Food & Function

Accepted Manuscript

This is an *Accepted Manuscript*, which has been through the RSC Publishing peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, which is prior to technical editing, formatting and proof reading. This free service from RSC Publishing allows authors to make their results available to the community, in citable form, before publication of the edited article. This *Accepted Manuscript* will be replaced by the edited and formatted *Advance Article* as soon as this is available.

To cite this manuscript please use its permanent Digital Object Identifier (DOI®), which is identical for all formats of publication.

More information about *Accepted Manuscripts* can be found in the **Information for Authors**.

Please note that technical editing may introduce minor changes to the text and/or graphics contained in the manuscript submitted by the author(s) which may alter content, and that the standard **Terms & Conditions** and the **ethical guidelines** that apply to the journal are still applicable. In no event shall the RSC be held responsible for any errors or omissions in these *Accepted Manuscript* manuscripts or any consequences arising from the use of any information contained in them.

Pasta supplemented with isolated lupin protein fractions reduces body weight gain and food intake of rats and decreases plasma glucose concentration upon glucose overload trial

Jessica Capraro[1*], Chiara Magni[1], Alessio Scarafoni[1], Rosita Caramanico[1,2], Filippo Rossi[3], Mauro Morlacchini[4], Marcello Duranti[1]

[1]Department of Food, Environmental and Nutritional Sciences (DeFENS), Università degli Studi di Milano, Via G. Celoria 2, 20133 Milan, Italy

[2]Consiglio per la Ricerca e la Sperimentazione in Agricoltura – Unità di Ricerca per la Selezione dei Cereali e la Valorizzazione delle Varietà Vegetali (CRA-SCV), Via Mulino 3, 26866 S. Angelo Lodigiano (LO), Italy

[3]Institute of Food Science and Nutrition (ISAN), Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122 Piacenza, Italy

[4]Research Centre on Livestock and Environment (CERZOO), Località Possessione di Fondo, San Bonico, Piacenza, Italy

*Corresponding Author:
Dr. Jessica Capraro
DeFENS
Via Celoria, 2
20133 Milano, Italy
phone: ++39 02 503 16823
fax number: ++39 02 503 16801
e-mail: jessica.capraro@unimi.it

Abstract

The supplementation of foods with biologically active compounds can be a powerful approach for improving diet and well being. In this study we separately included into pasta matrices a concentrate of γ-conglutin, a glucose-lowering protein from *Lupinus albus* seeds, an isolate of the other main lupin storage proteins and ovalbumin, with a ratio corresponding to 125 mg of pure protein in 100 g of pasta. With these products we fed rats made hyperglycaemic for 3 weeks. Among the most relevant changes measured in body and blood parameters were: *i.* a significant reduction of food intake of rats fed γ-conglutin concentrate supplemented pasta and a significant limitation in the body weight increase in rats fed α, β and δ-conglutins isolate supplemented pasta, while the food conversion indexes were unchanged; *ii.* a reduction of glycaemia upon glucose overload trial, especially in the γ-conglutin concentrate supplemented pasta fed animals at a dose of 45 mg/kg body weight. The correlations among the measured parameters are discussed. Overall, the results evidence the potentiality of supplementing traditional foods with exogenous nutraceutical seed proteins to control body weight gain and glycaemia.

Keywords: legume seed proteins; *Lupinus albus*; γ-conglutin; glycaemia; overweight; diabetes.

Abbreviations

ADG: Average Daily Gain; AUC: area under the curve; CI: conversion index; d.w.: dry weight.

1. Introduction

The role of foods and food components on the prevention and control of several diseases is increasingly been acknowledged worldwide. Therefore, the need of improving the nutritional/nutraceutical performance of foods to control expanding syndromes, such as diabetes, obesity, metabolic syndrome, overweight conditions and others, is crucial. One of the possible ways is the supplementation of traditional foods with biologically active compounds. However, effective examples in this area are not frequent and often technological/functional problems related to stability, efficiency, dosage of the compounds and their sensorial effects on foods prevent the implementation of potentially relevant formulas.

Nowadays, a critical sanitary area is the control of diabetes and obesity, which are already a public health emergency for their increasing incidence and prevalence throughout the world.[1] In this respect, the kinetics of glucose release and absorption after food ingestion and the resulting effects on the synthesis of and sensitivity to insulin, as well as the regulation of satiety, are gaining interest for the prevention of diabetes and obesity.

Lupin seeds and flours are among the natural foods which have been claimed to have anti-diabetic properties by the traditional medicine. As a matter of facts, the active principle responsible of the glucose-lowering activity has unequivocally been identified in the protein γ-conglutin, that in *Lupinus albus* seeds constitutes about 5% of total seed proteins, corresponding to about 2 g of γ-conglutin in 100 g of dry seeds or flour. Significant variations of γ-conglutin content amongst *L. albus* varieties have not been reported so far. Not only has this lupin protein repeatedly been shown to decrease glycaemia in animals and humans,[2,3] but its interaction with the putative target cells, including myocytes and hepatocytes,[4,5] proved to produce a number of effects which have collectively been classified as insulin-mimetic activity. Despite this potentiality, no study on the effects of γ-conglutin incorporated into a food matrix has been undertaken so far.

Pasta is a traditional food which, for many structural and technological reasons, can be considered an effective vehicle of bioactive proteins and peptides. Consequently, we have prepared pasta samples supplemented with a γ-conglutin concentrate or the remnant main lupin seed proteins isolate as well as with ovalbumin, as a control. With these samples, we have fed rats for three weeks and measured various body and blood parameters.

Thus, the objective of this work was to compare the effects of a diet based on pasta containing small amounts of specific lupin protein fractions on some physiological and biochemical parameters.

2. Material and methods

2. 1 Materials

White lupin seeds (*Lupinus albus*, var. Multitalia) were a kind gift of Agroservice S.p.A., Rocchetta, San Severino Marche, Italy.

Ovalbumin was purchased from Sigma Aldrich (Milan, Italy).

Chemicals were all reagent grade by Sigma Aldrich (Milan, Italy), unless specified otherwise.

2.2 Lupin proteins fractionation

Mature dry seeds of *Lupinus albus*, L. were used as a source of the protein fractions. Lupin proteins were fractionated as described by Sironi with some minor modifications.[6] Briefly, the seeds were milled to a flour which was sieved through a 80 mesh sieve. The proteins in the flour were extracted with distilled water containing 0.5 M NaCl in the ratio 1/10 w/v at 4°C for 3 hours. The pH was constantly kept between 7.0 to 9.0 by the addition of 0.1 M NaOH. The slurry was centrifuged at 10,000 x g at 4 °C for 30 min. The supernatant was adjusted to pH 4.5-5.5 with acetic acid for protein isoelectric precipitation. The resulting suspension was centrifuged as above. The pellet was washed twice with distilled water at pH around 5.0. The washed pellet was then freeze dried. This fraction consisted of the isoelectrically-precipitated main lupin proteins. The fraction soluble at pH 5.0 was adjusted to pH 7.0 with 0.1 M NaOH and 20 mM $ZnCl_2$ was added. The resulting suspension was centrifuged as above and the pellet recovered. The pellet was washed twice with distilled water and freeze dried. As it will be seen below, this fraction mainly consisted of γ-conglutin.

2.3 SDS-PAGE of the protein fractions

The protein samples were suspended in the sample buffer in the ratio 1:20 (w/v). The sample buffer consisted of 0.25 M Tris–HCl, pH 6.8, 7.5 mL/100 mL glycerol, 20 mg/mL SDS and 2.5 mL/100 mL 2-mercaptoethanol. After heating at 100°C for 5 min, each sample was loaded on the gel. SDS-PAGE was carried out on 7 x 8 cm 12% polyacrylamide gels, using a mini-Protean III cell (Bio-Rad). The gels were stained with Coomassie blue.[7] The gels were digitalised in an Epson Perfection V500 Scanner and analysed with ImageMaster 1-D Elite Software (GE Healthcare, Milan, Italy).

2.4 Western blotting

The proteins in gels were transferred to 0.45μ-pore nitrocellulose membranes (Protran, Whatman, Dassel, Germany) by using the TE 77 PWR Semidry Transfer Unit (Amersham Biosciences), according to Towbin, Staehelin and Gordon.[8] γ-Conglutin was immune-detected as already described by Magni.[9]

2.5 Pasta preparation and protein inclusion

Pasta supplied to the animals was a mix of commercial dry pasta and dry test pasta, including selected protein fractions from lupin seed and ovalbumin, as the control. Test pasta containing γ-conglutin concentrate was mixed with commercial pasta in a 1/4 ratio (w/w); α+β+δ-conglutins isolate and ovalbumin containing pasta were mixed with commercial pasta in a 1/8 ratio (w/w). All pasta samples were uncooked to allow easy grinding to a fine powder.

Test pasta was prepared according to standard protocols. Durum wheat semolina of good pasta-making performances (protein: 14.3 g/100g db; dried gluten: 13.2g/100 g) was supplied by Molino Grassi (Parma, Italy). Semolina and protein fractions (1g/100g semolina and 1g/100g each lupin protein fraction and commercial ovalbumin) were mixed with water (kept at 40°C) to produce a mixture with a final moisture of 34%. After mixing for 10 minutes, each blend was extruded and formed into macaroni shape (7 mm external diameter) using a continuous pilot-scale plant (20 kg/h; MAC 30, Italpast, Parma, Italy). Fresh pasta was finally dried in a pilot-scale drier (50 kg/h, Braibanti, Milan, Italy) using a low-temperature drying cycle (50°C max for 14h), according to Marti, Seetharaman and Pagani.[10]

2.6 Animal care and diets

Forty eight male Sprague Dawley rats weighting 195.7 ± 13.5 g were adapted to a standard diet, consisting of commercial pasta (80.5%), casein (10%), Arbocel (4%), minerals (3.5%), olive oil (1%) and vitamins (1%) for seven days. Tap water containing 10 % glucose was supplied *ad libitum* to the animals during the adaptation phase, in order to induce an hyperglycemic state and also during the subsequent three weeks experimental period with test pasta.

Between the adaptation phase and the test period, glucose administration was suspended for 12 hours and the glucose plasma levels were measured.

All procedures involving rats and their care were performed according to the Italian Law on animal tests (D.L. 116/1992).

2.6.1 Chronic treatment with test pasta

Test diet consisted of the above mentioned diet (adaptation diet) with the substitution of part of commercial pasta with a corresponding amount of test pasta samples. The animals (24) were divided into 3 homogenous groups, according to their blood glucose concentration. The animals were fed *ad libitum* with a diet containing pasta supplemented with lupin γ-conglutin concentrate, α+β+δ-conglutins isolate and ovalbumin, respectively, for three weeks. Glucose containing water

was freely available. Every day the food intake and the body weight of each animal were measured. At the end of the experimental period, the animals were fasted for 12 hours, sacrificed, and the blood was collected for the determination of glycaemia, insulin and other hormone concentrations.

2.6.2 Acute treatment with test pasta (Glucose Overload Trial)

At the end of the adaptation period, 24 untreated animals were divided into 3 homogenous groups, according to their blood glucose concentration. The animals were fasted for 20 hours. Then, 10 g of γ-conglutin, α+β+δ-conglutins and ovalbumin supplemented pasta samples were supplied to the animals, which entirely ate within 30 minutes. Immediately after this meal, 2 g/kg of D-glucose were administered to the animals by gavage. Before the meal and 30-60-90-120 minutes after glucose administration, blood glucose was determined.

2.7 Blood analysis

Glucose concentrations were measured using a kit, based on an enzymatic method of detection, purchased from R-Biopharm (Germany). Insulin, leptin and total ghrelin were assayed using ELISA commercial kits from Millipore Corporation (USA) and the microplate reader 3550 (BioRad, Milan, Italy).

2.8 Statistical analysis

The data obtained were analyzed by the SAS statistics program (SAS Inst., Cary, NC, USA) version 9.2, using the procedure of G.L.M. (General Linear Model) of the software and adopting the following experimental model:

$y = \mu + \alpha + \varepsilon$ where:

y = parameters (blood glucose, daily weight gain, food intake etc.)

μ = population means

α = effect of protein type (γ-conglutin, α+β+δ-conglutins, ovalbumin)

ε = experimental error

Analysis of variance was carried out on the data collected by using the above cited software.

The Bonferroni test was used to compare the means. The differences were considered significant at $P<0.05$. The relationships between variables was determined by means of Pearson's correlation coefficient, using the software PROC CORR of SAS 9.2.

3. Results

3.1 Electrophoretic analysis of the protein fractions included in the pasta samples

The two isolated lupin protein fractions and ovalbumin, which were included in the test pasta dough at a dose of 1g/100g, were submitted to SDS-PAGE under reducing conditions and the resulting profiles are shown in Figure 1. By comparing the electrophoretic patterns with the γ-conglutin and total proteins extract references and on the basis of previous separations and mass spectrometry analysis,[11] it was concluded that the lane B of Fig. 1 contained primarily γ-conglutin, while the lane A consisted of the main other lupin cotyledonary proteins, that is α+β+δ-conglutins, which, having an acidic pI, precipitated at pH 4.5-5.0 (see details under Methods, paragraph 2.2). These latter proteins consisted mainly in 11S- (legumin-like), 7S- (vicilin-like) and 2S-globulins, respectively. For a molecular characterization of these proteins see reference Duranti, Consonni, Magni, Sessa, and Scarafoni.[12]

Densitometric analysis of the lane B showed that the polypeptides corresponding to γ-conglutin, that is the bands of 30 and 17 kDa, amounted to about 50% of the total proteins in the lane. Therefore the actual amount of γ-conglutin in the test pasta samples was calculated to be 0.5 g/100 g. According to this finding and to the ratio 1:4 by weight of the mix between test pasta and commercial pasta, the concentration of pure γ-conglutin in the pasta mix supplied to rats with the diet was 0.125g/100g, corresponding to the same amounts of α+β+δ-conglutins and ovalbumin in the respective test pasta products.

3.2 Chronic effects of pasta intake on rat growth parameters and basal blood glucose levels

The effects on the rat body parameters measured after the adaptation period and the induction of a hyperglycemic condition of the three test pasta products are reported in Tables 1. Considering the amounts of added proteins in the pasta fed the animals each day and the average daily intake and body weight, it was calculated that the dosages of supplemented proteins were about 90 mg/kg.day.

The rat initial weights did not differ in the three groups, while differences were marked at the end of the 3 weeks trial: in particular a lower body weight was reached by rats fed α, β and δ-conglutins isolate compared to animals fed the ovalbumin supplemented pasta. The food intake of rats fed γ-conglutin concentrate supplemented pasta was also remarkably and significantly lower than that observed with ovalbumin supplemented pasta.

The constancy of the conversion index (CI) for all samples suggested a similar efficiency of the three groups in converting food to energy.

Table 2 reports the fasting glycaemia levels observed at the beginning and the end of the treatment. Glycaemia measured in rats fed lupin protein supplemented pasta for three weeks did not significantly differ from that measured with ovalbumin.

At the end of the treatment, no statistically significant changes of hormone levels between the lupin protein supplemented pasta and the ovalbumin supplemented pasta were monitored (Table 2), also due to the great variability.

3.3 Correlations between the measured parameters

All the measured parameters have been correlated by using the Pearson correlation coefficient in order to investigate the possible inter-dependences, as described under Methods and shown in Table 3.

After the chronic treatment, blood glucose, food intake, growth and leptin blood level of treated animals positively correlated: higher food intake induced higher growth and glycaemia; leptin is produced by adipose tissue, therefore higher growth induced higher release of leptin. Leptin and insulin also positively correlated, according to Amitani, Asakawa, Haruka, and Inui.[13] In our experiment both hormones were directly related and this could be due to the positive relationship between insulin and live weight. Since leptin is related to body weight, we could hypothesize that heavier animals have higher concentrations of insulin and leptin. Anyway the positive relationship between insulin and leptin observed in our experiment is a quite common finding.[14]

The interpretation of the positive relationship between ghrelin and leptin is more complex.[15] Usually the trends of these two hormones are opposite, but they are strongly affected by blood glucose. When glycaemia is high, ghrelin inhibits leptin, while at lower glucose concentration this does not happen.[16]

3.4 Glucose overload trial with test pasta samples

The animals (24) fed the adaptation diet for one week to induce hyperglycemia and fasted for 20 hours were submitted to glucose overload trial. During the 20 hour fasting, rats were allowed to drink tap water with no glucose added. Then, as detailed under Methods, 10 g of γ-conglutin concentrate, α+β+δ-conglutin isolate and ovalbumin supplemented pasta samples were given the animals, which entirely ate them within 30 minutes. Immediately after this meal, blood glucose was determined and 2 g/kg of D-glucose were administered to the animals by gavage. The amount of administered γ-conglutin in this experiment corresponded to about 45 mg/kg body weight. The glycaemia levels every 30 min for two hours of the glucose overload trial are reported in Table 4. Major changes were observed up to 60 min from the treatment, with statistically significant lower increments for γ-conglutin concentrate supplemented pasta. At 90 minutes all plasma glucose levels reached a maximum and then declined at 2 hours, steadily approaching the basal levels.

A global estimation of the glycaemia trend is depicted by the incremental areas under the curve (AUC) of the glucose overload trial, reported in Figure 2. As it can be seen, the plasma glucose reduction by γ-conglutin concentrate was apparent already at the first 30 min, though the differences were not statistically significant. Conversely, large and statistically significant differences were visible at 60 min and up to 90 minutes, where the AUCs induced by γ-conglutin concentrate included in pasta were dramatically lower than those obtained with pasta supplemented with ovalbumin. This finding is consistent with the glucose-lowering activity of lupin γ-conglutin previously measured with the purified protein administered by gavage in rats.[5] However, in the present work the protein was supplemented into a traditional food.

4. Discussion

In this work, the chronic and acute effects of lupin protein fractions physically entrapped into a common food matrix on selected body parameters and plasma glucose levels of rats was studied. Fasting blood glucose levels were into the same range as reported by previous works focusing on glycemic response to diets with different glycemic index or supplemented with γ-conglutin.[17, 2] An unforeseen finding of this work was the remarkable reduction of food intake and body weight increase induced by pasta supplemented with the lupin protein fractions with respect to the ovalbumin control. Regardless to the hypo-glicaemic activity, that is a peculiar property of γ-conglutin[2, 3], all lupin proteins showed a relevant effect on satiety and weight gain, although the mechanism of action is far from being understood. Although this result is somehow consistent with a former report by Lee *et al.*,[18] who monitored higher satiety and lower energy intake in human subjects fed with lupin enriched bread, the experimental plans of the two works strongly differed, beside being conducted on human subjects and rats. In particular, while these Authors included a lupin kernel flour into bread, thus providing also lupin fibers, of which the satiating role had already been assessed,[19] in our case only purified proteins and at very low dosage were used as supplements. Therefore, although a synergic effect between proteins and fibers could reasonably be expected, in our work the observed differences can be attributed to the protein fraction, only.

The further notable finding of this work concerned the decrease of acute glycaemic curve, especially at early times from the initial glucose load, and AUCs specifically induced by γ-conglutin concentrate supplemented pasta. This result confirms previous findings obtained with pure[2, 5] and raw[3] γ-conglutin, thus substantiating its role as plasma glucose-controlling natural agent. However, in the present work the biologically active protein has become a component of a food matrix, while in the previous works it was administered by gavage. Other teams have evidenced glycaemia-reducing effects of lupin flours in type II diabetic patients;[20] however, the Authors admitted that

their findings could have been affected by the lower carbohydrate content in the lupin meal with respect to the control. Still, pieces of evidence on the specific role played by lupin flours and its components in controlling plasma glucose concentrations are accumulating in the literature.[21,22]

The long term effect on glycaemia was less evident as compared to a previous work,[5] likely because γ-conglutin intake through the meal was less controlled in this than in that work, where the protein was administered daily by gavage.

To the best of our knowledge, this work represents the first report on the effects of a biologically active protein included in a food matrix. Indeed food processes (mechanical forces, high temperatures, pressures, etc.) could influence protein intrinsic properties and affect the interactions with other food components. However, it appears from our results that the physical entrapment of γ-conglutin into pasta matrix has not hampered its biological activity. It can be argued that the resulting diminished accessibility of the lupin protein to gastric enzyme and extreme low pH may have preserved γ-conglutin covalent structure and allowed its transit into duodenum in an intact and active form. As a matter of facts, γ-conglutin was shown to be fully resistant to digestive enzymes with optimum pH at neutral values.[23] In the present study, the heating effect on protein bio-activity could not be evaluated since pasta was administered to the animals without cooking. However, the presence of intact γ-conglutin in brine-cooked seeds has been detected (Capraro, unpublished results), although the protein decreased its solubility, probably due to interactions with other lupin proteins.

This work showed the feasibility of supplementing a traditional food with selected bioactive proteins, whose activities are not influenced by the inclusion. It can be stated that even low amounts of lupin proteins had unforeseen effects on body weight gain, satiety and glycaemia. These findings opens new perspectives to the exploitation of lupin γ-conglutin for the prevention and accompanying therapies of the many glucose-related diseases and, more in general, of the lupin proteins in weight controlling dietary programs.

Acknowledgements

The authors thank Dr. Antonio Gallo for his critic revising of the statistical analysis.

Jessica Capraro was supported by the grant "Dote Ricerca" of Regione Lombardia and European Social Found.

References

1 P.W. Sullivan, E.H. Morrato, V. Ghushchyan, and H.R Wyatt, *Diabetes Care*, 2005, **28**, 1599-1603.

2 C. Magni, F. Sessa, E. Accardo, M. Vanoni, P. Morazzoni, A. Scarafoni, and M. Duranti, *J Nutr Biochem*, 2004, **15**, 646-450.

3 J.C. Bertoglio, M.A. Calvo, J.L. Hancke, R.A. Burgos, A. Riva, P. Morazzoni, C. Ponzone, C. Magni and M. Duranti, *Phytoterapia*, 2011, **22**, 933-938.

4 I. Terruzzi, P. Senesi, C. Magni, A. Montesano, A. Scarafoni, L. Luzi and M. Duranti, *Nutr, Metab Cardiovas Diseases*, 2011, **21**, 197-205.

5 M.R. Lovati, C. Manzoni, S. Castiglioni, A. Parolari, C. Magni and M. Duranti, *Brit J Nutr*, 2012, **107**, 67-73.

6 E. Sironi, F. Sessa and M. Duranti, *Eur Food Res Technol*, 2005, **221**, 145-150.

7 C.M. Wilson, *Methods Enzymol*, 1983, **91**, 236-247.

8 H. Towbin, T. Staehelin and J.Gordon, *Proceedings of the National Academy of Science*, 1979, **76**, 4350-4354.

9 C. Magni, C. Ballabio, P. Restani, E. Sironi, A. Scarafoni, C. Poiesi and M. Duranti, *J Agric Food Chem*, 2005, **53**, 2275-2281.

10 A. Marti, K. Seetharaman and M.A. Pagani, *J Food Quality*, 2013, **36**, 133-138.

11 C. Magni, A. Scarafoni, A. Herndl, F. Sessa, B. Prinsi, L. Espen and M. Duranti, *Phytochemistry*, 2007, **68**, 997-1007.

12 M. Duranti, A. Consonni, C. Magni, F. Sessa and A. Scarafoni, *Trends Food Sci Tech*, 2008, **19**, 624-633.

13 M. Amitani, A. Asakawa, A. Haruka and A. Inui, *Front Neurosci*, 2013, **51**, 1-12.

14 M. Tsai, A. Asakawa, H. Amitani and A. Inui, *Indian J Endocrinol Metab*, 2012, **16**, S543-S548.

15 G.S. Barsh and M.W. Schwartz, *Nat Rev. Genet*, 2002, **3**, 589-600.

16 Otto-Buczkovska and A. Chabot, *Post□py higieny i medycyny doświadczalnej*, 2012, **6**, 795-798.

17 M. Kabir, W.R. Salwa, M. Champ, J. Luo, J. Boillot, F. Bruzzo and G. Slama, *J Nutr*, 1998, **28**, 35-43.

18 Y.P. Lee, T.A. Mori, S. Sipsas, A. Barden, I.B. Puddey, V. Burke, R.S. Hall and J.M. Hodgson, *Am J Clin Nutr*, 2006, **84**, 975-780.

19 B.J. Archer, S.K. Johnson, H.M. Devereux and A.L. Baxter, *Br J Nutr*, 2004, **91**, 591-599.

20 E.R. Dove, T.A. Mori, G.T. Chew, A.E. Barden, R.J. Woodman, I.B. Puddey, S. Sipsas and J.M. Hodgson, *Br J Nutr*, 2011, **106**, 1045-1051.

21 M. Fornasini, J. Castro, E. Villacrés, L. Narváez, M.P. Villamar, and M.E. Baldeón, *Nutr Hosp*, 2012, **27**, 425-433.

354 **Figure legends**

355

356 **Figure 1.** SDS-PAGE under reducing conditions of the protein fractions added to pasta. Lane A:
357 isoelectric precipitate; lane B: Zn^{++} precipitate (containing primarily γ-conglutin); lane C:
358 ovalbumin; lane M: marker proteins. γC: γ-conglutin reference, TPE: total protein extract reference.

359

360 **Figure 2**. Incremental areas under the curve (AUC) of the glucose overload trial. Plasma glucose
361 variations upon of the administration of pasta supplemented with lupin γ-conglutin concentrate
362 (black bars), α-, β- and δ-conglutins isolate (light grey bars) and ovalbumin (grey bars) to eight
363 animals for each group. Values are expressed as arbitrary units (a.u.). $^{a,b}P>0.05$ (Bonferroni Test).

364

Table 1

Growth parameters and food intake of rats fed pasta supplemented with lupin γ-conglutin concentrate, α+β+δ-conglutins isolate and ovalbumin for three weeks. For each of the three treatments, a separate set of eight rats was used. Data are presented as means ± standard deviation.

Treatment (n=8)	Initial weight (g)	Final weight (g)	ADG* (g/day)	Food intake (g/day)	CI§
γ-Conglutin concentrate	192.98 ± 20.17	345.50 ± 34.22[ab]	5.87 ± 1.28	18.46 ± 2.66[a]	3.22 ± 0.49
α+β+δ– Conglutins isolate	195.03 ± 10.41	331.83 ± 11.60[a]	5.26 ± 0.36	18.76 ± 0.89[ab]	3.57 ± 0.14
Ovalbumin	199.02 ± 8.97	370.20 ± 10.76[b]	6.58 ± 0.42	21.32 ± 1.41[b]	3.24 ± 0.21

* ADG: Average Daily Gain
§ CI = conversion index (food intake/growth)
[a,b] P>0.05 (Bonferroni Test)

Table 2

The fasting glycaemia and energy metabolism-related hormones levels in rats fed pasta supplemented with lupin γ-conglutin concentrate, α+β+δ-conglutin isolate and ovalbumin for three weeks For each of the three treatments, a separate set of eight rats was used. Data are presented as means ± standard deviation.

Treatment (n=8)	Initial glycaemia (mg/dL)	Final glycaemia (mg/dL)	Insulin (ng/mL)	Leptin (ng/mL)	Ghrelin (ng/mL)
γ-Conglutin concentrate	132.33 ± 9.95	131.83 ± 12.92	0.75 ± 0.68	1.59 ± 1.10	0.20 ± 0.19
α+β+δ-Conglutins isolate	133.83 ± 6.18	131.33 ± 22.66	0.59 ± 0.33	2.03 ± 0.46	0.15 ± 0.05
Ovalbumin	134.00 ± 6.20	135.17 ± 17.77	0.86 ± 0.08	2.14 ± 1.06	0.21 ± 0.09

Bonferroni Test: the effect of protein type did not result significant for any analyzed parameters ($P>0.05$).

Table 3

Correlation (r, Pearson's coefficient) between blood and growth parameters

	Glycaemia	Insulin	Ghrelin	Leptin	Weight	Intake	CI	ADG
Glycaemia	.	0.393†	NS	0.452*	0.521**	0.540**	NS	0.463*
Insulin		.	0.424*	0.625*	0.462*	NS	-0.500*	0.405*
Ghrelin			.	0.437*	NS	NS	NS	NS
Leptin				.	0.706**	0.567**	-0.582**	0.649**
Weight					.	0.937**	-0.752**	0.955**
Intake						.	-0.606**	0.907**
CI							.	-0.861**
ADG								.

CI = Conversion Index (feed/gain) ; ADG = Average Daily Gain
* $P < 0.05$
** $P < 0.01$
† $P = 0.064$

Table 4

Glycaemia variations upon glucose overload in hyperglycemic rats fed 10 g pasta supplemented with lupin γ-conglutin concentrate, α+β+δ-conglutin isolate and ovalbumin. For each of the three treatments, a separate set of eight rats was used. Data are expressed as means ± standard deviation.

Treatment (n=8)	Initial glycaemia (mg/dL)	Glycaemia (mg/dL) after glucose overload			
		30 minutes	60 minutes	90 minutes	120 minutes
γ-Conglutin concentrate	134.13 ± 21.60	158.75 ± 26.28	157.63 ± 12.32[a]	187.63 ± 32.83	154.50 ± 22.46
α+β+δ-Conglutins isolate	145.00 ± 25.86	182.75 ± 34.89	178.25 ± 20.36[a,b]	192.13 ± 40.39	155.00 ± 13.77
Ovalbumin	137.63 ± 17.91	173.25 ± 30.79	196.75 ± 35.16[b]	192.13 ± 47.30	158.50 ± 16.90

[a,b]P>0.05 (Bonferroni Test)

Figure 1

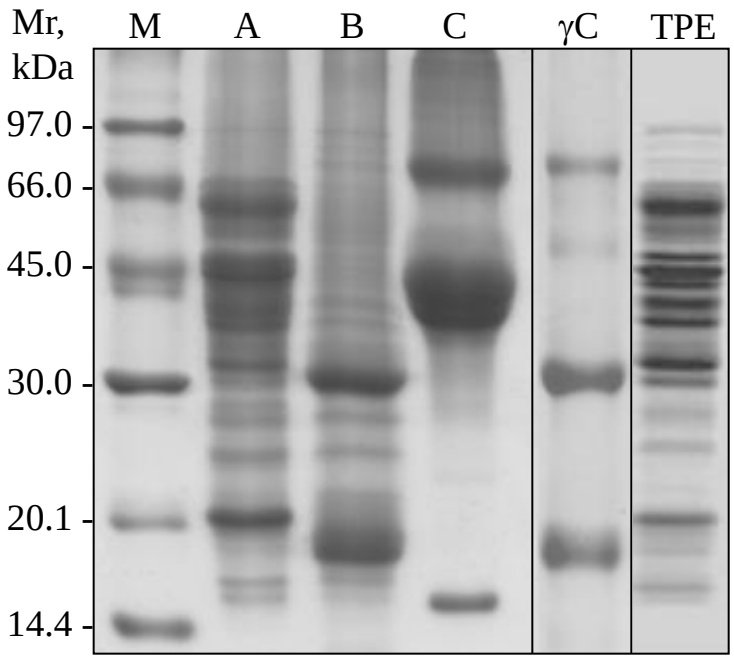
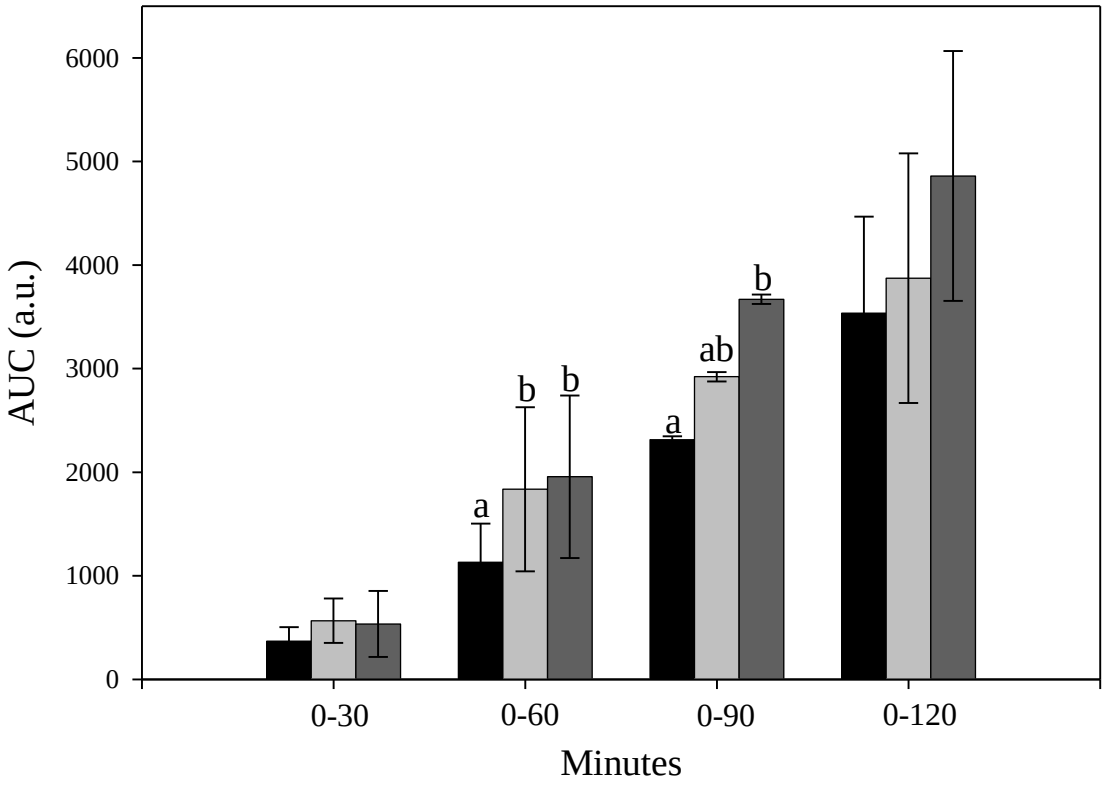


Figure 2



[a,b]P>0.05