Laboratory Animals (CENPALAB). The rats were distributed according to a random block design with three replications, six rats per treatment. They were free of bacteria, endoparasites and seven viruses (according to tests made by this institution). The treatments consisted of five levels of substitution (20, 40, 60, 80 and 100 %) starting from a basal diet of soybean-maize.

Cowpea and experimental diets composition was described by Aguirre *et al.* (2002).

Experimental procedure. After 15 d consuming the experimental diets, four hours after feed intake exactly, the animals were slaughtered by cervical dislocation to extract their accessory organs (liver, pancreas, both kidneys and the digestive tract). The latter was sectioned in stomach, duodenum, jejunum, ileum, cecum and final portion (colon + rectum) for their analysis. The accessory organs were weighed and expressed as absolute weight (g), weight relative to liveweight (LW) (g/g) and relative to metabolic weight (MW) ($g/g^{0.75}$). The sections of the digestive tract were weighed and measured with and without ileal content. A centimeter was taken from the duodenum, the jejunum and the ileum and before their manipulation, they were washed with salt solution adjusted at pH 7.2 for their preservation. These sections, together with the accessory organs, were preserved in phormaldehide solution at 10 %. Later, they were processed for their fixation in slides to be analysed in the microscope. Staining was carried out with hematoxilin (0.5 %) and eosine (1 %). A transversal cut was performed in the accessory organs and longitudinal and transversal cuts in the tubular organs (sections of the digestive tract). A Zeiss microscope with a photocamera attached was used.

Statistical analysis. The statistical analysis was in correspondence with the design selected. Duncan's (1955) test was used when necessary. The SPSS statistical package was used for regression and orthogonal polynomes analysis.

Results and Discussion

Table 1 shows the morphometry of the main digestive organs. None of the gastrointestinal tract sections was altered in weight or length, compared to the control, contrary to reports of Makinde *et al.* (1996) in studies with different species (pigs recently weaned). These authors found a decrease in the total length of the tract due to the intake of this legume. In this study, cowpea grain intake did not affect the microscopic traits of the digestive tract, thus, the anti-nutritional factors informed as direct cause of the hipo and hypertrophies of the tract (Cheeke and Kelly 1989 and Makinde *et al.* 1996) were not found and, if so, in very low concentrations.

In regards to accessory organs (table 2), only the pancreas weight was not affected by the different treatments, with normal values (Huissman and Tolman 1992) of approximately 0.70 g for rats of this liveweight. Liver and kidneys absolute weight was affected, decreasing (figures 1 and 2) as the percentage of cowpea in the diet increased. This performance in absolute weight can be explained by the productive results (Aguirre *et al.* 2002). The animals consuming more cowpea are larger and heavier and, thus, their organs should be heavier than those of smaller animals. Therefore, the weight relative to liveweight and to metabolic weight were determined as a means to uniform the results. There were no differences between treatments (table 2), being consistent with reports of other authors (Panigraphi *et al.* 1992).

Table 1. Morphometry of the digestive organs

Organs	Treatments (% of substitution of soybean by cowpea)						± SE
	Control	20 %	40 %	60 %	80 %	100 %	
Stomach							
Filled, g	16.41	15.59	14.70	14.75	16.36	15.09	0.43
Empty, g	1.17	1.07	1.13	1.18	1.22	1.03	0.06
Small intestine							
Length, cm	103.67	100.17	100.83	100.17	105.83	107.17	3.70
Filled, g	6.85	7.14	6.99	6.91	7.08	6.63	0.27
Empty, g	4.5	4.17	4.17	5.67	4.5	5.16	0.44
Cecum							
Length, cm	4.5	4.17	4.17	4.67	4.5	5.17	0.21
Filled, g	3.33	3.34	3.31	3.83	4.32	3.89	0.34
Empty, g	0.57	0.62	0.66	0.64	0.99	0.71	0.02
Rest of large intestine							
Length, cm	14.83	13.33	15.16	13.67	14.29	16.83	1-09
Filled, g	2.17	2.00	2.27	2.03	1.69	3.00	0.28
Empty, g	0.84	0.88	0.97	0.92	0.86	1.16	0.13

Table 2. Morphometry of the accessory organs

Weigth of the organs	Treatments (% of substitution of soybean by cowpea)						± SE
	Control	20 %	40 %	60 %	80 %	100 %	
Liver							
Absolute, g	5.65^{a}	5.41^{a}	5.47^{a}	5.60^{a}	4.98^{b}	4.93^{b}	0.16*
Relative to LW, g/g	0.032	0.034	0.034	0.035	0.032	0.034	0.002
Relative to MW, $g/g^{0.75}$	0.11	0.12	0.12	0.12	0.11	0.12	0.003
Pancreas							
Absolute, g	0.53	0.46	0.43	0.51	0.51	0.52	0.07
Relative to LW, g/g	0.003	0.003	0.003	0.003	0.003	0.003	0.004
Relative to MW, $g/g^{0.75}$	0.011	0.009	0.009	0.010	0.011	0.012	0.001
Kidneys							
Absolute, g	1.34^{a}	1.32^{a}	1.32^{a}	1.24^{ab}	1.16^{b}	1.19^{b}	0.03**
Relative to LW, g/g	0.008	0.008	0.008	0.007	0.007	0.007	0.0002
Relative to MW, $g/g^{0.75}$	0.03	0.03	0.03	0.03	0.03	0.03	0.0008

When analysing the histological cuts, the results were satisfactory up to 60 % inclusion of crude cowpea grains meal. Some alterations were found with higher levels. These microscopic analyses were conceived to find possible effects at cellular scale provoked by the anti-nutritional interactions. The changes in the brush border, the intestinal epithelial cells, the cell organization in the accessory organs, the vacuolization, congestion, proliferation, hypertrophy and hyperplasia affect the animal physiology at long term. This occurrs in an almost irreversible form and its consequences in the metabolic indices are not in a relatively short experimental period (15 d of experimental diets intake) as in this study. Thus, its observation allows to predict the appearance of further upsets.

No upsets of relevance were found in the intestine. The duodenum preserved its integrity. No upset was reported in the structure of the hairs or the calciform cells. In the

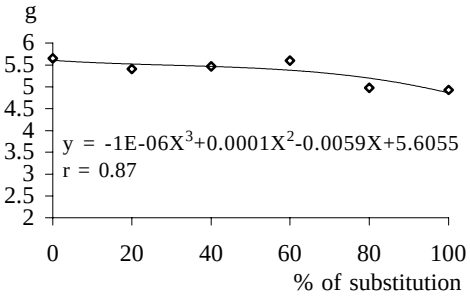

Figure 1. Curve of regression of the means of the absolute weight of the liver according to the percentage of substitution of soybean by cowpea ($P < 0.05$)

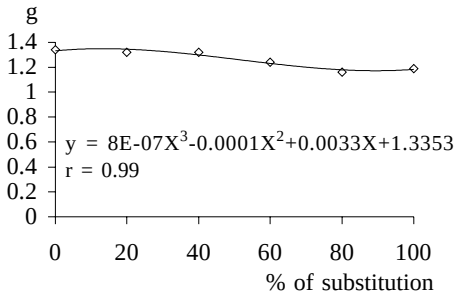

Figure 2. Curve of regression of the means of the absolute weight of the kidneys according to the percentage of substitution of soybean by cowpea ($P < 0.05$)

jejunum, a slightly proliferative swelling was observed, which could be associated with the presence of tannins (Ortiz *et al.* 1993). These authors reported a similar response in calciform cells of the ileum in rats due to the intake of tannins of kidney beans. However, the cell structure of the ileum was not altered in this study in any of the treatments.

There were no severe upsets of the cellular structure in the liver either in the treatments lower than 80 % substitution. However, in some regions, congestions and microhemorrages were found from this level. They could be due to the eutanasia method used and to the turbid tumefaction and the vacuolization that could be provoked by the presence of tannins (Ortiz *et al.* 1993).

No anomalies were found in the pancreas for the intake of the different levels of cowpea.

Some anomalies were observed in the kidneys in the treatments with 80 and 100 % substitution of soybean by cowpea. Vacuolar degeneration and degenerative processes were comparatively observed in the renal tubes. These results are consistent with those obtained in the organs weight. Renal upsets occurred at 80 % levels of substitution of soybean by cowpea. This could be due to anti-nutritional factors such as fitic acid (Díaz 2002) that provokes the precipation, among other upsets, of the metal salts by chelation in the kidneys, mainly divalent salts (Sakamoto *et al.* 1993).

The intake of crude cowpea grain meals as substitute of the commercial soybean cake did not affect, in morphology or histology, the main digestive organs related to protein metabolism in rats, although the upsets found in the kidneys and the liver substituting more than 60 % limit their inclusion. It is concluded that it is feasible to include up to this level in rations for rats, as model of monogastric animals.

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