Growth Performance of Rats Fed Jojoba Proteins. Possible Correlations with Trypsin Inhibitory Activity in Jojoba Proteins

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The effects of the water-soluble protein fraction of jojoba meal on growth and relative organ weight in rats were tested. Trypsin inhibitory activity (TIA) was measured in the untreated water-soluble jojoba proteins and in preparations after dry-heating (100 °C, 2 h) or methanol extraction (Soxhlet, 6 h). TIA was found in the jojoba protein fraction and could not be destroyed by dry-heating or methanol extraction of the jojoba proteins. In the first feeding experiment, growth performance and relative organ weight were compared of young rats fed ad libitum a diet containing either 18% soybean proteins (C), 6% soybean proteins (P6), or 6% soybean proteins supplemented with 12% jojoba proteins (JJ) or 12% toasted jojoba proteins (JJT). In a second 3 week experiment, growth and relative organ weight of young rats receiving a diet containing 6% soybean proteins supplemented with 12% methanol-extracted jojoba proteins (JJM) were compared for rats pair-fed with JJM with a diet containing 18% soybean proteins (PF). Although food intakes of C, JJ, and JJT rats were similar and P6 rats ate more than C rats, P6, JJ, and JJT rats showed growth retardation compared to C rats. JJM rats and PF rats showed a similar growth. The pancreas was hypertrophied in JJ and JJT rats, compared to C rats, and in JJM rats, compared to PF rats. Interscapular brown adipose tissue (BAT) was hypertrophied in P6, JJ, and JJT rats but normal in the JJM group. Jojoba proteins induce growth retardation. This is accompanied by pancreatic and BAT hypertrophy. As trypsin inhibition does not change but growth does ameliorate and BAT hypertrophy is abolished after methanol extraction, trypsin inhibition has no major influence on growth in the present experiments, while BAT hypertrophy is accompanied by growth reduction.

Keywords: *Simmondsia chinensis; jojoba meal; jojoba protein concentrate; rat*

INTRODUCTION

Due to the variable price of oil from the jojoba plant (*Simmondsia chinensis*) on the oil market, it would be very interesting if jojoba meal, the byproduct of the oil production, could be marketed (Wisniak, 1988). Jojoba meal has been rejected as animal feed because it reduces food intake and causes emaciation in mammals and chickens (Verbiscar et al., 1981; Ngou Ngoupayou et al., 1982). In mammals, these effects are mainly ascribed to simmondsin and its derivatives (Elliger et al., 1973), which are known to induce satiation in rats (Cokelaere et al., 1995a,b). Other authors described the presence of heat labile trypsin inhibitors (Samac and Storey, 1981) and condensed tannins with anti-trypsin activity (Sanchez-Lucero and Price, 1988). Recently, Abbott and et al. (1994) described a method of concentrating the water-soluble proteins present in jojoba meal. The procedure, based on ultrafiltration, yields a protein-rich powder, free of simmondsins.

The aims of the present work were (1) to quantify the trypsin inhibitory activity (TIA) present in the jojoba protein fraction and (2) to investigate the effect of this protein fraction on the growth performance of rats, when offered as the major protein source, with or without dry-heat treatment or methanol extraction to destroy or eliminate possible trypsin inhibitors.

MATERIALS AND METHODS

Jojoba protein concentrate was prepared as described by Abbott et al. (1994). The protein content, measured using the Lowry method, as adapted by Rodríguez-Vico et al. (1989), was 78%. Condensed tannins, measured according to the vanillin binding method (Randalph, 1995), were present at the level of 2.75%. The jojoba proteins were used (1) as such, (2) after heat treatment (100 °C, 120 min, dry-heating), or (3) after extraction with methanol (Soxhlet, 6 h, dried at 50 °C for 4 h).

Trypsin Inhibition. Trypsin inhibition was measured according to the method of Schwert and Takenaka (1955) (1) in jojoba proteins, (2) in toasted jojoba proteins, and (3) in methanol-extracted jojoba. To 2.75 mL of substrate (*N*α-benzoyl-L-arginine ethyl ester HCl, 0.3 mg/mL) were added 0.15 mL of jojoba protein solution (5−20 mg/mL, final concentration 0.25−1 mg/mL assay) and 0.2 mL of trypsin (0.01 mg/mL). Changes in optical density (OD) were followed for 10 min at 22 °C at 540 nm on a Beckman DU-70 spectrophotometer and compared to the OD change for the control (no addition of jojoba protein). TIA was expressed as percent inhibition per milligram of jojoba protein under the given experimental conditions.

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S0021-8561(97)00218-5 CCC: $14.00

Table 1. Relative Organ Weights of Rats Receiving a Diet of 6% Soybean Proteins Supplemented with 12% Untreated Jojoba Protein (JJ) or with 12% Toasted Jojoba Proteins (JJT) Compared to Rats on 6% (P6) and 18% Soybean Protein Diets (C)[a]

organ/treatment	liver	spleen	heart	kidney	BAT	pancreas
JJ	2.94 ± 0.16	0.24 ± 0.03	0.37 ± 0.03	0.38 ± 0.02	0.170* ± 0.01	0.57* ± 0.03
JJT	3.21 ± 0.13	0.25 ± 0.04	0.38 ± 0.02	0.40 ± 0.02	0.180* ± 0.03	0.66* ± 0.05
P6	2.90 ± 0.19	0.20 ± 0.02	0.36 ± 0.01	0.34 ± 0.05	0.188* ± 0.02	0.32 ± 0.05
C	3.16 ± 0.11	0.22 ± 0.04	0.35 ± 0.02	0.37 ± 0.02	0.135 ± 0.03	0.34 ± 0.02

[a] Values are expressed as percentage of total body weight and given as the mean of eight replicates ± SEM; *, significantly different from the C group ($p < 0.05$).

Growth of Rats Fed Jojoba Meal. Thirty-two young male Wistar rats (160−180 g; raised at the central laboratory animal facility of the Katholieke Universiteit Leuven, Heverlee, Belgium) were held under standard laboratory conditions (22 °C, 60% relative humidity, light from 8 a.m. to 8 p.m., water ad libitum). They were divided into four groups of eight and fed (1) a semisynthetic basal rat diet containing 6% soybean protein (Carfil, Oud Turnhout, Belgium) (low-protein group, P6) or the same diet supplemented with (2) 12% soybean protein (control group, C), (3) 12% jojoba protein (JJ group), or (4) 12% toasted jojoba protein (JJT group). All groups had free access to food. The added amounts of jojoba protein concentrates were corrected for protein content (i.e. 78% protein in the concentrate), such that the C, JJ, and JJT groups all received a diet containing 18% protein and 11.24 MJ/kg of metabolizable energy.

Daily food intake and growth were followed for 3 weeks; rats were then fasted overnight and sacrificed using ether. The heart, spleen, liver, kidney, and pancreas were removed and weighed. Interscapular brown adipose tissue (BAT), easily discernible from the adhering white adipose tissue and muscle tissue, was carefully freed from its surrounding tissues and weighed.

Influence of Methanol Extraction of Jojoba Protein Concentrate on Growth. Sixteen young male Wistar (166−174 g) rats were kept under standard laboratory conditions and divided in two groups of eight. The first group (JJM) received the above-described basal diet, supplemented with 12% methanol-extracted jojoba proteins, with the added amount of protein concentrate again being corrected for protein content. Members of the second group were offered the basal diet supplemented with 12% soybean proteins and served as the control for the JJM group, being fed exactly the same daily amount of food eaten by the JJM group (pair-fed group, PF). Pair-feeding was installed to prevent any effect of differences in food intake on growth. The same parameters were monitored as in the previous experiment.

The results were expressed as the mean ± SEM. Organ weights were expressed as percentage of total body weight. Results were analyzed statistically by ANOVA, followed by a Tukey test for differences between groups, using Microstat software (Ecosoft, Inc., 2.0, 1984); p values < 0.05 were considered statistically significant.

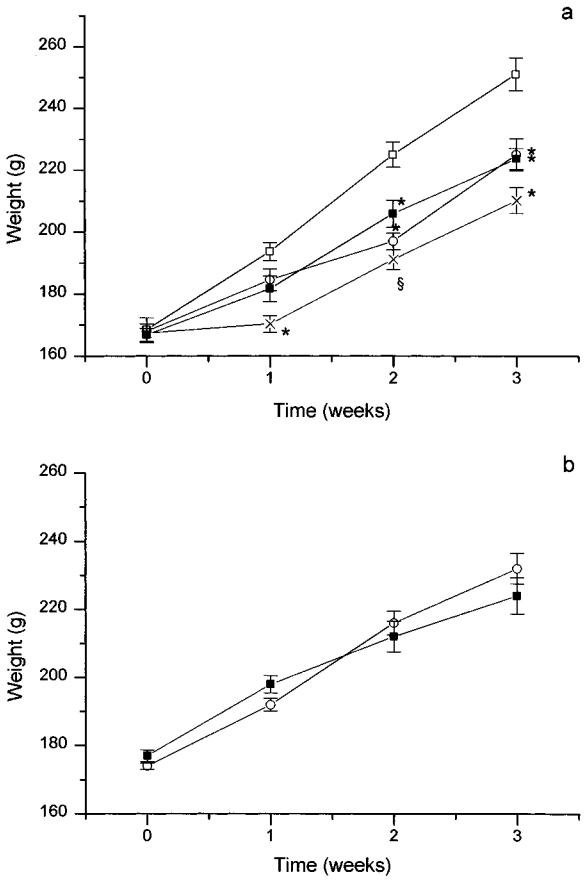

Figure 1. (a) Growth curves of control animals, receiving a diet containing 18% soybean protein C (□), 6% soybean protein P6 (○), jojoba protein (12% untreated jojoba proteins, added to a 6% soybean protein diet) JJ (×), or toasted jojoba protein (12% toasted jojoba proteins added to a 6% soybean protein diet) JJT (■). (b) Growth curves of rats fed methanol-extracted jojoba protein (12% methanol-extracted jojoba protein added to a 6% soybean protein diet) JJM (■) and their pair-fed controls receiving 18% soybean proteins PF (○). Values are the mean of eight replicates; vertical bars denote the SEM; *, significantly different from C ($p < 0.05$); §, significantly different from C and JJT ($p < 0.05$).

RESULTS

Trypsin Inhibition. The jojoba protein concentrate showed a linear concentration-dependent inhibition of trypsin activity (r^2 = 0.99, 0.98, and 0.99 for the untreated, toasted, and methanol-extracted jojoba proteins, respectively) (data not shown). Toasting or methanol extraction of the jojoba proteins did not significantly alter the TIA, this being 60.3 ± 4.1%/mg of untreated jojoba meal, 53.9 ± 4.3%/mg of toasted jojoba meal, and 51.5 ± 3.8%/mg of methanol-extracted jojoba protein.

Growth of Rats on Jojoba Proteins. The mean daily food intakes per rat were very similar for the JJ, JJT, and C rats (18.3 ± 0.3, 18.5 ± 0.8, and 18.4 ± 0.3 g, respectively). The daily food intake in the P6 group (20.6 ± 0.4 g) was significantly higher than in the other groups. Compared to the C group, the P6, JJ, and JJT rats showed growth retardation (Figure 1a). No significant differences in relative organ weights were seen between the treatment groups (Table 1). The relative BAT weight found for the control groups was in the same range as found by others (Rothwell et al., 1984). Compared to the C group, the BAT and pancreas in the JJ and JJT rats showed hypertrophy. Toasting the protein fraction did not abolish the trophic effect on the pancreas or BAT. Compared to C, P6 rats showed no pancreas hypertrophy but did show distinct BAT hypertropht (Table 1).

Influence of Methanol Extraction of the Jojoba Protein Concentrate on Growth. The mean daily food intake for the JJM and PF rats was 18.0 ± 0.4 g. The growth performance of the JJM rats did not differ

Table 2. Relative Organ Weights of Rats Receiving a Diet of 6% Soybean Proteins Supplemented with 12% Methanol-Extracted Jojoba Proteins (JJM) Compared to Controls Fed a Diet with 18% Soybean Proteins (PF)[a]

organ/treatment	liver	spleen	heart	kidney	BAT	pancreas
JJM	2.85 ± 0.17	0.21 ± 0.03	0.34 ± 0.03	0.39 ± 0.05	0.127 ± 0.03	0.64* ± 0.05
PF	2.74 ± 0.10	0.23 ± 0.04	0.34 ± 0.02	0.33 ± 0.01	0.125 ± 0.02	0.35 ± 0.04

[a] Values are expressed as percentage of total body weight and given as the mean of eight replicates ± SEM; *, significantly different from the PF group ($p < 0.05$).

significantly from that of their pair-feds (PF) (Figure 1b). No difference in liver, kidney, heart, spleen, and BAT weights was seen between the JJM and PF rats, but the JJM rats showed hypertrophy of the pancreas (Table 2).

DISCUSSION

The present results indicate that water-soluble jojoba proteins display TIA, confirming the results of other authors (Samac and Storey, 1981; Sanchez-Lucero and Price, 1988), who demonstrated the presence of TIA in both the proteins and tannins present in jojoba meal. In our experiments, the TIA in the water-soluble jojoba proteins was not destroyed by either toasting or methanol extraction. Since the presence of moisture can be important in the denaturation of enzymes (Wolf et al., 1994) and toasting (as performed in this paper) is dry-heating, it is quite possible that proteinaceous trypsin inhibitors might not be denatured during our heat processing. The effect of moisture was not studied for the practical reason that it is not possible to mix jojoba proteins with normal rat chow after wet heating, as this results in the formation of a gel. Condensed tannins were also present in the protein concentrate and could obviously be responsible for at least part of the heat-stable and non-methanol-extractable TIA (Asquith et al., 1983; Savelkoul et al., 1992; Ahmed et al., 1991).

Due to the lower protein concentration in their food, the growth of the P6 rats was evidently lower than that of the C rats. Although the protein and calorific intakes of the JJ, JJT, and C groups were similar, reduced growth was seen after feeding either the heated or nonheated water-soluble jojoba protein fraction (JJ and JJT groups), compared to the C group. Several hypotheses can be put forward to explain this effect. Jojoba proteins may be less digestible than soybean proteins, as has been described for complete jojoba meal when fed to rabbits (Ngou Ngoupayou et al., 1985) or lambs (Swingle et al., 1985). In addition, the observed pancreatic hypertrophy suggests that there may be an increased protein loss due to pancreatic stimulation. The observed pancreatic enlargement can be explained by the presence of trypsin inhibitory substances, as demonstrated in the previous experiment. Trypsin inhibitory substances are known to induce pancreatic enlargement through stimulation of cholecystokinin release (Fölsch et al., 1974). As described for nonheated soybean flour (Booth et al., 1960; Fölsch and Creutzfeldt, 1985), pancreatic enlargement induced by trypsin inhibitors can lead to an enhanced secretion of pancreatic enzymes, resulting in an increased loss of protein through the pancreas, which is not completely restored by absorption of amino acids from digested enzymes (Khayambashi and Lyman, 1966; Melmed and Bouchier, 1969). This, together with lower protein digestibility, may result in lower protein availability for growing rats, resulting in growth retardation.

Toasting or methanol extraction did not destroy either the TIA or the pancreatic hypertrophic effect of the protein fraction used. However, methanol extraction abolished the induction of BAT hypertrophy and almost restored the growth rate to pair-fed levels. This indicates that BAT, together with the above-mentioned factors, plays a key role in the growth retardation seen in jojoba protein-treated rats. In young rats, BAT is a highly metabolic active tissue, converting a large part of thyroxine (T4), produced in the thyroid gland, to the biological active thyroid hormone, triiodothyronine (T3), thus stimulating metabolism (Leonard et al., 1982; Fernandez et al., 1987). An increase in BAT induces a higher maintenance metabolism, leaving less energy for growth (Swick and Gribskov, 1983). In previous experiments, we observed increased T3 concentrations in jojoba meal-treated animals with a high protein demand, such as fast-growing young rats (Cokelaere et al., 1993a) and pregnant rats (Cokelaere et al., 1993b). This observation can be explained by the present observed BAT hypertrophy. BAT can be increased under various conditions such as low temperature, glucagon administration, high fat intake, or protein shortage (Kopecky et al., 1986; Billington et al., 1987; Swick and Gribskov, 1983). The increase in BAT weight seen in the P6 rats confirms the finding that protein shortage stimulates BAT growth, while the BAT hypertrophy seen in the JJ and JJT groups suggests a relative protein shortage, as discussed above, plus a higher energy requirement for maintenance metabolism. In JJM rats, the BAT increase was not seen, and growth was comparable to that of the control group, suggesting a better protein availability and energy use. Since this was not due to a decreased TIA, we suggest that it results from methanol extraction of an unknown antinutritional substance and/or from improved protein digestibility due to the methanol treatment. The residual small, statistically insignificant decrease in growth seen in the JJM group compared to the PF group (Figure 1b) can easily be explained by the TIA remaining in the JJM meal, resulting in the observed pancreatic hypertrophy. Taken together, the results suggest that protein loss by hyperstimulation of the pancreas itself is of minor importance in the observed growth retardation.

We suggest that the growth retardation seen in jojoba protein-treated growing rats is related to protein shortage induced (1) by the lower digestibility of jojoba proteins compared to soybean proteins and (2), to a lesser extent, by pancreatic hyperstimulation, due to the presence of non-methanol-extractable, heat-stable trypsin inhibitors. The use of jojoba proteins as a major protein source is accompanied by BAT hypertrophy, suggesting relative protein shortage and probably inducing a higher energy use. Feeding methanol-extracted jojoba proteins abolished the increase in BAT and resulted in improved growth, most probably by reducing the relative protein shortage. These effects of water-soluble jojoba proteins may partly explain the negative growth effect of jojoba meal feeding.

ABBREVIATIONS USED

BAT, interscapular brown adipose tissue; C, control rats fed a diet containing 18% soybean proteins; JJ, rats

fed a diet containing 6% soybean proteins and 12% jojoba proteins; JJM, rats fed a diet containing 6% soybean proteins and 12% methanol-extracted jojoba proteins; JJT, rats fed a diet containing 6% soybean proteins and 12% dry-heated jojoba proteins; PF, rats pair-fed with JJM rats; P6, rats fed a diet containing 6% soybean proteins; TIA, trypsin inhibitory activity; T3, triiodothyronine; T4, tetraiodothyronine.

ACKNOWLEDGMENT

We thank M. Landuyt for technical assistance and E. Vanderbeke from AVEVE for determination of the tannin levels.

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Received for review March 18, 1997. Accepted July 14, 1997.[⊗] This work was funded by the research board of the Katholieke Universiteit Leuven, OT/95/29.

JF970218D

[⊗] Abstract published in *Advance ACS Abstracts*, September 1, 1997.