Ann Zootech (1990) 39, 53-61

Original article

Amino acids and starch digestibility and true metabolizable energy content of raw and extruded jackbeans *(Canavalia ensiformis)* in adult cockerels

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(Received 12 September 1989; accepted 15 December 1989)

Summary — Four experiments were conducted with adult cockerels to determine digestibility of amino acids, starch digestibility and true metabolizable energy in ground raw (RJB) and extruded (EJB) jackbean seeds. Three feeding methods were used: *ad libitum* feeding, dry and wet force feeding. Both RJB and EJB were tested alone or at 3 levels of inclusion, 10, 30 and 50%, in combination with maize starch or yellow maize. The results indicated that extrusion significantly increased amino acid digestibility from 73.6% in RJB to 94.0% in EJB. Similarly, starch digestibility was found to be 65.2% and 96.0% for RJB and EJB respectively, using a force feeding technique of pure bean meal. True metabolizable energy content of EJB varied from 2900–3200 kcal/kg on a dry matter basis, and was 400–600 kcal/kg higher than that of RJB depending on the method used. Toxicity of JB proved to strongly decrease voluntary food intake and justify the use of different experimental approaches to ascertain the metabolizable energy value. These data confirmed the potential value of JB as a feedingstuff for poultry and also indicate that extrusion may represent a practical method for improving digestibility of its nutrients by 20% for amino acids and 30% for starch.

amido acid / starch / metabolizable energy / digestibility / cockerels / extrusion / jackbean / *Canavalia ensiformis* / poultry

Résumé — Digestibilité des acides aminés et de l'amidon, évaluation de la teneur en énergie métabolisable des graines de *Canavalia ensiformis* crues ou extrudées chez le coq adulte. *Quatre expériences ont été réalisées sur coqs adultes pour mesurer la digestibilité des acides aminés, de l'amidon et la teneur en énergie métabolisable de graines broyées de* Canavalia ensiformis *crues (RJB) ou extrudées (EJB). Trois méthodes d'alimentation ont été utilisées :* ad libitum, *gavage sec, gavage humide. Les deux types de* Canavalia *ont été testés soit purs, soit en mélange avec de l'amidon de maïs ou du maïs à trois taux d'inclusion : 10, 30 et 50%. Les résultats ont montré que l'extrusion augmentait significativement la digestibilité des acides aminés : de 73,6% (RJB) vs 94% (EJB). Parallèlement, la digestibilité de l'amidon a été évaluée à 65,2% (RJB) et 96,0% (EJB) en utilisant une technique de gavage sec des farines de* Canavalia *pures. L'énergie métabolisable «vraie» mesurée de EJB variait selon les essais de 2 900 à 3 200 kcal/kg (par rapport à la matière sèche)*

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soit de 400 à 600 kcal/kg plus élevée que celle de RJB. La toxicité des graines de Canavalia *a fortement diminué l'ingéré alimentaire volontaire des coqs, ce qui justifie le recours à différentes techniques pour évaluer leur concentration en énergie métabolisable. Ces résultats confirment la valeur nutritionnelle potentiellement élevée de* Canavalia *pour l'alimentation des volailles et indiquent que l'extrusion améliore la digestibilité des nutriments qu'elle contient de 20% pour les acides aminés et 30% pour l'amidon.*

acide aminé / amidon / énergie métabolisable / digestibilité / coqs / extrusion /* Canavalia ensiformis / *volaille

INTRODUCTION

Canavalia ensiformis, the "Jackbean" (JB), is a legume adaptable to a wide range of agronomical and climatic conditions. Yields of seed as high as 4t/ha have been reported by the National Academy of Sciences (1979) in tropical countries. The proximal chemical composition of JB seeds has been studied (Leon *et al*, 1989) and it appears to be suitable for monogastric animal nutrition: *ie* (% dry matter, DM) 31.3% of crude protein (N x 6.25), 35.7% of starch, 20.1% of cell wall, 3.5% of lipids and 3.0% of ashes. Its composition is similar to that of field beans. Its amino acid composition shows low content of lysin and sulphur containing amino acids.

However, JB is known to contain several toxic and antinutritional factors. The best known is concanavalin A (Con A), a lectin (Hague, 1975; Liener, 1986) which has been reported to interfere with nutrient absorption (Jaffé, 1980). Canavanine, a structural analogue of arginine, is present in the seed at more than 3.0% of DM (Rosenthal, 1972; Léon *et al*, 1989). Canatoxin has recently been identified as a non hemagglutinating toxic protein distinct from concanavalin A (Carlini and Guimaraes, 1981).

JB seeds are of poor nutritive value unless heated (Borchers and Ackerson, 1950). Regarding the nutrient bioavailability of this seed very little is known (Liener, 1986). Practical experiments on broilers and laying hens show a severe reduction of food intake, growth rate and feed conversion for dietary levels as low as 10% raw JB seeds (Montilla *et al*, 1981; Vierma and Montilla, 1983; Vierma, 1984; Carabano *et al*, 1985; Leon *et al*, 1989). Partial detoxification by heat treatment of the seeds, mainly by autoclaving (Montilla *et al*, 1981; Vierma and Montilla, 1983; Vierma, 1984) has been reported. D'Mello measured the metabolizable energy (ME) content of JB seeds soaked in water and autoclaved and reported an average value of 2 850 kcal/kg DM in chicks (D'Mello *et al*, 1985).

Four experiments were conducted to determine the digestibility of amino acid and starch and the metabolizable energy content of raw and extruded JB seeds. Our goal was to determine whether or not JB seed represents a real potential for poultry nutrition before proceeding to a detailed study of its toxicity (Leon *et al*, 1990, unpublished results).

MATERIALS AND METHODS

The low "palatability" and high toxicity of JB seeds led us to test its digestibility by different methods: dry and semi-liquid force feeding and *ad libitum* feeding, regimes and different concentrations of JB in the test diet (10, 30, 50 and 100%) associated with 2 different diluents (maize starch or maize).

Animals and diets

Adult Isa Brown cockerels, aged between 30 and 60 weeks, depending on the experiment, were taken from a group of 72 birds housed in individual metabolic cages inside environmentally controlled rooms (20 °C). At the beginning of each test the cockerels were allocated to the treatments according to their live weight (3–4 kg).

A batch of 100 kg of JB seeds from the variety "original" grown in the Faculty of Agronomy of Venezuela, was sent to France and half of it was extruded on an industrial extruder by Société Dievet (Parc Industriel d'Incarville, 27100 Le Vaudreuil, France). Temperature at the output of the extruder was 130 °C. The complete chemical composition of the seeds had been determined previously (Léon *et al,* 1989).

Pure JB, raw (RJB) or extruded (EJB) was ground and fed alone in experiment 2. In experiments 1 and 3 it was diluted with maize starch (50/50, w/w). In experiment 4 it was diluted with yellow maize, at 2 levels of JB inclusion (10 and 30%, w/w). Protein free diets (maize starch or starch + glucose, 50/50, w/w) were used in experiment 1.

Experimental design

Experiment 1 was set up to determine amino acid digestibility. Two techniques were applied to 3 diets: a protein free diet, a RJB/maize starch diet and an EJB/maize starch diet according to a factorial design 2 x 3 (6 treatments).

1) Single dry force feeding (Sibbald, 1982) of 60 g of each JB diet, or 30 g of the protein free diet (maize starch) per bird, between 2 periods of starvation of 48 h.

2) Semi-liquid force feeding meals were administered 3 times during 48 h. The feeding period was preceded and followed by 1 period of starvation of 24 h. Intake and water dilution are reported in table I. The diets were mixed with water before each meal. The dry matter content of the pastes was measured at several times during the test and found to be stable and comparable with the calculated values.

Each one of the 6 treatments was applied to 6 individually controlled cockerels. Feces were collected daily during the last 48 h in the dry force fed group and during 72 h in the semi-liquid force fed group. Feces were freeze dried, weighed, ground and individually analyzed for nitrogen after uric acid elimination (Terpstra and Dehart, 1974) and then pooled by groups of 3 cockerels for amino acid, starch and energy analysis.

Experiment 2 was designed to determine starch digestibility. Fifty grams of pure RJB or pure EJB were force fed in a dry form to 9 cockerels for each treatment before and after a starvation period of 48 h. Feces were individually collected for 48 h and processed for starch, nitrogen and energy analyses after freeze drying, weighing and grinding.

Table I. Semi-liquid force feeding procedure. * *n* : 6 birds/diet.

Diet	*Composition (w/w)*	*Water addition final paste (%)*	*Average intake of the dry diet* * *(g/cockerel/48 h)*	*Standard deviation (g)*
Protein free	Starch + glucose	35.1	181.1	1.5
Raw JB	RJB + starch	51.7	172.8	3.7
Extruded JB	EJB + starch 50/50	62.1	166.6	2.8

Experiment 3 studied individual variability of metabolizable energy values. The same dry force feeding procedure and the same diets as in Experiment 1 or 2 were used, except that feces were individually analyzed for gross energy and nitrogen.

Experiment 4 metabolizable energy was evaluated using *ad libitum* fed cockerels. A standard procedure (Picard *et al,* 1985) was used with 6 individual cockerels for each treatment during 4 successive periods, namely, 48 h adaptation to the diet — 24 h starvation — 48 h *ad-libitum* test feeding period–24 h starvation, with total feces collection during the last 72 h.

Analytical methods

Dry matter content was measured after heating the samples at 103 °C for 6 h. In Exp 1, the non-uric nitrogen of feces was measured according to the procedure of Terpstra and Dehart (1974). Starch was analyzed by the amyloglucosidase dimethyl-sulfoxide procedure as described by Conan and Carré (1989).

Amino acid determinations were performed on 4 replicate samples for feeds and on 2 for feces. Hydrolysis of protein was performed using HCl (6 N) at 100 °C for 24 h. Amino acids in the hydrolysate were separated and quantified using a LKB 4400 amino acid analyzer. Gross energy measurements were made by the use of a Gallenkamp adiabatic calorimeter.

Statistical methods

Due to the limited number of treatments and the very large differences observed, only the means and standard deviations are presented for Exp 1—3. In exp 4, an analysis of variance was carried out for the basal diets and the variance error of ME values was calculated for each level of JB inclusion (Yoshida, 1972).

RESULTS

Amino acid digestibility (exp 1)

Since no differences in amino acid digestibility were detected between semi-liquid and dry procedures, the average results for each JB diet are given in table II. Digestibility coefficients were corrected for the endogenous losses measured in the feces of the cockerels receiving the protein free diets. The average sum of the amino acid analyzed in the excreta of the animals receiving the protein free diets was 261 mg/cockerel/24 h with the dry feeding procedure *vs* 286 mg/cockerel/24 h with the semi-liquid feeding procedure. These results suggest that the differences in amount and moisture content of the feed given did not exert a large effect on endogenous amino acid excretion.

Data shown in table II revealed that a significant improvement in amino acid availability was obtained when JB was extruded. On average, the digestibility of the protein fraction of EJB was 94%. These results indicate that extrusion may offer a practical method for improving the nutritive value of JB for poultry diets. The data also show that canavanine, a non-protein amino acid, is readily digestible for the adult bird from both RJB and EJB. Since this amino acid makes an important contribution to the total N content of JB (Léon *et al,* 1989), it causes overestimation of the actual digestibility value of the protein present in RJB.

Starch digestibility

A preliminary trial was set up to evaluate the digestibility of starch in RJB and EJB (exp 1). These 2 JB meals were force fed in combination with corn starch (50:50, by weight) with or without water addition. A wide variation was found between the duplicates for a given sample of RJB. Therefore, a second experiment was conducted on 9 cockerels per JB sample without the addition of corn starch (exp 2).

Table II. True amino acid digestibility of raw and extruded Jackbean seeds. * True : corrected for the endogenous losses (amino acid excreted by the protein free fed group); ** SEM = Standard error of mean. The number of replicates is 4 (pooled sample of three cockerels) by average for amino acids and 12 (individual measurements) by average for nitrogen digestibility.

	*True * amino acid digestibility (%) ($\bar{X}$ ± SEM) ***	
Amino acid	*Raw JB*	*Extruded JB*
Aspartic acid	74.8 ± 5.3	93.1 ± 1.5
Threonine	69.8 ± 6.0	92.2 ± 0.9
Serine	75.7 ± 5.1	95.3 ± 0.4
Glutamic acide	74.1 ± 5.7	95.5 ± 0.7
Alanine	71.1 ± 7.1	92.9 ± 0.5
Valine	68.5 ± 6.6	94.1 ± 0.8
Isoleucine	72.4 ± 6.2	95.3 ± 1.1
Leucine	73.8 ± 5.4	94.3 ± 0.7
Tyrosine	73.9 ± 6.2	95.6 ± 1.1
Phenylalanine	74.0 ± 5.5	94.9 ± 0.6
Lysine	73.7 ± 5.9	93.5 ± 2.0
Histidine	75.3 ± 4.9	91.3 ± 0.6
Arginine	76.9 ± 4.9	92.9 ± 3.0
Sum of analyzed AA	73.6 ± 5.6	94.0 ± 0.7
Canavanine	97.1 ± 0.9	95.5 ± 2.0
True * digestibility of N after uric acid elimination in the feces (%)	79.3 ± 2.2	91.5 ± 1.1

The results clearly show a significant improvement of starch digestibility of JB after extrusion (65.2 ± 3.2% *vs* 96.0 ± 0.4%, respectively). This effect is in agreement with that observed for amino acid digestibility.

Metabolizable energy values ME (exp 1–4)

Table III shows the effect of JB level in the diet on food intake. Both EJB and RJB induced a severe food intake decrease when its concentration in the diet was higher than 10%. This is the major toxic effect of JB on birds. Table IV shows the influence of the inclusion level on ME determination. At low inclusion levels (10%) the residual error of the mean calculated for JB ME values is large, 363 kcal/kg DM (table IV). This illustrates the difficulties usually found in assaying the ME value of a toxic feed ingredient such as JB.

Table IV summarizes the metabolizable energy ME values obtained from the 4 ex-

Table III. Experiment 4. Food intake (g/cockerel/48 h). * SD = standard deviation of 6 individually controlled cockerels by treatment.

% JB in the diet	*Raw Jackbean ($\overline{X}$ ± SD) **	*Extruded Jackbean ($\overline{X}$ ± SD)*
0 (maize)	181.3 ± 25.7	
10	160.7 ± 29.9	164.6 ± 53.1
30	69.6 ± 28.8	73.7 ± 34.1

Table IV. Evaluation of the metabolizable energy content of Jackbean seeds using different techniques. * Corrected for endogenous energy losses and for N balance = 0. ** When possible the results are given as S + SEM calculated from Yoshida (1972). *** One low value eliminated.

Experiment	*True * metabolizable energy (kcal/kg DM of seed) ** RJB*	*EJB*	*Difference*
1) Estimation from diets containing 50% JB + 50% maize starch (*n* = 6 cockerels/treatment)			
- dry force feeding (60 g/bird)	2 419	2 880	461
- wet force feeding (180 g DM/bird)	2 738 ***	3 209	471
2) Estimation from pure Jackbean seed (*n* = 9 cockerels/treatment)			
- dry force feeding (50 g/bird)	2 771 ± 85	3 183 ± 82	412
3) Estimation from diets containing 50% Jackbean + 50% maize starch (*n* = 6 cockerels/treatment)			
- dry force feeding (60 g/bird)	2 395 ± 282	2 990 ± 30	595
4) Estimation from diets containing Jackbean (10 or 30%) + maize *ad libitum* feeding (table III)			
Level of inclusion 10%	2 067 ± 363	2 488 ± 363	421
Level of inclusion 30%	2 485 ± 110	2 984 ± 110	499

periments. All data are expressed in terms of true metabolizable energy corrected for zero nitrogen balance (Sibbald, 1982).

The force feeding experiments gave results consistent with the 30% level of exp 4 (table IV), namely, 2 500 kcal ME/kg DM for RJB and 3 000 kcal ME/kg DM for EJB. However, in exp 2 when JB samples were force fed alone, this led to higher ME values (2771 *vs* 3183 kcal/kg DM for RJB and EJB, respectively). In all cases the difference between raw and extruded Jackbean ranged between 400 and 600 kcal ME/kg DM.

DISCUSSION

The above results confirm the high potential nutritive value of JB seeds after heat treatment. Extrusion improved the amino acid digestibility by 20%, the starch digestibility by 30% and the ME values of JB seeds by 500 kcal ME/kg DM. D'Mello *et al* (1985) measured a ME content of 2 850 kcal/kg of autoclaved JB seeds which is within the range of our results between RJB and EJB. The nutritive value of extruded Jackbeans is similar to that of the field beans (Guillaume, 1978; Lacassagne *et al,* 1988).

In general, seed legumes having no major lectin toxicity, such as field beans, show a large improvement in digestibility following heat treatment (Guillaume, 1978; Lacassagne *et al,* 1988; Conan & Carré, 1989). However, under our conditions, EJB remained toxic as demonstrated by the drastic reduction in food intake observed upon feeding 30% of JB to the birds (table III). It is possible that the extrusion process resulted in structural modifications of the starch and protein fractions of JB which led to improvements in digestibility of these fractions. The presence of protease inhibitors in Jackbean seeds has been reported (Liener and Kakade, 1980), which may have been destroyed by extrusion.

The high digestibility of canavanine in both raw and extruded JB suggests that the antinutritional factors present in RJB may exert their actions by interfering predominantly with the digestion of the protein and starch components of JB with minor impact on absorption of free nutrients such as canavanine.

The exact mode of action of extrusion cannot be elucidated in the present study. The major thermolabile antinutritional factor present in RJB is concanavalin A. Lectins are reported to affect nutrient utilization by different mechanisms including binding to the glycoproteins and glycolipids of the digestive tract mucosa (Jaffé, 1980; Liener, 1986), inhibition of the activity of enzymes of the brush border of enterocytes (Rouanet *et al,* 1983) and/or by interaction with the adherence of enteric bacteria to the intestinal wall (Jayne and Williams, 1973).

A precise evaluation of the nutritive value of a raw material (such as *Canavalia*) containing antinutritive factors raises some specific methodological difficulties (Picard *et al,* 1985). In this case, the ME determination should be accompanied by other analyses such as digestibility of nitrogen after uric acid elimination and starch which usually yield more precise values. Also, when using force feeding or *ad libitum* feeding with low food intake, there are other factors that may affect the accuracy of the results such as: the use of abnormally high concentrations of the raw material in the diet (Lessire *et al,* 1985), or variability of the correction factors (for endogenous losses or/and nitrogen balance). All these factors should be taken into account for the correct interpretation of the results.

The variability of the ME results reported in table IV could be explained by methodological factors as well as effects of the toxicity of JB seeds. Force feeding procedures may affect the digestive function of the birds especially when a pure or highly concentrated diet in the toxic compounds is force fed. Maize starch digestibility is inaccurately supposed to be completely digested in experiments 1 and 3. The measurement of ME content in JB seed using various techniques is a way to avoid a too dangerous error which could be associated with a single test procedure. More accurate data will be necessary when the seed is correctly detoxified by an appropriate technology.

The observed effect of extrusion on the ME content of JB seeds can be further discussed taking into account the measured digestibility values of starch and crude proteins for RJB and EJB, the gross energy content of starch (4.1 kcal/g) and the ME content of digestible crude protein (4.3 kcal/kg).

A theoretical calculation of the difference of ME concentration between EJB and RJB based on major nutrients (starch and proteins) could be:

ME extrusion effect:

$$[4.1 \times (0.96-0.652) \times 357 + 4.3\ (0.915-0.793) \times 313] = [451 \text{ kcal/kg DM} + 163 \text{ kcal/kg DM}] = 614 \text{ kcal/kg DM}$$

where:

4.1 = gross energy content of starch (kcal/g); 0.96 = digestibility of starch from EJB; 0.652 = digestibility of starch from RJB; 357 = starch content is JB seed (g/kg DM); 4.3 = ME content of crude protein (kcal/g); 0.915 = digestibility of crude protein from EJB; 0.793 = digestibility of crude protein from RJB; 313 = crude protein content of JB seed (g/kg DM).

The result (614 Kcal/kg DM) is slightly higher than the directly measured ME differences between EJB and RJB which vary between 412 and 595 Kcal/kg DM (table IV). Extrusion improves the ME content of JB primarly by improving the digestibility of the starch fraction (73% of the effect).

ACKNOWLEDGMENTS

The authors thank Dr Legoy from Dievet for extruding the Jackbean seeds; Protinal; Proagro; Polar and Mendoza Foundations (Venezuela); the French Embassy in Venezuela and Centre d'Etude et de Formation des Ingénieurs for supporting this research.

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