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Muscle and liver protein metabolism in rats fed raw or heat-treated pea seeds

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Abstract

Raw or extruded pea (*Pisum sativum*, cv. Ballet) diets with or without supplementary amino acids were fed for 15 days to young growing rats and the effects on tissue weights, liver and muscle protein metabolism and hormone levels monitored. Body weight gain, liver and gastrocnemius muscle weights and protein contents were reduced and some key hormones altered when rats were fed unsupplemented raw pea diets. This appeared to be a result of amino acid deficiencies in the diet, the action of antinutritional factors and the refractory nature of the reserve proteins and other seed components. However, this did not in itself improve the nutritional performance of the rats due to the overriding effects of the amino acid deficiencies in the pea diets. After supplementation, extruded peas supported much higher rates of growth and skeletal muscle deposition than did supplemented raw peas. Despite this, the weight gains remained less than achieved on a high quality control diet. Protein synthesis and degradation rates in skeletal muscles and total protein contents were similar to control values. The lower growth rate did not appear to be due to impaired deposition of skeletal muscle. Deposition of other body components, possibly lipids, may have been lowered by supplemented extruded pea diets. Liver protein levels were reduced in rats fed supplemented raw peas and blood corticosterone was elevated. In conclusion, extrusion treatment of peas in combination with amino acid supplementation appeared to abolish the negative effects of peas on skeletal muscle deposition.

Keywords: Extrusion-cooking; Pisum sativum; Liver; Muscles; Protein turnover; Hormone; Rat

1. Introduction

Legumes and among them peas, play an important role in the traditional diets of many regions throughout the world. Pea seeds are low in fat and are excellent sources of protein, dietary fiber and a variety of micronutrients and bioactive compounds [1]. This nutrient composition meets the requirements of the prudent diet, recommended for the prevention and treatment of chronic degenerative Western diseases [2]. Recent studies have shown that legume consumption can have beneficial effects by lowering the incidence of certain cancers [3,4], protecting against osteoporosis [5], lowering serum cholesterol [6] or reducing body lipid accumulation [7] among others. However, there are a number of components in legumes that may exert a negative effect on the nutritional quality of the proteins. Among these factors are protease inhibitors and lectins [8]. Protease inhibitors exert their antinutritional effect by reducing or preventing digestion of nutrients and possibly impairing body metabolism, growth and health [9]. Lectin, by virtue of its ability to bind to carbohydrate receptors of gut epithelial cells, not only interferes with nutrient absorption but may also be taken up systemically and affect hormone balance and lipid and muscle metabolism [10].

As a protein source, most legumes do not meet the sulfur amino acid requirements of humans and some animal species. This could therefore compromise the efficiency with which their proteins can be utilized and their health-promoting value since sulfur amino acids have an important role in antioxidant defense and immune systems and influence of genetic function [11]. This deficiency could be overcome by supplementing legume-based diets with sulfur amino acids or plant materials rich in sulfur amino acids.

In recent years, extrusion cooking has been widely applied in the production of nutritious foods. It can be described as a high temperature, low liquid, and short time

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processing technique [12,13]. This thermal processing procedure, which denatures and inactivates antinutritional factors and increases protein quality and availability, has become one of the most popular processes developed by the food and feed industry [14]. Extrusion treatment of dry beans is relatively new and provides an economically and convenient alternative to cooking and canning [15,16].

The aims of the present study were to investigate in feeding trials with rats the effects of diets based on raw or extruded peas with or without supplementary amino acids on: 1) organ growth, 2) the fractional rates of protein synthesis (k_s), degradation (k_d), and accretion (k_g) in liver and hindlimb muscles and 3) blood hormones.

2. Material and methods

2.1. Legume flour and processing

Pea seeds (*Pisum sativum* L. cv. Ballet) cultivated in Navarra (Spain), were used for all studies. Peas were finely ground (0.5 mm) and extrusion cooking was performed in a Clextral X-5 model BC 45 twin-screw extruder (F-42100 Firminy, France). The extrusion temperature at the outlet die was 145°C. The moisture content in the extruder barrel was constant at 25%. The extruder was operated at 100 rpm and the feeder was set to deliver 21.5 kg h^{-1}. The extrudates were allowed to cool to room temperature, re-ground to pass a 0.5 mm sieve and then stored at 4°C until analysis. All chemicals and reagents were purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI, USA) and Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Chemical analysis

For assessment of enzyme inhibitor levels, appropriate dilutions of seed extracts were incubated with a known amount of trypsin, chymotrypsin or α-amylase to allow formation of the enzyme-inhibitor complex. Residual enzyme activity was then determined using the appropriate substrate for the enzymes. Trypsin inhibitors were determined using α-N-benzoyl-DL-arginine-p-nitroanilidehydrochloride (BAPNA) as substrate [17]. Chymotrypsin inhibitors were analyzed using benzoyl-L-tyrosine ethylester (BTEE) as substrate [18]. α-amylase inhibitor activity was assessed using starch as substrate [19].

Hemagglutination assays were carried out by a serial dilution method using trypsin-treated rabbit erythrocytes [17]. One unit of hemagglutinating activity (HU) was defined as that contained in the amount of sample in the last dilution that caused 50% agglutination of the blood cells.

Protein digestibility in vitro was determined in pea flour suspensions (1 mg N ml^{-1}) using a multienzyme system consisting of 1.6 mg trypsin (14,600 U mg^{-1}), 3.1 mg α-chymotrypsin (48 U mg^{-1}) and 1.3 mg peptidase (102 U g^{-1}) [20]. Starch digestibility in vitro was determined in flours suspensions (50 mg ml^{-1} of 0.2 M phosphate buffer, pH 6.9) using 0.5 ml of pancreatic amylase (1260 U mg^{-1}) suspension (0.4 mg ml^{-1} of 0.2 M phosphate buffer, pH 6.9) [21].

Table 1
Composition of the diets

Ingredient (g kg^{-1})	Diet		
	Casein control	Raw peas	Extruded peas
Casein	131.0	—	—
Raw peas meal	—	569.7	—
Extruded peas meal	—	—	560.1
Corn starch	605.2	335.1	333.3
Sucrose	114.3	24.3	23.7
Olive oil	70.0	65.2	65.2
Cellulose	50.0	31.1	29.6
Mineral mix*	35.0	35.0	35.0
Vitamin mix†	10.0	10.0	10.0
L-Cys	3.0	—	—
Choline	2.5	2.5	2.5
Protein (g kg^{-1})	110.0	110.0	110.0
IVPD	930.0	750.0	802.0
IVSD	—	245.0	296.0
Energy (MJ kg^{-1})	17.4	16.5	16.5

* Amount in diet (mg kg^{-1}): Ca, 5000; P, 1561; K, 3600; Na, 1019; CI, 1571; S, 300; Mg, 507; Fe, 35; Cu, 6.0; Mn, 10.0; Zn, 30.0; Cr, 1.0; K, 0.2; Se, 0.15; F, 1.0; B, 0.5; Mo, 0.15; Si, 5.0; Ni, 0.5; Li, 0.1; V, 0.1.

† Amount in diet (mg kg^{-1}): thiamine, 6; riboflavin, 6; pyridoxine, 7; niacin, 30; calcium pantothenate, 16; folic acid, 2; biotin, 0.2; vit. B_{12}, 0.025; vit. A, 8; vit. E, 0.15; vit. D_3, 1000 I.U.; vit. K, 0.75.

IVPD In vitro protein digestibility; IVSD In vitro starch digestibility.

2.3. Animals and diets

Male rats of the Wistar Han (Charles River) strain were weaned at 19 days of age and given a stock diet (Harlan Teklad, Madison, Wisconsin) for 7 days. Rats matched by weight (85.0 ± 1.1 g), were housed individually in polypropylene and stainless steel metabolism cages.

Rats were adapted by giving them a casein control diet for 3 days and then fed for 15 days with test or control diets (Table 1). The rats were housed under controlled conditions of room temperature (22 ± 1°C), relative humidity (50 ± 5%), ventilation (at least 15 complete changes of air h^{-1}), and artificial light-dark cycle (12 h light/dark period). Water was freely available at all times. Total protein (N × 6.25) in casein, pea flours and diets was determined by a Kjeldahl procedure [22].

In the first experiment, forty rats were divided into 4 groups of 10 animals. Two groups were given free access to raw or extruded pea diet. The remaining groups were given casein (control) and were pair-fed to the daily intake of rats given either raw or extruded pea diet. In the second experiment, thirty rats were divided into 3 groups. Two groups had free access to raw or extruded pea diets that had been supplemented with amino acids to the target requirements

Table 2
Body weight gain and food intake

	Diets (expt 1)*				Pooled SD	Diets (expt 2)			Pooled SD
	C	RP	C	EP		C	SRP	SEP	
Weight gain, g	60.6[b]	44.6[c]	67.7[a]	46.1[e]	2.1	125.8[a]	91.5[c]	110.3[b]	3.4
Food intake, g	158.5[b]	158.9[b]	170.2[a]	168.2[a]	3.0	270.6[a]	261.0[a]	263.1[a]	7.4
Gain/Feed, g/g	0.38[a]	0.28[b]	0.40[a]	0.27[b]	0.10	0.47[a]	0.35[c]	0.42[b]	0.07

* C, control (casein); RP, raw peas; EP, extruded peas; SRP, supplemented raw peas; SEP, supplemented extruded peas.

Statistical comparisons were done separately for experiments 1 and 2. For each experiment, means in a row with different superscripts differ significantly ($p \leq 0.01$).

for rats and the control group was fed casein (control) diet. Animal weights and food intakes were monitored daily.

2.4. Determination of liver and muscle k_s, k_d and k_g

The k_s was determined by a flooding dose method [23] with some modifications [24]. Ten rats per group were intraperitoneally injected with 1 ml/100 g body weight of a solution containing 1.84 MBq/ml of L-[ring-2,6–^{3}H(N)] phenylalanine (1702 GBq/mmol specific activity and 37.0 MBq/ml concentration) combined with unlabeled phenylalanine to reach a final concentration of 150 mM. Ten minutes exactly after phenylalanine administration, rats were decapitated, and the trunk blood drained and collected. Liver and gastrocnemius, soleus and extensor digitorum longus muscles bilaterally excised, were weighed and frozen in liquid nitrogen. Muscles weighing less than 50 mg were pooled with their corresponding contralateral hindlimb from the same animal. Organ and tissues were homogenized and the phenylalanine specific radioactivity free and protein-bound was determined [25]. The k_s, expressed as percentage of protein synthesized per day, was calculated from the following equation: $k_s = 100 \cdot S_B/S_A \cdot t$; where S_A and S_B are the specific radioactivities of free and protein-bound phenylalanine, respectively, and t is the incorporation time in days.

The k_g values were estimated from the variation of protein content at successive days. Three rats per group were sacrificed for liver and muscle protein content determination at days 13, 14, 16 and 17 of the experiment. The growth rate, in grams of protein per day, was divided by the mean protein mass at that time point and multiplied by 100 to obtain the k_g, expressed as percentage of protein per day. The k_d values were calculated from the relationship $k_d = k_s\text{-}k_g$.

2.5. Protein, DNA and RNA analysis

Liver and muscles (100 mg) were homogenized in 10 ml ice-cold bideionised water using an Ultraturrax blender at 24,000 rpm at 0°C. Subsequently, the homogenates were diluted, and an aliquot taken for protein determination by Bradford method [26]. The remainder was precipitated with 0.6 N perchloric acid at 0°C. The soluble supernatant was discarded. Pellets were homogenized twice in 0.2 N perchloric acid and supernatants discarded. The residue was solubilised in 0.3 N KOH and incubated at 37°C for 60 min, and the protein and DNA was then precipitated by addition of an equal volume of 0.2 N perchloric acid. The RNA content of the supernatant was estimated by the orcinol reaction [27]. The protein-DNA precipitate was resuspended in 0.8 N perchloric acid and heated at 90°C for 15 min. The DNA content of the supernatant was measured using diphenilamine reagent [28].

2.6. Hormonal analysis

Plasma and serum were separated from the blood samples by centrifugation at 1,500 g for 10 min at 4°C and the stored at −20°C. Glucagon in plasma was measured by using a double-antibody radioimmunoassay kit (Diagnostic Products). Intra- and interassay coefficients of variation were 8.5 and 10.1%, respectively. Serum insulin was measured with a radioimmunoassay kit for rats (Amersham). Intra- and interassay coefficients of variation were 4.7 and 9.7%, respectively. Serum thyroxine (T_4) and 3,5,3′-triiodothyronine (T_3) levels were estimated by using commercially available RIA kits (CIS Bio International). Intra- and interassay coefficients of variation were lower than 4 and 5%, respectively. Serum testosterone was determined by using a radioimmunoassay kit (Amersham). Intra- and interassay coefficients of variation were 5.5 and 8.9%, respectively. Serum corticosterone was estimated with a RIA kit validated for rodents (DRG Instruments). Intra- and interassay coefficients of variation were 4.6 and 6.7%, respectively.

2.7. Statistical analysis

One-way analysis of variance (ANOVA), followed by Fisher's least significant difference (LSD), was used to determine the differences among treatments. Differences were considered significant with a P value at the 5% level. Computations were performed with the StatView/Apple Macintosh version 4.01 non-FPU (Abacus Concepts, 1992–1993) statistical package.

Table 3
Relative organ and muscle weights

Organ or tissue	Diets (expt 1)*				Pooled SD	Diets (expt 2)			Pooled SD
	C	RP	C	EP		C	SRP	SEP	
Liver (g/100 g bw)	3.93[a]	3.18[b]	3.67[c]	3.18[b]	0.12	3.55[a]	3.18[a]	3.38[ab]	0.14
Gastrocnemius (mg/100 g bw)	568[a]	494[b]	566[a]	512[b]	15	539[a]	521[b]	552[a]	6
Tibialis anterior (mg/100 g bw)	200[a]	174[b]	194[a]	179[b]	5	180[a]	180[a]	185[a]	4
Soleus (mg/100 g bw)	44[a]	42[a]	46[a]	45[a]	2	43[ab]	41[b]	46[a]	2
Extensor digitorum longus (mg/100 g bw)	46[a]	42[a]	45[a]	43[a]	2	43[a]	42[a]	47[b]	1

* C, control (casein); RP, raw peas; EP, extruded peas; SRP, supplemented raw peas; SEP, supplemented extruded peas.

Statistical comparisons were done separately for experiments 1 and 2. For each experiment, means in a row with different superscripts differ significantly ($p \leq 0.01$).

3. Results

3.1. Chemical analysis

Raw peas contained significant amounts of trypsin (3.2 ± 0.0 g/kg) and chymotrypsin (2.6 ± 0.2 g/kg) inhibitors and lectin (32×10^4 HU/kg) but no detectable levels of α-amylase inhibitor. The protease inhibitors and lectin were however completely inactivated or eliminated by extrusion cooking. Protein digestibility *in vitro* (IVPD) of pea flours was increased significantly (from 750 ± l to 802 ± l g/kg, $P < 0.01$) by extrusion. However, even for extruded peas the value remained lower than that for casein (930 g/kg). Starch digestibility *in vitro* (IVSD) was significantly ($P < 0.01$) improved (from 245 ± l to 296 ± 3 g/kg) as a result of heat treatment.

3.2. Rat growth

Food conversion efficiency and growth by rats was significantly reduced when raw peas provided the sole source of protein in the diet (Table 2). Extrusion pre-treatment of the seed meals led to a slight increase in food intake but no improvement in weight gain or feed conversion efficiency. Supplementation of diets based on raw or extruded peas with individual amino acids [raw: Met, lIe, His, Trp, Thr; extruded: Met, lIe, His, Trp, Val] up to the essential amino acid requirements for rats greatly increased consumption of these diets by rats. Indeed, intakes were similar to those of rats given free access to control diet (Table 2). Food conversion efficiencies and weight gains were also enhanced (Table 2). However, while the changes in these parameters were quite small for diets based on supplemented raw peas (SRP), they were marked for the supplemented extruded pea (SEP) diets. Thus, extrusion treatment did significantly improve the nutritional quality of peas. However, these beneficial changes were not apparent with unsupplemented extruded peas due to the overwhelming effects essential amino acid deficiencies on consumption and utilization of the seed meals.

3.3. Organ weights

The relative (g/100 g body weight) wet weights of the gastrocnemius and tibialis anterior hindlimb muscles ($p \leq 0.05$) and the liver ($p \leq 0.01$) were greatly decreased in rats fed RP or EP (Table 3). Supplementation with amino acids appeared to abolish the negative effects of pea diets on the tibialis anterior muscles. Gastrocnemius muscle weights remained low in rats given SRP but were close to control values in rats fed SEP. Liver weights were also reduced by SRP. However, unlike the gastrocnemius muscles, liver

Table 4
Protein turnover in gastrocnemius

	Diets (expt 1)*				Pooled SE	Diets (expt 2)			Pooled SE
	C	RP	C	EP		C	SRP	SEP	
Protein content (mg)	176.5[a]	124.7[b]	177.3[a]	126.4[b]	3.1	226.4[a]	207.2[b]	225.4[a]	5.6
DNA (mg)	0.78[a]	0.70[a]	0.79[a]	0.70[a]	0.04	0.97[a]	0.98[a]	0.98[a]	0.06
RNA (mg)	1.23[a]	0.85[b]	1.26[a]	0.83[b]	0.03	1.50[a]	1.28[b]	1.54[a]	0.05
k_s (% · d^{-1})	14.0[a]	12.8[a]	13.8[a]	13.2[a]	0.8	15.2[a]	13.7[b]	14.8[a]	0.4
k_s/RNA (g protein · gRNA, d^{-1})	20.3[a]	18.9[a]	19.8[a]	20.3[a]	1.7	23.1[a]	22.6[a]	22.0[a]	1.3
k_d (% d^{-1})	12.2[a]	11.7[a]	12.0[a]	12.0[a]	0.9	13.2[a]	12.1[a]	13.0[a]	0.5
k_g (% d^{-1})	1.8[a]	1.1[b]	1.8[a]	1.2[b]	0.1	2.1[a]	1.7[b]	1.8[ab]	0.1

* C, control (casein); RP, raw peas; EP, extruded peas; SRP, supplemented raw peas; SEP, supplemented extruded peas.

Statistical comparisons were done separately for experiments 1 and 2. For each experiment, means in a row with different superscripts differ significantly ($p \leq 0.01$).

Table 5
Protein turnover in soleus muscle

	Diets (expt 1)*				Pooled SE	Diets (expt 2)			Pooled SE
	C	RP	C	EP		C	SRP	SEP	
Protein content (mg)	11.3[a]	8.8[b]	11.7[a]	9.2[b]	0.6	226.4[a]	207.2[b]	225.4[a]	5.6
DNA (mg)	0.13[a]	0.12[a]	0.13[a]	0.12[a]	0.01	0.97[a]	0.98[a]	0.98[a]	0.06
RNA (mg)	0.14[a]	0.13[a]	0.14[a]	0.13[a]	0.01	1.50[a]	1.28[b]	1.54[a]	0.05
k_s (% · d^{-1})	16.2[a]	14.8[b]	16.0[a]	15.2[ab]	0.1	15.2[a]	13.7[b]	14.8[a]	0.4
k_s/RNA (g protein · gRNA, d^{-1})	25.6[a]	16.5[b]	27.0[a]	17.7[b]	0.2	44.2[a]	27.9[b]	35.3[c]	0.3
k_d (% d^{-1})	11.7[a]	11.0[b]	11.4[ab]	11.1[b]	0.2	11.4[a]	11.1[a]	11.3[a]	0.2
k_g (% d^{-1})	4.5[a]	3.8[b]	4.6[a]	4.1[ab]	0.2	4.8[a]	4.6[a]	4.8[a]	0.2

* C, control (casein); RP, raw peas; EP, extruded peas; SRP, supplemented raw peas; SEP, supplemented extruded peas.

Statistical comparisons were done separately for experiments 1 and 2. For each experiment, means in a row with different superscripts differ significantly ($p \leq 0.01$).

weights were not fully restored to control levels when rats were given SEP. Soleus and extensor digitorum longus muscle wet weights did not appear to be consistently affected by pea-based diets.

3.4. Muscle and liver composition and protein turnover

Gastrocnemius muscles of rats given RP or EP contained around 30% less protein and RNA than comparable control muscles (Table 4). DNA levels did not however differ between the test and control groups. Protein accretion (k_g) rates were lowered (about 0.6–0.7%/day less than control values) in both test groups and k_s was also slightly, although not significantly, reduced. There was little difference between RP and EP in their effects on these muscles.

Supplementation of raw or extruded pea diets with individual amino acids led to an increase in the protein and RNA in gastrocnemius muscles and higher rates of protein accretion (k_g) and synthesis (k_s) (Table 4). However, significant differences in the effects of SRP and SEP were now evident. Thus, while protein, RNA, k_g and k_s in gastrocnemius muscles remained below control values with rats fed SRP, these parameters were higher and generally similar to control levels with rats fed SEP.

Pea diets appeared, in part, to affect soleus and extensor digitorum longus muscles in a manner similar to that seen in the gastrocnemius muscles. Thus, protein levels, K_s and K_g were reduced when RP, EP or SRP diets were fed to rats but these parameters were close to control values when rats were given SEP (Tables 5–6). Nonetheless, unlike with the gastrocnemius muscles, protein degradation rates (K_d) in extensor digitorum longus muscles were also slightly reduced by RP, EP or SRP. In addition, RNA levels in extensor digitorum longus and soleus muscles were unaltered by pea-based diets.

RP and EP diets both affected liver composition and protein turnover in a similar manner (Table 7). Livers from rats fed RP or EP contained considerably less protein, RNA and DNA (around 30–36% less in total) than did livers from control animals. The protein accretion (k_g) rates were also greatly reduced (between 1.6–2.0%/day less). However, protein synthesis (k_s) and degradation (K_d) in the liver were high (>50%/day) and did not seem to be significantly altered by RP or EP diet.

Liver protein, RNA and DNA levels were elevated in all rats fed supplemented pea or control diet *ad libitum* (Table 7). The increases were however less marked in rats given SRP or SEP. Thus, livers from these animals contained less (around 14–24%) protein and RNA than livers from controls. The DNA contents also tended to be lower as did liver

Table 6
Protein turnover in extensor digitorum longus muscle

	Diets (expt 1)*				Pooled SE	Diets (expt 2)			Pooled SE
	C	RP	C	EP		C	SRP	SEP	
Protein content (mg)	15.0[a]	12.1[b]	15.2[a]	12.1[b]	0.6	19.8[a]	16.4[a]	18.6[a]	0.71
DNA (mg)	0.12[a]	0.10[a]	0.11[a]	0.12[a]	0.01	0.14[a]	0.14[a]	0.14[a]	0.01
RNA (mg)	0.12[a]	0.11[a]	0.13[a]	0.11[a]	0.01	0.15[a]	0.13[a]	0.15[a]	0.01
k_s (% · d^{-1})	12.3[a]	8.0[b]	12.2[a]	8.5[b]	0.2	14.9[a]	12.7[b]	14.4[a]	0.4
k_s/RNA (g protein · gRNA, d^{-1})	15.1[a]	9.3[b]	14.9[a]	9.8[b]	0.5	20.0[a]	15.8[b]	18.7[a]	0.8
k_d (% d^{-1})	10.6[a]	6.7[b]	10.4[a]	7.1[b]	0.2	13.1[a]	11.2[b]	12.7[a]	0.4
k_g (% d^{-1})	1.7[a]	1.3[b]	1.8[a]	1.4[b]	0.1	1.8[a]	1.5[b]	1.8[a]	0.1

* C, control (casein); RP, raw peas; RP, raw peas; EP, extruded peas; SRP, supplemented raw peas; SEP, supplemented extruded peas.

Statistical comparisons were done separately for experiments 1 and 2. For each experiment, means in a row with different superscripts differ significantly ($p \leq 0.01$).

Table 7
Protein turnover in liver

	Diets (expt 1)*				Pooled SE	Diets (expt 2)			Pooled SE
	C	RP	C	EP		C	SRP	SEP	
Protein content (mg)	1.06[a]	0.75[b]	1.07[a]	0.79[b]	0.04	1.32[a]	1.00[b]	1.07[b]	0.06
DNA (mg)	28.70[a]	19.59[b]	28.71[a]	19.75[b]	1.57	40.21[a]	34.26[a]	36.25[a]	2.55
RNA (mg)	43.20[a]	27.70[a]	43.34[a]	28.27[b]	2.67	52.92[a]	43.56[b]	46.35[b]	1.23
k_s (% · d^{-1})	58.0[a]	58.6[a]	57.7[a]	58.1[a]	2.0	53.6[a]	55.1[a]	54.1[a]	1.4
k_s/RNA (g protein · gRNA, d^{-1})	14.1[a]	16.0[a]	14.0[a]	16.1[a]	0.7	13.5[a]	12.9[a]	12.5[a]	0.7
k_d (% d^{-1})	53.5[a]	56.2[a]	53.3[a]	54.3[a]	2.0	48.8[a]	51.2[a]	49.7[a]	1.4
k_g (% d^{-1})	4.4[a]	2.4[b]	4.4[a]	2.8[b]	0.2	4.8[a]	3.9[b]	4.4[ab]	0.2

* C, control (casein); RP, raw peas; RP, raw peas; EP, extruded peas; SRP, supplemented raw peas; SEP, supplemented extruded peas.
Statistical comparisons were done separately for experiments 1 and 2. For each experiment, means in a row with different superscripts differ significantly ($p \leq 0.01$).

k_g. In contrast, k_s and k_d did not appear to be altered by SRP or SEP. Again, there was little difference between the raw or heat-treated pea diets in their effects on the liver even after supplementation with amino acids.

3.5. Hormones

Glucagon levels in blood were slightly increased in rats fed RP or EP (Table 8). None of the other hormones analyzed were consistently affected by these diets although triiodotyronine was increased with RP and tetraiodotyronine by EP. There were no differences in glucagon, insulin, tetraiodotyronine and testosterone blood concentration between rats given free access to SRP, SEP or control diet (Table 8). However, triiodotyronine levels were slightly increased when rats were fed SRP and corticosterone was elevated in rats given SRP or SEP.

Blood corticosterone was high in control rats given a regulated amount of food daily and testosterone was low (Table 8). Thus, dietary intake restriction significantly affected the circulating levels of both of these hormones. None of the other hormones studied appeared to be significantly altered by intake restriction.

4. Discussion

Raw pea proteins were poorly utilized by rats. This appeared to be due to a combination of factors including amino acid deficiencies, the presence of anti-nutritional compounds and possibly the refractory nature of the reserve proteins and other seed constituents such as starch. Heat-treatment of the peas inactivated or eliminated the major anti-nutritional factors (lectins and enzyme inhibitors) from the seeds and greatly increased protein and starch digestibility in vitro as with other seeds [17,29,30]. However, this did not appear to lead to any significant improvement in the efficiency with which the peas were utilized *in vivo*. This was due to amino acid deficiencies, primarily in the sulfur amino acids, in the pea diets that had major and overriding effects on utilization of both raw and heat-treated pea proteins. Once these deficiencies had been eliminated, by supplementation of the pea diets with individual amino acids up to the target requirements for rats, free intake of pea diets was increased by approximately 65% and weight gain on pea diets by about 105% (raw) and 140% (extruded) respectively. Heat-treatment did therefore improve pea protein availability *in vivo*. However, the improvements were moderate compared to those achieved through supplementation

Table 8
Hormone levels

	Diets (expt 1)*				Pooled SE	Diets (expt 2)			Pooled SE
	C	RP	C	EP		C	SRP	SEP	
Glucagon (pg/ml)	106.0[b]	129.0[a]	104.1[b]	121.7[b]	4.9	98.3[a]	103.0[a]	99.0[a]	4.3
Insuline (μU/ml)	7.5[a]	6.4[a]	7.5[a]	6.6[a]	0.5	7.5[a]	6.5[a]	6.9[a]	0.3
Triiodotyronine (ng/ml)	1.10[b]	1.38[a]	1.13[b]	1.23[b]	0.06	0.98[b]	1.18[a]	1.00[b]	0.07
Tetraiodotyronine (ng/ml)	5.23[b]	5.80[b]	5.25[b]	6.10[a]	0.24	5.58[a]	5.03[a]	5.43[a]	0.25
Testosterone (ng/ml)	0.41[a]	0.31[a]	0.42[a]	0.35[a]	0.05	0.64[a]	0.50[a]	0.61[a]	0.06
Corticosterone (ng/ml)	324.5[a]	358.3[a]	320.0[a]	355.1[a]	20.4	213.3[a]	304.2[b]	282.6[b]	19.3

* C, control (casein); RP, raw peas; EP, extruded peas; SRP, supplemented raw peas; SEP, supplemented extruded peas.
Statistical comparisons were done separately for experiments 1 and 2. For each experiment, means in a row with different superscripts differ significantly ($p \leq 0.01$).

of pea diets with essential amino acids alone. This is consistent with previous findings [11].

Muscle protein synthesis and deposition were greatly reduced if rats were fed either raw or extruded peas. This was primarily due to the amino acid deficiencies in the diets since both parameters were greatly elevated after supplementation of the pea diets with individual amino acids up to the target profile for rats. However, rates of muscle protein synthesis and total muscle protein content in rats fed supplemented raw peas remained lower than for controls given free access to a high quality diet. This impairment may have been due to reduced nutrient availability [1] to the muscles as a result of interference with gut and systemic metabolism by antinutritional factors [9,10] and poor digestion of reserve proteins and other seed components due to their refractory nature.

Extrusion treatment of peas in combination with supplementation with amino acids (SEP) appeared to abolish the negative effects of peas on skeletal muscle deposition. Thus, protein synthesis (K_s) rates and protein content in gastrocnemius and extensor digitorum longus muscles from rats fed supplemented extruded peas were similar to control levels. These parameters were also greatly enhanced in soleus muscles of rats fed SEP. Despite these improvements in muscle deposition, body weight gains by rats fed SEP were still lower ($+7.4 \pm 0.2$ g/day) compared to that of control animals ($+8.4$ g/day). Assuming skeletal muscle to be 152 g/kg body wet weight [31], SEP-fed rats should have gained 16.8 g skeletal muscle over the study ($+1.1$ g/day) while controls should have deposited 19.0 g ($+1.3$ g/day). Overall, the data suggests that poorer than expected weight gains by SEP-fed rats are not due to reduced deposition of skeletal muscle. Although the relative weights of muscle from rats fed SEP did not statistically differ from controls, the means for all the muscles studied were higher in rats fed SEP than in controls. This would be consistent with accretion of other body components being impaired slightly more than muscle deposition. For example, if lipid deposition is reduced, muscle weight as a proportion of body weight increases [32]. Muscle K_s was decreased in fasted chickens previously fed on the soya-bean diet unsupplemented with lysine or methionine [33]. Administration of leucine alone induced a decrease in the rate of myofibrillar degradation, whereas the serum insuline concentration was constant [34].

Liver protein accretion (K_g) and protein content were also adversely affected by pea diets. Supplementation of pea diets with amino acids increased both liver K_g and protein content. Nonetheless, the values remained well below those obtained for control livers. In addition, unlike for muscle metabolism, extrusion treatment of the peas did not appear to lead to an increase in liver protein content. The reasons for this remain unclear. However, the liver was very metabolically active with high protein turnover rates (K_s approximately 54%/day and k_d around 50%/day), whereas turnover rates in muscle were much slower (K_s approximately 15%/day and k_d around 12%/day). Protein availability *in vitro* was greatly improved by extrusion treatment of peas but remained slightly below that for a high quality protein, such as casein. Because of its high demand for amino acids to support turnover, it is possible that the liver was more severely affected by a slight short-fall in protein availability than skeletal muscle where protein turnover was much slower.

A number of hormones were affected by inclusion of raw or extruded peas in the diet. However, with the exception of triiodotyronine (SRP diet) and corticosterone (SRP and SEP diets), these treatment differences were abolished after supplementation of raw or extruded pea diets with amino acids. Blood corticosterone levels remained high in rats fed SEP compared to those in controls. This did not appear to be a stress response to restriction of food intake. However, if nutrient availability from SEP is slightly below optimal, it may be a response to a cumulative short-fall in nutrients. Alternatively, it may have been triggered by heat-stable bioactive factors [35] in the pea diet.

Very high levels of corticosterone lead to a catabolic state where breakdown of body reserves can occur [36,37]. SEP caused a small increase in circulating corticosterone that may have been enough to slightly reduce accretion of lipid and glycogen reserves without affecting muscle deposition thereby slowing weight gain by the animals. In addition, because of the high protein turnover in the liver, elevated corticosterone may have been sufficient to interfere with protein deposition in that tissue.

In summary, muscle protein synthesis and deposition were greatly reduced if animal were fed either raw or extruded peas. Protein synthesis, rate, availability and protein content in muscles from rats fed supplemented extruded peas were similar to control levels. The rat model has shown that extrusion improved protein synthesis rate. Consumption of these extruded legumes, incorporated in the diet, will be beneficial to protein metabolism and hormonal levels. These beneficial effects may improve the nutritional status among populations who consume diets rich in legumes.

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