Protein metabolism and productive performance of rats consuming raw cowpea (*Vigna unguiculata*) grain meal to substitute commercial soybean cake

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Thirty six male Sprague-Dawley rats of 100 ± 5 g live weight were selected, according to a completely randomised design with three replications and six rats per treatment, to study the physiological effect of the consumption of raw grain meals of *Vigna unguiculata* var INIFAT-93, as substitute of the commercial soybean cake, on protein metabolism and productive performance. Treatments consisted of six experimental diets, a maize and commercial soybean cake control and five rations in which the latter was substituted by raw grain meal of cowpea at 20, 40, 60, 80 and 100 %. Out of the indicators measured, neither the apparent biological value (ABV) nor the nitrogen balance (NB) were altered in any case, ranging between 50-54 % and 2-2.4 g, respectively. However, there was a decrease in the apparent faecal nitrogen digestibility (AFND) and the protein efficiency ratio (PER) of 10 and 20 %, respectively, in all treatments, compared to the control. In the first case, the values were from 81 to 71 % and, in the second, from 2.2 to 1.7. Carcass weight and average daily gain decreased starting up in 80 % substitution of the soybean cake by cowpea from 71.2 to 58.9 g and from 3.9 to 3.0 g/d, respectively. All severe alterations were found at levels higher than 80 % substitution of soybean cake by cowpea. Therefore, it is concluded that the substitution at the inferior level (60 %) in diets for rats, as model for physiological studies of non-ruminant species, is feasible.

Key words: *protein quality, productive performance, rats, cowpea, soybean, protein metabolism of monogastrics*

Cowpea (*Vigna unguiculata*) is a promissory legume for the feeding of farm animals. This is due to its good agricultural yields both as foliage and grains; its adequate bromatological composition and low contents of anti-nutritional factors that are common to the rest of the species of this family (Díaz and Padilla 1997).

The variety INIFAT-93 has been developed at the National Institute of Basic Research of Tropical Agriculture (INIFAT) through genetic improvement. It shows a potential yield of 700-1100 kg/ha of grains with an average protein composition of 24 % raw protein (Díaz and Padilla 1997), specific growth and resistance to the main pest and diseases affecting this crop (INIFAT 1996 and Díaz *et al*. 1997). It has a poor concentration of anti-nutritional factors as protease inhibitors, tannin (León1994), lectin (D´Mello 1997) and phytic acid (Arora 1995). Regarding its protein composition, it has been reported that its quality is adequate (Grant *et al.* 1995 and Aguirre 1997) in comparison to soybean, although some authors state that it is deficient in sulphurous amino acids, as the majority of the legumes.

All these characteristics confirm its introduction in the feeding of non-ruminant species. Thus, experiments on the protein quality of its raw grain meal were carried out (Aguirre *et al*. 1998). The satisfactory results obtained propitiated subsequent studies. The objective of this experiment was to study the effect of the substitution of the commercial soybean cake by the raw grain meal of cowpea on the protein metabolism of rats taken as experimental model of the digestive physiology of non-ruminant species.

Materials and Methods

The *Vigna unguiculata* variety INIFAT-93 harvested in areas of the Institute of Animal

Science was used for this experiment. The grains were sun-dried for two days after the harvest to reduce their humidity content from 10 to 12 % and later stored at temperatures between 4 and 10 °C and with relative humidity lower than 45 %. For the preparation of the diets, the grains were finely ground in a hammer mill to attain an average particle size of 0.5 mm. The proximal analysis of five lots of meals showed (% DM): DM 90.27, CP 23.33, CF 6.08, ashes 4.23, Ca 0.26, P 0.47 and GE 14.92 MJ/kg.

Six experimental diets, a control and five substitution levels were prepared. Their composition is shown in table 1. The control consisted of a soybean-maize conventional diet, designed to cover the rat requirements in this study, according to Reeves *et al.* (1993). The other five treatments consisted of the substitution of increasing levels of a commercial soybean cake by cowpea meal, based on the protein content of both sources: 20, 40, 60, 80 and 100 %. The diets were balanced according to the protein content and the protein/energy ratio. The rest of the indicators were also found in close ranges. All were determined according to AOAC (1990), except TP that was determined by the method of Bernstein (1970), cited by Meir (1986). GE was determined in an adiabatic Gallenkamp calorimeter.

Animals. Thirty-six albino male Sprague-Dawley (conventional) rats of 100 ± 5 g live weight supplied by the National Centre of Laboratory Animals (CENPALAB) were selected. Animals were free of eleven bacteria (tested), endoparasites and seven viruses (tested), according to this center.

Experimental procedure. The animals were housed for 20 d in individual metabolism cages (Eggum 1973). The first five days were for the adaptation to the diets and the following 15 d for sampling (Donkoh *et al*. 1994). The diet was supplied at a level of 10 g of DM/d and the water *ad libitum.* The daily sampling consisted of measuring diet rejection and the collection of faeces and urine for the determination of the nitrogen content. Urine was collected in a 50 mL flask with a 5 % H_2SO_4 solution

Table 1. Composition of the experimental diets (g/kg)

Ingredients	Treatments (% of soybean substitution by cowpea)					
	Control	20 %	40 %	60 %	80 %	100 %
Maize	540.97	464.67	389.25	313.78	238.29	162.86
Soybean	341.53	273.22	204.92	136.61	68.31	0
Cowpea	0	144.61	288.33	432.11	575.90	719.64
Soybean oil	70	70	70	70	70	70
Mineral mixture[1]	35	35	35	35	35	35
Vitamin mixture[1]	10	10	10	10	10	10
Choline bitartrate	2.5	2.5	2.5	2.5	2.5	2.5
Proximal analysis, %						
DM	87.05	87.29	87.51	89.41	89.57	88.19
CP	19.38	18.83	18.46	18.87	19.26	19.91
TP	18.70	17.82	18.25	17.33	17.72	17.75
CF	3.35	4.97	4.47	5.26	4.49	5.46
Ether extract	4.89	5.36	5.07	7.02	7.10	6.20
Ash	5.72	5.82	5.66	6.95	5.53	5.47
Ca	0.30	0.30	0.30	0.42	0.52	0.59
P	0.48	0.50	0.55	0.57	0.52	0.59
GE, MJ/kg	16.64	15.98	16.20	15.74	15.65	15.96
Protein/energy	1.16	1.18	1.14	1.20	1.23	1.24

[1]According to Reeves *et al.* (1993)

(throughout the whole period) to avoid nitrogen losses as ammonia. The amounts of nitrogen consumed (Nc, mg) and excreted in the urine (Nu, mg) and in the faeces (Nf, mg) and the nitrogen balance (NB), apparent faecal nitrogen digestibility (AFND, %) and the apparent biological value (ABV, %) were calculated according to the equations described in the literature (Eggum 1973 and Aguirre *et al*. 1998).

The animals were weighed at the beginning of the sampling (five days) and at 15 d they were weighed again and slaughtered by cervical dislocation. The PER (Aguirre *et al*. 1998) and productive indicators such as DM consumption (g), DM conversion, protein conversion, average daily gain (g/d) and carcass weight (g) were calculated.

Statistical analysis. A random block design was used. Six rats, one for each treatment, formed each block and this was replicated six times. Duncan's (1955) multiple range test was used for mean comparison when necessary. The statistical package SPSS was used for the regression and orthogonal polynomial analyses.

Results and Discussion

Table 2 shows the results obtained for the indicators of protein quality. The substitution of soybean by cowpea did not affect the NB of the animals in any treatment. All were maintained in the normal range described in the literature for rats consuming a medium quality protein, that is, from 2.0-2.6 (Eggum 1973, Bravo *et al.* 1994 and Fernández-Quintela *et al*. 1998). Similar performance was observed in the apparent biological value (ABV). All values are in the normal range for these proteins, approximately between 50 and 55 % (Eggum 1973, Nti and Phahar 1995 and Fernández-Quintela *et al*. 1998).

However, cowpea inclusion in the diet provoked a decrease in AFND ($P < 0.001$) compared to the control and to the most common values described for average protein quality, that is, from 80-90 % (Bravo *et al*. 1994, León 1994, Nti and Phahar 1995, Juskiewricz and Zdunczyk 1997 and Occeña *et al*. 1997). This could be due to the protein structure (resistance to certain enzymatic attack, for example) and the presence of anti-nutritional factors (tannins, for example) trapping, inhibiting or hindering their maximum degradation (Liener 1989).

On analysing the results of the nitrogen use contributed by the diet, an increase in the Nf excretion (figure 1) can be observed with a maximum associated with the treatment of 80 % substitution of soybean by cowpea. This indicates that the cowpea protein, under these conditions, is less digestible than that of the soybean, that is, as the inclusion percentage in the diet increases, the animal attains to absorb less dietary protein that is directly reflected in the drop of the AFND. On analysing the PER values (table 2) the above mentioned is confirmed. These also decrease ($P < 0.05$) compared to the control and they are lower considering the values (2.00-2.20) reported by Júskiewiez and Zdúnczyk (1997), Occeña *et al*. (1997) and Fernández-Quintela *et al*. (1998), on increasing the inclusion levels

Table 2. Indicators of the protein quality

Indicators	Treatments (% of soybean substitution by cowpea)						SE ±
	Control	20 %	40 %	60 %	80 %	100 %	
NB, g	2.37	2.09	2.06	2.28	2.38	2.20	0.12
ABV, %	54	49.83	50.17	52.83	54.83	50.83	2.65
AFND, %	81.34^{a}	75.47^{b}	73.39^{b}	73.70^{b}	71.65^{b}	72.02^{b}	1.19***
PER	2.18^{a}	2.02^{ab}	1.95^{ab}	1.90^{ab}	1.88^{b}	1.71^{b}	0.09*

^{abc} Different letter in the same row differ significantly at $P < 0.05$ (Duncan 1955)
*$P < 0.05$ ***$P < 0.001$

of cowpea. In view of the fact that the nitrogen retention was similar for both sources (ABV), this PER decrease, that is, in the live weight gain at the expense of the protein consumed, confirms that the protein quality of the cowpea is lower than that of the soybean, that is, in spite of being retained in a similar way, it promotes less growth. The explanation for this performance could be given by a disproportion in the amino acid pattern according to the animal requirements: the protein does not completely supplement one or various amino acids necessary for tissue synthesis and/or their rechanging.

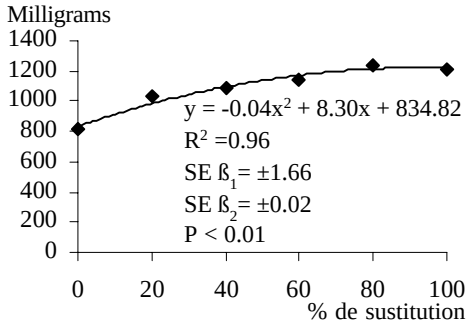

Figura 1. Means of the regression curves of the nitrogen excreted in the feaces (Nf) according to the soybean percentage by cowpea

The results of the indicators of the productive performance are set out in table 3, according to those previously discussed of protein use. In the first place, the increase in cowpea inclusion did not affect the diet consumption, neither the dry matter nor the protein conversions, although in this latter, a slight decrease ($P < 0.05$) was observed, and there is a reduction in the performance according to the reductions of the ADG and carcass weight indicators. Makinde *et al*. (1996) described a similar effect by cowpea consumption (in recently weaned piglets) and they proposed that it was due to the presence of anti-nutritional factors, mainly tannins. This study is another evidence that the cowpea protein has a lower quality than that of the soybean, at similar ingestion levels of both sources. The animals fed soybean have a superior performance concerning body weight gain.

In general, the reductions in the indicators are of poor magnitude, even when the maximum substitution is made (100 %), that is, that even if there are quantitative differences, they are small and the protein quality between both sources is quite similar. This was very relevant, since in the 100 % substitution treatment the cowpea is included in 72 % of the diets, that is, almost the total amount of the diet is made up by cowpea and no sudden changes were found in the use of the protein concerning a very high quality source such as the commercial soybean cake that is only found at 34 % in the control. However, at levels higher than 80 % substitution of the soybean by cowpea there were possible severe negative effects on the nitrogen use. Therefore, it is concluded that the substitution of up to 60 % of commercial soybean cake by cowpea meal is physiologi-cally feasible in diets for rats, as model for non-ruminant animals.

Table 3. Indicators of the productive performance

Indicators	Treatments (% of soybean substitution by cowpea)						SE ±
	Control	20 %	40 %	60 %	80 %	100 %	
DM intake, g	9.39	9.28	9.20	9.55	9.42	9.06	0.12
DM conversion,	2.43	2.72	3.18	2.84	2.80	3.00	0.21
Protein conversion	0.47	0.51	0.59	0.54	0.54	0.60	0.04
ADG, g/d	3.96^{a}	3.52^{ab}	3.33^{b}	3.43^{ab}	3.41^{b}	3.08^{b}	0.16*
Carcass, g	71.22^{a}	68.99^{ab}	67.85^{ab}	67.78^{b}	65.62^{bc}	58.87^{c}	1.67***

^{abc} Different letters in the same row differ significantly at $P < 0.05$ (Duncan 1955)
*$P < 0.05$ ***$P < 0.001$

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