

Nutritional value of the proteins of soybeans roasted at a small-scale unit level in Africa as assessed using growing rats

Charles Luanga Ouédraogo^{a*}, Etiennette Combe^b, Jean-Paul Lallès^c,
René Toullec^c, Serge Trèche^d, Jean-François Grongnet^{a**}

^a Laboratoire des sciences animales, École nationale supérieure agronomique de Rennes, 65, rue de Saint-Brieuc, 35042 Rennes cedex, France

^b Unité d'étude du métabolisme azoté, centre de recherche en nutrition humaine, Inra, 63122 Saint-Genès-Champanelle, France

^c Laboratoire du jeune ruminant, Inra, 65, rue de Saint-Brieuc, 35042 Rennes cedex, France

^d Laboratoire de nutrition tropicale, Institut français de recherche scientifique pour le développement en coopération, 911, avenue Agropolis, 34032 Montpellier cedex 1, France

(Received 11 December 1997; accepted 5 February 1999)

Abstract — Increasing the roasting time of soybeans from 15 (RSF15) to 25 (RSF25) min led to an important decrease in the antitryptic activity and immunoreactivity of the storage globulins, but it did not seem to greatly affect the concentrations of the indispensable amino acids in the protein. The RSF15 and RSF25 flours were used as the only protein sources in balanced diets for growing rats, and they were compared to a diet based on casein in a pair-feeding experiment. When roasted as usually happens at the workshop level (RSF15), soybean flour induced a significant hypertrophy of the pancreas. Increasing the roasting time by up to 25 min considerably improved the nutritional value of the soybean protein, without apparent consequence on the levels of free amino acid pools in the plasma and muscles. The data also indicated that the tissues of the small and large intestines of the young rat were sensitive to the hyperactivity of the intestinal microflora, and also possibly to the residual activity of some antinutritional factors. © Inra/Elsevier, Paris.

soybean / roasting / protein / growing rat / free amino acid pools / nutritional utilization

Résumé — Utilisation nutritionnelle, évaluée chez le jeune rat, des protéines des graines de soja torréfiées à petite échelle, en Afrique. La prolongation de 15 min (RSF15) à 25 min (RSF25)

* Present address: Département de productions animales, Institut national de l'environnement et de recherches agricoles, BP 7192, Ouagadougou 08, Burkina-Faso

** Correspondence and reprints

E-mail: grongnet@roazhon.inra.fr

Fonds Documentaire IRD



010022282

Fonds Documentaire IRD
Cote : B*22282 Ex:1

du temps de torréfaction à 100 °C des graines de soja entraîne une diminution importante de l'activité antitrypsique et de l'immunoréactivité des globulines de réserve, mais ne semble pas modifier notablement les teneurs en acides aminés indispensables des protéines. Utilisées comme seules sources de protéines dans la ration équilibrée du rat en croissance, ces farines ont été comparées à la caséine dans un essai in vivo où l'ingéré du groupe témoin caséine avait été ajusté à celui des groupes RSF. Traitée selon la procédure en vigueur dans les ateliers (RSF15), la farine de soja a induit une hypertrophie significative du pancréas. La prolongation du temps de torréfaction des graines de soja jusqu'à 25 min a permis d'améliorer considérablement la valeur nutritionnelle des protéines de soja, sans dommage apparent pour les niveaux des pools d'acides aminés libres circulants et tissulaires. Les résultats ont aussi montré que les tissus de l'intestin grêle et du gros intestin du jeune rat sont sensibles à l'hyperactivité de la microflore intestinale ainsi que, peut-être, à l'activité résiduelle de certains facteurs anti-nutritionnels. © Inra/Elsevier, Paris.

soja / torréfaction / protéine / jeune rat / pool d'acides aminés libres / utilisation nutritionnelle

1. INTRODUCTION

Legume grains are of interest in human and animal nutrition because of their high protein content (from 20 to 40 %) and relative cheapness. In this respect, soy is the most frequently used. After defatting, soybean cakes have long provided the major protein fraction in the diet of farm animals in the USA and Europe. What is new, however, is the increasing use of fullfat soy in developing countries and especially in Africa. In these countries, it is routinely incorporated into complementary foods for very young children [25]. Protein-energy malnutrition which generally appears between 6 and 24 months of age still affects more than 40 % of pre-school children in developing countries [4]. Consequently, improving the quality of complementary foods seems to be a good way of improving the nutritional status of children.

Another potential use of soybean in the third world relates to the development of modern milk production in addition to traditional breeding systems. There is an increasing demand for milk replacers for young animals because feeding fresh milk to young goats or calves is considered to be a waste of money by most breeders. This is supported by the fact that the selling price for fresh milk is very attractive.

Fullfat soybean must be treated technologically before it can be incorporated into complementary foods or milk replacers in order to reduce the antinutritional properties, which are high in raw soybean, to a reasonable level. Antitryptic factors limit digestion [14] and may cause pancreas hypertrophy [9]. Allergic symptoms and a high concentration of circulating antibodies have also been reported in babies [5] and calves [12, 22]. To prevent these symptoms, glycinin and β -conglycinin, which are the main globulins of soybean, must be sufficiently denatured [22]. Also, in preruminant calves, the apparent digestibility of soybean nitrogen is negatively correlated to the level of immunoreactive glycinin and β -conglycinin [11]. The small scale and the poor level of development of most of the African workshops manufacturing such legume grain products do not make it possible to consider treatments other than simply roasting, or possibly boiling before oven-drying. If correctly applied, roasting will decrease the antitryptic activity to acceptable levels even though antigenic activities do not reach levels that are totally innocuous to sensitive subjects. Overheating, however, will adversely affect the availability of lysine and therefore the biological value of protein. That is the reason why a careful treatment is necessary.

The aim of the present work was to determine whether the practical roasting conditions frequently encountered in Africa, i.e. roasting at 100 °C for 15 min (because of traditional recommendations) actually provide nutritionally satisfactory products, as assessed using growing rats. A longer treatment (25 min) was also introduced in this study in order to demonstrate that an improvement in the nutritional value of roasted soybean flour can be easily achieved.

2. MATERIALS AND METHODS

2.1. Diets and animals

Soybeans produced locally were roasted in a Declerck-type roaster (Declerck, Brussels) in a small-scale unit based in Brazzaville (Congo). After a slow increase (48 min) up to 100 °C, the temperature was kept constant for 15 min, which is the duration in use in traditional workshops, or it was prolonged by a further 10 min. Then, the soybeans were dehulled in a grinder with cylinders, manually winnowed, and ground using a hammer grinder (0.5-mm sieve). The fullfat

roasted soybean flours obtained were called RSF15 and RSF25, with regards to the time (15 or 25 min) they were kept at 100 °C. Diets for growing rats were formulated on the basis of the composition of the experimental flours, or casein for the control diet, in order to provide 17 % crude protein (CP = N × 6.25) and 3 800 kcal per kg of dry matter (DM). The soybean flours and casein were the only source of proteins in the experimental and control diets, respectively (table I). The supplementation in essential amino acids (AA) and minerals was calculated on the basis of the AA and mineral compositions of the protein sources (table II), and the AA requirements of the growing rat [18]. Minerals and trace elements came from a standard mixture described elsewhere [2]. Powdery components were mixed (45:55) with water in order to obtain a dough which prevents the rats from wasting the food. The homogenized diets in a semi-liquid form were kept at 4 °C for a maximum of 4 d.

Twenty-four Sprague-Dawley male rats (Iffa-Credo, Saint-Germain-l'Arbresle, France) weighing 78.4 ± 3.7 g (mean ± SD) were used in this experiment. On arrival, they were placed in individual cages in a room with lighting from 7 to 19 h and kept at 22 °C and 60 % relative humidity. The rats were divided into three homogeneous groups and were given one of the diets

Table I. Composition of the diets (g·kg⁻¹).

| Ingredients | Diet | | |
|------------------------------------|--------|--------|--------|
| | Casein | RSF15 | RSF25 |
| Fullfat soybean flour | — | 434.6 | 437.0 |
| Casein | 198.5 | — | — |
| Agar agar | 25.2 | 26.5 | 26.4 |
| Wheat starch | 585.66 | 487.45 | 485.25 |
| Maize oil | 77.4 | — | — |
| Maize vitaminized oil ¹ | 19.4 | 18.9 | 18.9 |
| Vitamin supplement ² | 20.3 | 18.9 | 18.8 |
| Mineral mixture ³ | 71.6 | 12.9 | 12.9 |
| Cystine | 1.94 | 0.49 | 0.49 |
| L-methionine | — | 0.26 | 0.26 |

¹ Retinol (20 000 IU/100 mL) and ergocalciferol (10 000 IU/100 mL).

² Supplied/kg dry matter: B₁₂ 3 mg; folic acid 0.1 mg; E 3 g; K₁ 0.5 g; B₁ 0.8 g; nicotinic acid 3 g; B₆ 0.8 g; B₂ 1.5 g; Ca pantothenate 3 g; hexol cyclohexane 50 g; para-amino-benzoic acid 50 g.

³ Supplied/kg dry matter: NH₄ citrate, Fe III, 343 mg; MnCO₃ 83.6 mg; CuSO₄·5H₂O 39.4 mg; ZnO IV, 7 H₂O 132 mg; NaF 11 mg; KI 0.26 mg; CoCO₃ 0.20 mg; SeO₂ 0.14 mg; Al₂(SO₄)₃·Cr SO₄, 24 H₂O 1.75 mg.

RSF 15: flour from soybeans roasted at 100 °C for 15 min.

RSF 25: flour from soybeans roasted at 100 °C for 25 min.

Table II. Amino acid composition of soybean flours and casein (g/16 g N).

| Amino acid | Casein ¹ | RSF15 | RSF25 |
|---------------|---------------------|-------|-------|
| Aspartic acid | 7.50 | na | na |
| Threonine | 4.55 | 3.69 | 3.63 |
| Serine | 6.00 | 4.92 | 4.93 |
| Glutamic acid | 25.00 | 17.87 | 15.30 |
| Proline | 10.90 | na | na |
| Glycine | 1.90 | 3.96 | 3.81 |
| Alanine | 3.20 | 3.94 | 3.97 |
| Valine | 6.95 | 4.63 | 4.65 |
| Cystine | 0.50 | 1.40 | 1.37 |
| Methionine | 3.10 | 1.59 | 1.61 |
| Isoleucine | 5.40 | 4.70 | 4.68 |
| Leucine | 9.70 | 7.51 | 7.20 |
| Tyrosine | 5.95 | 3.53 | 3.42 |
| Phenylalanine | 5.45 | 5.50 | 5.35 |
| Histidine | 3.10 | 2.49 | 2.51 |
| Lysine | 8.40 | 5.81 | 5.70 |
| Arginine | 3.85 | 7.28 | 7.57 |
| Tryptophan | 1.10 | na | na |

¹ Casein, Union des caséineries coopératives de Charente Maritime, France [Dry Matter (DM) 98.45 % and crude protein 92.74 % DM].

na: not available.

RSF 15: flour from soybeans roasted at 100 °C for 15 min.

RSF 25: flour from soybeans roasted at 100 °C for 25 min.

based on soybeans (RSF15, RSF25) or casein for 14 d. After 3 d of adaptation, refusals were measured every morning for 11 d. Dry matter intake in the casein group was restricted to the same level as the average intake by the rats given the soybean diets. Every rat was weighed on the day of arrival, and then every 2 d for the last 11 d of the experiment. Digestibility measurements were made on five rats per group during the first 5 d following the adaptation period. The cages of these five rats were equipped with a device separating the faeces from the urine. Urine was collected daily under 1 mL of HCl 0.1 N. Collections were stored at -20 °C until analysis.

2.2. Sample preparation and laboratory analysis

After the 14 d period, the rats were killed by intraperitoneal injection of sodium pentobarbital (0.5 mL/100 g body weight; Sanofi, France). The carotid arteries were sectioned, the blood was collected in a heparinized tube, centrifuged at

4 °C, and the plasma was kept at -20 °C until analysis. The *gastrocnemius* and *plantaris* muscles (right and left) were promptly dissected on ice, frozen rapidly in liquid nitrogen, and stored at -20 °C. The pancreas, the small intestines and the large intestines were removed and weighed. The amount of gut contents was calculated as the difference between the full and empty organ, as obtained by rinsing with cool saline (NaCl 0.9 %). The preparation of samples and AA analyses were carried out according to methods already described [3]. Briefly, faecal dry matter (DM) was determined by freeze-drying and nitrogen (N) was assayed using the Kjeldahl method (Kjeltec Auto-Analyzer 1030, Tecator). The anti-tryptic activity in the soybean flours was analysed according to the method of Liu and Markakis [16]. The residual glycinin and β -conglycinin immunoreactivities were determined by dot-blotting [13] using hyperimmune sera directed against the native globulins [26]. The AA concentrations of the soybean flours and casein were determined by ion-exchange chromatography following hydrolysis in 0.5 N HCl

for 24 and 48 h [17]. Methionine and cystine were determined on samples that had been oxidized in performic acid. The free AA concentrations in pooled samples of plasma and muscles were determined after extraction with 0.6 M trichloroacetic acid and purification by ion exchange chromatography. The AA were eluted by 4 N NH_4OH , concentrated and finally resuspended in 0.1 M HCl. The AA concentrations were measured on the AA analyser. Norleucine was used as an internal standard. From previous determinations in individual samples, concentration variability was assumed to be 15 % for most AA, 25 % for threonine and 20 % for the sulphur AA [1]. Anti-soy antibody titres were determined in plasma by the passive haemagglutination test using a saline extract of a commercial heated soybean flour as the coating antigen mixture [10].

2.3. Statistical analysis

The results between treatments were compared using analysis of variance, and the differences between means were assessed by the Scheffé test [24] unless otherwise mentioned.

3. RESULTS

3.1. Chemical characteristics of soybean flours

Prolonging the roasting duration at 100 °C from 15 to 25 min was found to affect several

components of soybeans. Only minor changes were noted in the AA composition: less than 2 % for lysine and threonine, 2–3 % for cystine and phenylalanine, 3 % for tyrosine and 4 % for leucine (table II). The effects of prolonging the roasting time appeared, however, for antitryptic activity and immunoreactivity of the soybean storage globulins (table III). The residual antitryptic activity was still 41 % of the value of raw soybean flour in RSF15, i.e. when the soybean flour was treated following the protocol in use in the workshops, but this activity decreased to 13 % of the initial value in RSF25. Similarly, the immunoreactive glycinin and β -conglycinin represented 31 and 34 % of the initial values in RSF15, and 7 and 6 % in RSF25, respectively.

3.2. Feed intake, growth and feed efficiency

In this study, DM and CP intakes were not significantly different in the three groups (table IV). In contrast, growth rates and the ratio of weight gain to DM or CP intakes were significantly lower with the RSF15 as compared to the casein diet ($P < 0.01$). The ratio of weight gain to DM intake was also significantly lower in the RSF25 than in the casein group ($P < 0.01$), while growth rates

Table III. Composition and antinutritional activities of soybean flours.

| Constituent | Soybean flours | | |
|--|----------------|-------|-------|
| | Raw | RSF15 | RSF25 |
| Dry matter (DM) (%) | 89.3 | 93.3 | 92.8 |
| Fat (% DM) | 18.7* | 22.0 | 21.0 |
| Crude protein (CP = N \times 6.25) (% DM) | 41.5 | 42.5 | 40.9 |
| Trypsin inhibitor activity (TUI ¹ /mg CP) | 151 | 62 | 19 |
| Immunoreactive glycinin (mg/g CP) | 271 | 85 | 19 |
| Immunoreactive β -conglycinin (mg/g CP) | 173 | 59 | 11 |

DM = dry matter; N = nitrogen; CP = crude protein.

* Determined before dehulling.

¹ Trypsin units inhibited.

RSF 15: flour from soybeans roasted at 100 °C for 15 min.

RSF 25: flour from soybeans roasted at 100 °C for 25 min.

Table IV. Feed intake and rat growth.

| Ingredients | Diet | | |
|-----------------------|--------------------------|--------------------------|-----------------------------|
| | Casein (n = 8) | RSF15 (n = 8) | RSF25 (n = 8) |
| DM intake (g/d) | 14.0 ± 0.9 | 14.4 ± 1.1 | 14.8 ± 1.2 |
| CP intake (g/d) | 2.55 ± 0.17 | 2.47 ± 0.18 | 2.45 ± 0.20 |
| Growth rate (g/d) | 7.18 ± 0.88 ^A | 5.69 ± 0.64 ^B | 6.85 ± 0.95 ^{A, B} |
| Growth rate/CP intake | 2.82 ± 0.23 ^A | 2.31 ± 0.16 ^B | 2.78 ± 0.19 ^A |
| Growth rate/DM intake | 0.51 ± 0.04 ^A | 0.40 ± 0.03 ^B | 0.46 ± 0.03 ^C |

^{A, B, C} means (± SD) with different superscript letters are different ($P < 0.01$). Values obtained over 11 consecutive days.

DM: dry matter; CP: crude protein.

RSF 15: flour from soybeans roasted at 100 °C for 15 min.

RSF 25: flour from soybeans roasted at 100 °C for 25 min.

and the ratio of the weight gain to CP intake were not different between these groups.

3.3. Apparent digestibility and metabolic utilization

Nitrogen urinary excretion was not different ($P = 0.07$) within the groups (*figure 1*). The faecal and total excretions of N were significantly higher in both soybean groups than in the casein group ($P < 0.01$). The highest faecal and total N excretions were found in the RSF15 group. The apparent digestibility of DM and N, and net protein utilization (NPU) were significantly different ($P < 0.01$) between the three groups

(*table V*). The highest values were observed with casein and the lowest with RSF15. The faecal excretion of N was significantly higher ($P < 0.01$) with the soy-based diets than with the casein, the highest excretion being observed with RSF15. The retention of absorbed N (NR) was lower with the RSF15 diet than with the casein diet ($P < 0.05$), but no difference was recorded between the RSF25 and the casein group (*table V*).

3.4. Relative weights of digestive contents, intestinal tissues and pancreas

The fresh digestive contents of the small intestine were significantly heavier ($P < 0.01$) in the two soybean groups than in the casein group (*table VI*). The fresh digestive contents of the large intestines were significantly heavier ($P < 0.05$) in the RSF25 group than in the casein group. When the two soybean groups were compared, the fresh digestive contents were significantly heavier in the small intestines of the RSF15 than in the RSF25 group ($P < 0.01$), but they were not different in the large intestines. No significant difference was found between the RSF25 and the casein group. The relative weights of the fresh and dry tissues of the small intestines and large intestines were significantly heavier in the two soybean groups than in the casein group ($P < 0.01$).

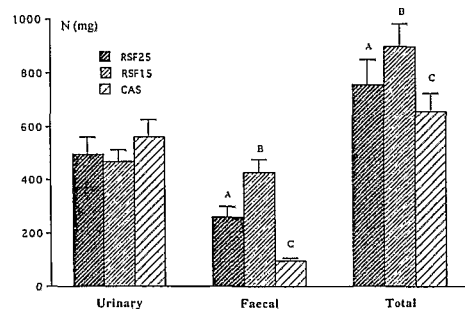


Figure 1. Repartition of nitrogen excretion (values with a different superscript are different, A, B, C: $P < 0.01$).

Table V. Apparent digestibility of nutrients and nitrogen balance.

| Ingredients | Diet | | |
|--|---------------------------|--------------------------|-----------------------------|
| | Casein (<i>n</i> = 5) | RSF15 (<i>n</i> = 5) | RSF25 (<i>n</i> = 5) |
| Apparent digestibility (%) | | | |
| Dry matter | 92.5 ± 0.50 ^A | 88.1 ± 0.85 ^B | 90.4 ± 0.89 ^C |
| Nitrogen | 94.7 ± 0.56 ^A | 74.7 ± 1.20 ^B | 85.1 ± 1.21 ^C |
| Net protein utilization (%) ¹ | 64.1 ± 3.38 ^A | 47.1 ± 1.36 ^B | 56.3 ± 1.50 ^C |
| Nitrogen retention (%) ² | 67.7 ± 3.38 ^a | 63.1 ± 1.67 ^b | 66.2 ± 1.86 ^{a, b} |

^{A, B, C, a, b} means (± SD) with different superscript letters are different (upper cases $P < 0.01$, lower cases $P < 0.05$).

Values obtained over five consecutive days.

¹ $100(I - F - U)/I = \text{NPU}$; ² $100(I - F - U)/(I - F) = \text{NR}$.

I = N intake; F = faecal N; U = urinary N.

RSF 15: flour from soybeans roasted at 100 °C for 15 min.

RSF 25: flour from soybeans roasted at 100 °C for 25 min.

Table VI. Relative weight of gut contents, intestinal and pancreatic tissues (% of body weight).

| Ingredients | Diet | | |
|-------------------------|----------------------------|-----------------------------|----------------------------|
| | Casein (<i>n</i> = 8) | RSF15 (<i>n</i> = 8) | RSF25 (<i>n</i> = 8) |
| Gut fresh contents | | | |
| Small intestines | 0.85 ± 0.14 ^A | 2.64 ± 0.43 ^B | 1.54 ± 0.26 ^C |
| Large intestines | 1.98 ± 0.30 ^a | 2.30 ± 0.37 ^{a, b} | 2.40 ± 0.46 ^b |
| Small intestine tissues | | | |
| Fresh | 2.29 ± 0.16 ^A | 3.35 ± 0.43 ^B | 2.96 ± 0.20 ^C |
| Dry | 0.51 ± 0.03 ^A | 0.76 ± 0.09 ^B | 0.67 ± 0.04 ^C |
| Protein | 0.449 ± 0.073 | 0.487 ± 0.049 | 0.430 ± 0.021 |
| Large intestine tissues | | | |
| Fresh | 0.77 ± 0.06 ^A | 1.09 ± 0.09 ^B | 1.07 ± 0.11 ^B |
| Dry | 0.17 ± 0.02 ^A | 0.20 ± 0.02 ^B | 0.21 ± 0.02 ^B |
| Protein | 0.058 ± 0.013 ^A | 0.122 ± 0.007 ^B | 0.118 ± 0.013 ^B |
| Pancreas | 0.31 ± 0.06 ^A | 0.43 ± 0.07 ^B | 0.32 ± 0.08 ^A |

^{A, B, C, a, b} means (± SD) with different superscript letters are different (upper cases $P < 0.01$, lower cases $P < 0.05$).

RSF 15: flour from soybeans roasted at 100 °C for 15 min.

RSF 25: flour from soybeans roasted at 100 °C for 25 min.

The tissues of the small intestines were significantly heavier in the RSF15 group than in the RSF25 ($P < 0.05$), but this was not the case for the tissues of the large intestines. The relative weight of the pancreas was

34–39 % higher in the RSF15 group than in the casein and RSF25 groups ($P < 0.01$). No significant difference was found between the RSF25 and the casein group. The relative protein masses of the small intestines were

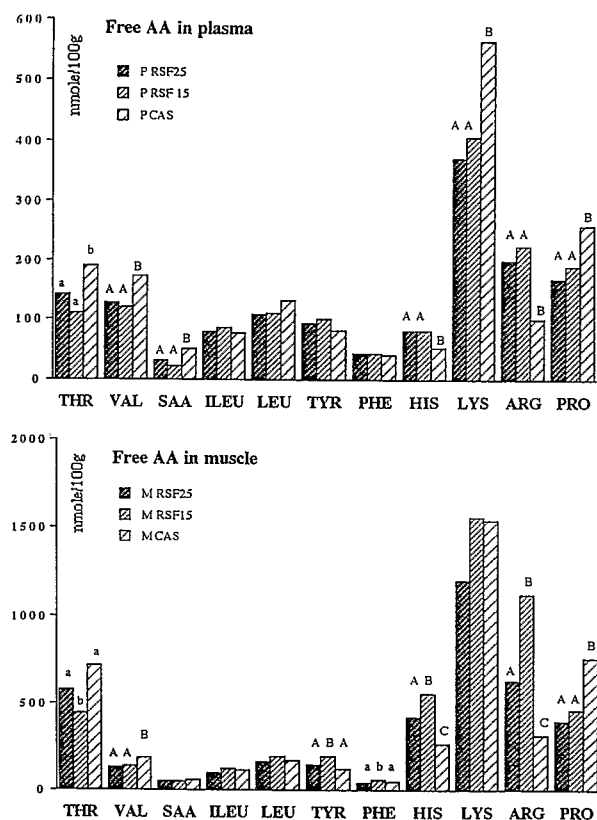


Figure 2. Free amino acid concentrations in plasma and muscle (SAA: sulphur amino acids; values with a different superscript (a, b, c: $P < 0.05$; A, B, C: $P < 0.01$) are considered different.

not significantly different among the three groups. In tissues of the large intestines, they were, however, significantly heavier in the two soybean groups than in the casein group ($P < 0.01$).

3.5. Free AA concentrations of the plasma and the muscles

The concentrations of isoleucine, leucine, tyrosine and phenylalanine in the plasma were not different in the experimental groups (figure 2). The concentrations of the other essential AA were not different between the two soybean groups. The concentrations of arginine and histidine were higher in the soybean groups than in the casein group ($P < 0.01$). The concentrations of threonine,

valine, sulphur AA and lysine were lower in the soybean groups than in the casein group ($P < 0.05$).

The concentrations of sulphur AA, isoleucine, leucine and lysine in the muscles were not different in the three groups. The concentrations of threonine and phenylalanine were similar in the RSF25 and the casein groups, but threonine concentrations were higher and phenylalanine concentrations lower than in the RSF15 group ($P < 0.05$). The concentrations of valine were not different in the two soybean groups, and they were lower than in the casein group ($P < 0.01$). The concentrations of tyrosine, histidine and arginine were higher in the RSF15 than in the RSF25 group ($P < 0.01$). The concentrations of his-

tyrosine and arginine were higher in the RSF25 than in the casein group ($P < 0.01$), but the concentration of tyrosine was not different between them.

3.6. Immune responses

The titres of anti-soy antibodies determined in the plasma were below the detection limits of the passive haemagglutination test for all the dietary treatments.

4. DISCUSSION

4.1. Nutritional value of the soybean diets

4.1.1. Diet composition

Prolonging the soybean roasting time did not greatly impair their AA composition, but it was clearly effective in decreasing their antinutritional activities and denaturing their globulins. Our results showed that the antitryptic activity was diminished three-fold, and the immunoreactivities were lowered five-fold, when the RSF25 was compared to the RSF15 group. A proper inactivation of these antinutritional factors was mentioned as a prerequisite to the nutritional utilization of soybean in the review by Liener [15], but even in the RSF25 flour, the levels of immunoreactive globulins were still high enough to induce immune-mediated disorders in preruminant calves [11, 12].

4.1.2. N digestibility, feed efficiency and other ratios

The nutritional value of these roasted soybean flours, as assessed by the comparison of apparent digestibility, feed efficiency and protein utilization ratios of the soybean diets, was greatly improved by increasing the roasting time. Such an effect which was previously reported [19, 21] may be related to various factors. First, the antitryptic activ-

ity, which is detrimental to protein digestion and causes pancreas hypertrophy in rats, was drastically reduced here. Second, a reduction in the antinutritional effects of lectins and tannins may have occurred here as well [6, 27] but they were not assessed. Third, thermal treatment is also known to improve protein digestibility by increasing the access of proteins to enzymatic attacks [7, 28]. This is also consistent with the observation that the apparent digestibility of soyabean nitrogen in preruminant calves was negatively correlated with residual glycinin and β -conglycinin immunoreactivities which can be viewed as measures of protein denaturation [11]. This is supported here by the observation that increasing the roasting time further reduced the level of residual glycinin and β -conglycinin immunoreactivities. Hot ethanol extraction of soybean flour is a more effective treatment for abolishing the so-called antigenicity of soybean products [23], but it may be unrealistic to envisage it in small units in African conditions. Finally, the digestibility of N from the roasted soybean flours prepared in the present study was still lower than that of casein, probably because of the presence of α -galactosides and fibre in soy. Indeed, these components are responsible for increased excretion of faecal N when diets based on legumes such as faba beans, lentils and chick peas are fed to growing rats [1, 3].

4.1.3. AA concentrations in plasma and muscles

Changes in free AA concentrations in the plasma and the muscles of rats fed balanced diets where the only protein was the studied protein, have been proposed as a way of estimating the degree of fulfilment of the essential AA requirements [20]. It was assumed that a lack in the availability of an essential AA might be detected through an imbalance of the concentration of the free essential AA pools in the blood and the muscle. In this experiment, where the dietary

intake of the essential AA was adjusted to (or above) the requirements of growing rats, our results for the free AA concentrations did not show any sign of severe imbalance in the plasma or in the muscle. The free AA concentrations in the plasma corresponded to the characteristics of AA intake: there was an abundance of arginine in the soybeans, and an abundance of lysine, tyrosine, leucine and valine in the casein. Results from the two soybean groups showed that plasma free AA concentrations were not affected by prolonging the time of roasting. This result was confirmed when studying the free AA pools of the muscle: no sign of severe AA imbalance was detected when the rats were fed the soybean diets, therefore suggesting no major unavailability of AA through Maillard reactions. The question may, however, be raised about the origin of modifications of the pools of free tyrosine, histidine and arginine in the muscle of the rats fed RSF15.

4.2. Physiological effects of soybeans

4.2.1. Gut contents and faecal excretion

Prolonging the time of roasting did not allow the apparent digestibility of DM and N and the NPU ratio of the RSF25 diet to reach that of the casein diet. This is partly due to an increased faecal excretion. It is comparable to the results obtained for other legumes [1–3]. But the apparent digestibility takes the total loss of material (including endogenous and microbial) more into account than the dietary origin which has not been truly digested. Recent data obtained using labelled legumes have shown that their true digestibility can be as high as that of the controls [8]. Moreover, the soybean diets are especially rich in fibre, sucrose and stachyose, and to a lesser extent raffinose (Kawamura, cited in [21]). These oligosaccharides are known to provide microflora substrates for fermentation.

4.2.2. Hypertrophy of gut tissues

We propose that the hypertrophy of the tissues of the large intestine found in the two RSF groups may come from the increased activity of the microflora. This has already been related to feeding of legumes (cooked beans, lentils, chick peas, peas) which bring together proteins and polysaccharides [2]. While soybean does not provide starch, its fibre and α -galactosides can be used as efficient substrates by the microflora of the large intestine. Metabolites of bacteria are known to play an active role in the metabolism of intestinal tissues.

Hypertrophy of the tissues of the small intestine and pancreas may also be related to some residual antinutritional activities such as antitryptic activity present in the experimental diets. In contrast, immune reactions may not be involved, owing to the lack of detection of anti-soy antibodies in the plasma. Moreover, the comparison of the free AA pools of the muscle between the two soybean groups has shown a lower concentration of threonine and higher concentrations of histidine and arginine in the RSF15 group; the RSF25 group differed by the level of antitryptic activity and immunoreactivity of storage globulins.

5. CONCLUSION

The results of the present study clearly show that roasting soybeans at 100 °C for 15 min, as usually carried out in traditional workshops in Africa, still provides a flour which contains too high levels of antitryptic activity and immunoreactive storage proteins. It is possible, however, to substantially reduce the level of these antinutritional factors, and consequently to improve the nutritional quality of such products, simply by increasing the roasting time to 25 min, without decreasing the apparent bioavailability of lysine. Our experiment with growing rats demonstrated the beneficial effect of

this extended roasting time on pancreas weight, N digestibility, feed efficiency and protein utilization. However, the residual activity of antinutritional factors probably affected some parameters of protein metabolism in the tissues, such as the free pools of threonine, histidine and arginine in the muscles and the hypertrophy of the small intestine. The hyperactivity of the microflora and that of the tissues of the large intestines, are more related to the presence of specific substrates such as fibre and oligosaccharides brought by the soybeans.

REFERENCES

- [1] Combe E., Cvirn M., Utilisation nutritionnelle des protéines de la lentille chez le jeune rat, *Ann. Nutr. Metab.* 39 (1995) 16–27.
- [2] Combe E., Naudin-Lebecque A., Pion R., Influence de la variété et de la cuisson du pois (*Pisum sativum*) sur la nature des composés azotés des fèces de rats, *Ann. Zootech.* 37 (1988) 233–254.
- [3] Combe E., Achi T., Pion R., Utilisations digestive et métabolique comparées de la fève, de la lentille et du pois chiche chez le rat, *Reprod. Nutr. Dev.* 31 (1991) 631–64.
- [4] de Onis M., Monteiri C., Akré J., Clugston G., The worldwide magnitude of protein-energy malnutrition: an overview from the WHO global database on child growth, *Bull. World Health Org.* 71 (1993) 703–712
- [5] Fries G.F., Studies on the allergenicity of soybean, *Ann. Allergy* 29 (1971) 1–7.
- [6] Grant G., Van Driessche E., Legume lectins: Physicochemical and nutritional properties, in: Van Der Poel A.F.B., Huisman J., Saini H.S. (Eds.), *Recent Advances of Research in Antinutritional Factors in Legume Seeds*, Wageningen Pers, Wageningen, 1993, pp. 219–233.
- [7] Herkelman K.L., Cromwell G.L., Stahly T.S., Pfeiffer T.W., Knabe D.A., Apparent digestibility of amino acids in raw and heated conventional and low-trypsin-inhibitor soyabean for pigs, *J. Anim. Sci.* 70 (1992) 818–826.
- [8] Huisman J., Heinz T., van der Poel A.F.B., van Leeuwen P., Souffrant W.B., Versteegen M.W.A., True digestibility and amounts of endogenous protein measured with the ¹⁵N-dilution technique in piglets fed on peas (*Pisum sativum*) and common beans (*Phaseolus vulgaris*), *Br. J. Nutr.* 68 (1992) 101–110.
- [9] Kakade M.L., Hoffa D.E., Liener I.E., Contribution of trypsin inhibitors to the deleterious effects of unheated soyabeans fed to rats, *J. Nutr.* 103 (1973) 1772–1778.
- [10] Kilshaw P.J., Sissons J.W., Gastrointestinal allergy to soyabean protein in preruminant calves. Allergenic constituents of soyabean products, *Res. Vet. Sci.* 27 (1979) 366–371.
- [11] Lallès J.P., Tukur H.M., Toullec R., Miller B.G., Analytical criteria for predicting apparent digestibility of soy protein in preruminant calves, *J. Dairy Sci.* 79 (1996) 475–484.
- [12] Lallès J.P., Dréau D., Salmon H., Toullec R., Identification of soyabean allergens and immune mechanisms of dietary sensitivities in preruminant calves, *Res. Vet. Sci.* 60 (1996) 111–116.
- [13] Lallès J.P., Tukur H.M., Toullec R., Méthodes immunochimiques de dosage de la glycine et de la β-conglycine dans les dérivés de soja. Prédiction de la digestibilité de l'azote et de l'immunogénicité du soja chez le veau, *Ann. Zootech. Paris* 46 (1997) 193–205.
- [14] Liener I.E., Antinutritional factors in legume seeds: state of the art, in: Huisman J., Van Der Poel A.F.B. (Eds.), *Recent Advances of Research in Antinutritional Factors in Legume Seeds*, Pudoc, Wageningen, 1989, pp. 6–13.
- [15] Liener I.E., Implications of antinutritional components in soybean foods, *Crit. Rev. Food Sci. Nutr.* 34 (1994) 31–67.
- [16] Liu K., Markakis P., An improved colorimetric method for determining antitryptic activity in soya bean products, *Cereal Chem.* 66 (1989) 415–422.
- [17] Moore S., Spackmann D.M., Stan W.M., Chromatography of amino acids on-sulfonated polystyrene resins, *Anal. Chem.* 30 (1958) 1185–1190.
- [18] National Research Council, Nutrient requirement of the laboratory rat, in: *Nutrient Requirement of the Laboratory Animals*, 3rd ed., Washington DC, 1978, pp. 7–37.
- [19] Nitsan Z., Liener I.E., Studies of the digestibility and retention of nitrogen and amino acids in rats fed raw or heated soy flour, *J. Nutr.* 106 (1976) 292–299.
- [20] Pion R., Arnal M., Grizard J., Utilisation métabolique des acides aminés, *Med. Nutr.* 15 (1979) 109.
- [21] Salunkhe D.K., Kadam S.S., *CRC Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology, and Utilization*, vol. III, CRC Press Inc, Boca Raton, Florida, 1989.
- [22] Sissons J.W., Effects of soyabean products on digestive processes in the gastrointestinal tract of preruminant calves, *Proc. Nutr. Soc.* 41 (1982) 53–61.
- [23] Sissons J.W., Smith R.H., Hewitt D., The effect of giving feeds containing soya-bean meal treated or extracted with ethanol on digestive processes in the preruminant calf, *Br. J. Nutr.* 42 (1979) 477–485.

- [24] Snedecor G.W., Cochran W.G., in: Boelle H., Camhaji E., Méthodes statistiques, 6th ed., Association de Coordination Technique Agricole, Paris, 1971.
- [25] Trèche S., Analyses des expériences de production de farines infantiles en Afrique, in: Trèche S., de Benoist B., Benbouzid D., Vester A., Delpeuch F. (Eds.), L'alimentation de complément du jeune enfant, Orstom, collection colloques et séminaires, Paris, 1995, pp. 225-235.
- [26] Tukur H.M., Lallès J.P., Mathis C., Caugant I., Toullec R., Digestion of soyabean globulins, glycinin, α -conglycinin and β -conglycinin, in the preruminant and the ruminant calf, Can. J. Anim. Sci. 73 (1993) 891-905.
- [27] Vallet J., Rouanet J.M., Besançon P., Dietary grape seed tannins: effects on nutritional balance and on some enzymic activities along the crypt-villus axis of rat small intestine, Ann. Nutr. Metab. 38 (1994) 75-84.
- [28] Van Der Poel A.F.B., Effect of processing on antinutritional factors and protein nutritional value of dry beans (*Phaseolus vulgaris* L.). A review, Anim. Feed. Sci. Technol. 29 (1990) 179-208.