

Maillard Product Consumption and Nitrogen Digestibility in Young and Adult Rats

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Abstract

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We investigate the effects of consumption of MRPs from the glucose-lysine model system heated 15°C-90 min on protein digestibility and its utilisation in young (3-week) and adult (12-week) rats. Nitrogen faecal excretion significantly increased after MRP consumption, especially during the third week. Protein digestibility was lower in both age groups studied, but the utilisation was unaffected. Parallely, the nitrogen retention and its net utilisation for the entire experimental period did not vary. In young rats the faecal nitrogen exceeded the amount of ingested nitrogen coming from MRPs, suggesting that digestibility of undamaged nitrogen was affected. The same action is suspected in adult animals, but the results were not quantitatively conclusive, and therefore the effect should be moderate in this period.

Keywords: protein; net protein utilisation; Maillard reaction products; young rats; adult rats

Food processing may improve the nutritional value of proteins because it denatures the native structure and destroys trypsin inhibitors and other anti-nutritional factors present in raw foods (GONZALEZ-VEGA *et al.* 2011). However, heat treatment often induces the Maillard reaction (MR) development, which may affect the nutritional quality of proteins by the destruction of essential amino acids or by a reduction in their availability (FRIEDMAN 2003).

Different studies have established that the formation of Maillard reaction products (MRPs) in browned foods, such as the crust of a heated mincemeat loaf (OSTE & SJODIN 1984) or the bread crust (SIMONATO *et al.* 2002), reduces protein digestibility. In the same line, SEIQUER *et al.* (2006) reported diminished protein digestibility after consumption of a diet rich in MRPs by a group of male adolescents aged 11–14 years. Not only the amino acid damage during heating appears to be responsible for this effect, but also that heat-induced changes in protein caused by MR may inhibit or impair the action of digestive enzymes

and reduce their proteolytic activity (OSTE *et al.* 1986). Additionally, several years ago it was postulated that the presence of MRPs could also affect the digestibility of unaltered proteins (MOUGHAN *et al.* 1996), i.e. those not involved in the reaction.

The purpose of this study was to investigate the effects of the consumption of MRPs from a glucose-lysine model system heated 150°C-90 min on digestibility and utilisation of the total dietary protein in young and adult rats.

MATERIAL AND METHODS

Sample preparation. Glucose (Merck, Darmstadt, Germany) and lysine (Sigma Chemical, St. Louis, USA) were used to prepare the sample. Equimolar mixtures of glucose, lysine and HCl (GL) (40% moisture) were heated in open recipients in an oven (Selecta 2000210; J.P. Selecta, Barcelona, Spain) at 150°C for 90 min to obtain the GL90 sample. After heating, the reaction was stopped by cooling in an ice bath and the products were then removed, fro-

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zen, lyophilised, and stored at 4°C as described by DELGADO-ANDRADE *et al.* (2004) until required for preparing the diet.

Preparation of diets. The AIN-93G purified diet for laboratory rodents (Dyets Inc., Bethlehem, USA) was used as the control diet. The GL90 sample was added to the AIN-93G diet to reach a final concentration of 3% (GL90 diet). The individual analysis of the GL90 diet revealed no modification of the overall nutrient composition compared with the control diet (AIN-93G). The mean \pm SD nutrient content of the diets was as follows: moisture (g/kg) 81.4 ± 0.08 ; protein (g/kg) 176.6 ± 3.1 ; fat (g/kg) 78.1 ± 0.9 . Furosine and hydroxymethylfurfural (HMF) levels were analysed in both diets: furosine (mean \pm SD) 28.8 ± 0.5 and 1787.08 ± 7.31 mg/kg fresh matter for control and GL90 diets; HMF (mean \pm SD) 0.44 ± 0.06 and 5.15 ± 0.08 mg/kg fresh matter for control and GL90 diets, respectively.

Biological assays. Thirty weanling Wistar rats weighing 40.77 ± 0.29 g (mean \pm SE) were used in the study. Twenty were randomly distributed into two groups (10 animals per group) and each group was assigned to one of the dietary treatments. The animals were individually housed in metabolic cages in an environmentally controlled room under standard conditions. The rats had *ad libitum* access to their diets and demineralised water (Milli-Q Ultrapure Water System; Millipore Corp., Bedford, USA). The remaining ten animals were sacrificed by anaesthesia overdose on day 0 to analyse their initial nitrogen body content, determined as 1163 ± 20 mg (mean \pm SE).

The animals were fed the different diets for 88 days. The nitrogen balance for the entire experimental period, termed the 'global balance', was calculated from the difference between the final and the initial nitrogen body content. Food intake was monitored weekly for each rat during this period and then nitrogen intake was calculated. Six animals from each group were sacrificed by anaesthesia overdose on day 88 to determine their individual final nitrogen whole-body content.

In the third and in the twelfth week of the experimental period two additional nitrogen balances were performed on all the animals. The first one aimed to study the nitrogen balance in young rats, while the second one pretended to know the situation in adult animals. In either of them, faeces and urine from each animal were collected daily and stored separately as a 1-week pool. The faeces were individually weighed, lyophilised and then homogenised. The urine was collected on 0.5% HCl (v/v), filtered (Whatman Filter

Paper No. 40, ashless; Whatman, Cambridge, UK) and diluted to an appropriate volume.

All management and experimental procedures carried out in this study were in strict accordance with current European regulations (86/609 E.E.C.) regarding laboratory animals. The Bioethics Committee for Animal Experimentation at our institution (EEZ-CSIC) approved the study protocol.

Analytical techniques. After sacrifice of the animals for the global balance, whole bodies were weighed, lyophilised, ground and then homogenised. Total nitrogen was analysed at least in duplicate in aliquots of all samples (urine, faeces, diets, and whole body) using the Kjeldahl procedure with mineralisation (Block Digestor Selecta S-509; J.P. Selecta, Barcelona, Spain), distillation units (Büchi Laboratoriums Technik AG, Flawil, Switzerland), and titration units (MetromAG, Herisau, Switzerland). Pools of faeces, urine, and diet were used as an internal control to assess precision. The interassay CV for nitrogen was 0.64% in urine, 0.84% in faeces, and 3.02% in the diet.

The following indices were calculated using the data for nitrogen intake (I) and its faecal (F) and urinary (U) excretion in the third and in the last week of the assay: apparent absorption (A) = I – F; apparent retention (R) = A – U; apparent absorption efficiency or digestibility (% A/I or D) = A/I \times 100; apparent biological value (BV) = R/A \times 100; apparent net protein utilisation (NPU) = R/I \times 100. Since all the indices were calculated in apparent form, henceforth the term 'apparent' will be omitted. The parameters calculated for the global balance were global nitrogen retention (final – initial body content) and global %R/I.

Measurement of colour in faeces. The colour of the faecal material was determined using a Chroma Meter CR-400 optical sensor (Konica Minolta Sensing, Inc., Osaka, Japan) according to the CIE Lab scale (Committee 1974). The system provides the values of three colour components, L^* (black-white component, luminosity) together with the chromaticity coordinates, a^* (+red to –green component) and b^* (+yellow to –blue component). The samples were placed in a 34 mm optical glass cell. The sample was illuminated with D65-artificial daylight (10° standard angle) in accordance with the manufacturer's instructions. Each colour value reported was the mean of three determinations at 22–24°C. The yellowing index (YI) was calculated from the equation $YI = 142.86 b^*/L^*$.

Statistical analysis. All data were statistically tested by the one-way analysis of variance (ANOVA), followed by Duncan's test to compare means that showed a

significant variation ($P < 0.05$). Analyses were performed using Statgraphics Plus, Version 5.1 (2001).

RESULTS AND DISCUSSION

Data concerning the nitrogen balance during the third and the twelfth week of assay are included in Tables 1. Nitrogen intake was unaffected by MRPs consumption regardless of the week of the experiment. The nitrogen faecal excretion significantly increased in animals fed the GL90 diet in both periods analysed ($P < 0.001$), more markedly in the third-week balance, although subsequent changes in the absorption did not reach statistical significance in any case. The scientific literature describes the existence of non-absorbable and insoluble melanoidins (final MRPs) in faeces of rats fed these compounds (HOMMA & FUJIMAKI 1981), a fact supported in our trial by the dark-brown colour of the faecal material from animals fed the GL90 diet compared with the control group at the end of the trial. Their CIELab coordinates (L^* , a^* , and b^*) were measured in these faeces and were as follows: L^* 74.90 ± 0.48 vs. 45.73 ± 0.29 ; a^* 1.73 ± 0.26 vs. 8.10 ± 0.07 ; b^* 21.30 ± 0.67 vs. 17.11 ± 0.20 for control and GL90 groups, respectively. Based on them the yellowing index (YI), a global marker of the presence of MRPs, can be calculated: $YI = 142.86b^*/L^*$. It was established as 40.39 ± 1.46 for the control group vs. 53.45 ± 0.40 for the GL90 group, indicating the significantly higher presence of MRPs in the latter group ($P < 0.05$).

As a consequence of the higher nitrogen faecal excretion, digestibility was significantly diminished after model MRPs consumption (Figure 1) in both periods considered. Similarly, assays performed in our research group on male adolescents aged 11–14 years reported lowered protein digestibility after consumption of a MRP rich diet (SEIQUER *et al.* 2006). Other authors have also documented decreases in nitrogen digestibility after feeding rats a diet based

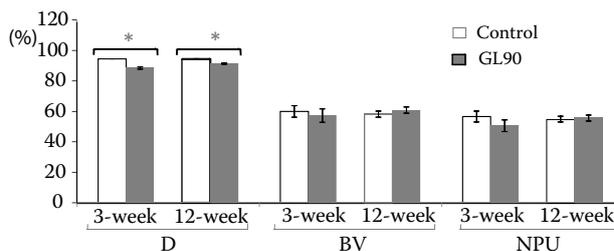


Figure 1. Nitrogen biological indices during the third and the twelfth week in rats fed different diets (mean \pm SE, $n = 10$)

D – apparent digestibility; BV – biological value; NPU – net protein utilisation; symbol on the bars indicates significant differences between groups ($P < 0.05$)

on commercial ultra-high temperature milk stored for 6 months as unique source of protein vs. a diet made of pasteurised milk (ALKANHAL *et al.* 2001). In the same line SARRIÁ *et al.* (2000) demonstrated the worse nitrogen digestibility of a diet elaborated with in-bottle-sterilised infant formula compared with a conventional powder formula. Based on the food intake, the proportion of MRPs added to the diet and their nitrogen content, the amount of nitrogen consumed coming from the MRPs within the GL90 diet may be calculated. In the third-week balance, the total amount of nitrogen ingested coming from MRPs was 14.6 mg/day. Since the difference in the nitrogen faecal excretion between groups was 21 mg/day, even supposing that the total nitrogen from MRPs is excreted, there is an additional nitrogen excretion that should come from the basal nitrogen of the AIN-93G diet. This finding is in agreement with the statements of MOUGHAN *et al.* (1996) suggesting that the intake of Maillard derivatives decreases not only the digestibility of altered nitrogen but also the total nitrogen digestibility of the diet. However, applying the same calculations in the twelve-week balance, due to a lower increase in the nitrogen faecal excretion compared with the third week, we cannot

Table 1. Nitrogen balance (mg/day) during the third and twelfth week in rats fed the assayed diets

Diets	Intake	Faeces	Urine	Absorption	Retention
Third week (young animals)					
Control	368.7 \pm 13.6	20.5 \pm 1.5 ^A	140.7 \pm 14.8	348.3 \pm 13.1	207.6 \pm 12.5
GL90	370.4 \pm 20.3	41.5 \pm 1.6 ^B	142.0 \pm 17.1	328.9 \pm 19.4	186.9 \pm 18.2
Twelfth week (adult animals)					
Control	396.8 \pm 12.4	22.3 \pm 1.1 ^A	155.2 \pm 6.6	374.5 \pm 11.8	219.3 \pm 12.2
GL90	397.0 \pm 26.6	34.6 \pm 2.8 ^B	143.0 \pm 12.9	362.4 \pm 24.0	219.4 \pm 14.8

Values are means \pm SE, $n = 10$; Different letters within a column indicate significant differences between groups ($P < 0.05$)

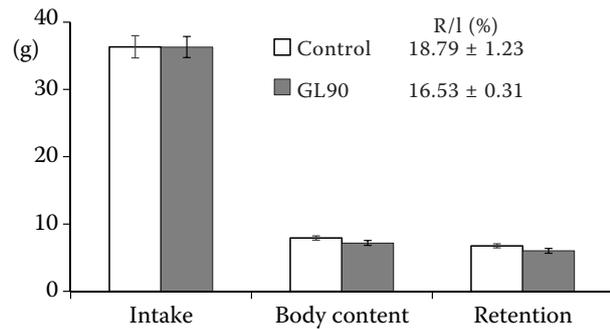


Figure 2. Nitrogen global balance in rats fed different diets (mean \pm SE, $n = 6$). No significant differences were found between groups ($P > 0.05$)

assure that the digestibility of unaltered nitrogen was affected by MRP consumption.

To understand the decreases in protein digestibility it must also be considered that: (i) some MRPs can impair the activity of diverse enzymes, such as trypsin, chymotrypsin, aminopeptidase N, glycyl-leucine dipeptidase, amylase, lipase, etc., important contributors of nutrient digestion (SCHNEEMAN & DUNAIF 1984); (ii) MRPs can compete for transporters and absorption places with several amino acids (MOUGHAN *et al.* 1996); (iii) and favour the elimination of those with endogenous origin (VAN BARNEVELD *et al.* 1994). As a consequence, the efficiency of the whole digestive process could be compromised (O'BRIEN & MORRISSEY 1989), a fact that seemed to affect more deeply younger animals.

The nitrogen urinary excretion was unaltered in both studied periods, as well as its retention, although a non-significant 10% decrease was detected during the third week of the assay (Table 1). At this moment the BV and the NPU tended to decrease after MRP intake, while no modifications were detected in these indexes during the twelfth week of the trial (Figure 1). In the above-mentioned study by ALKANHAL *et al.* (2001) the BV and NPU found after feeding rats the diet containing the ultra-high temperature milk stored for 6 months were lower than those of the diet based on pasteurised milk by 9 and 12%, respectively. The lack of concordance between these data and our results could be due to the study design. In the experiment of ALKANHAL *et al.* (2001) all the protein consumed had been damaged during the heat treatment, while in our case only a fraction was affected, so that there was intact protein enough to meet necessities during the animal growth. Against the authors who referred the negative effects on protein biological indexes, SARRIÁ *et al.* (2000) reported higher BV of the diet based on the

in-bottle-sterilised infant formula vs. that containing the powder formula, as well as stability in the NPU.

Considering the entire experimental period (Figure 2), animals from both groups consumed the identical amount of nitrogen, leading to similar nitrogen body content and body retention. The global %R/I for the trial was unaffected by MRP consumption. Despite that MRPs impaired the protein digestibility, the AIN-93G seemed to contain protein enough to support the minimal nitrogen requirements of the animals, allowing appropriate values of retention even during an intensive growth period.

CONCLUSIONS

The intake of the assayed glucose-lysine MRPs decreases protein digestibility due to a higher nitrogen faecal excretion. In young rats, the faecal nitrogen excretion clearly exceeds the total amount of ingested nitrogen coming from MRPs, demonstrating that browning products also compromise the digestibility of undamaged nitrogen. The same action is suspected to happen in adult rats, but the result is quantitatively less clear and, in this case, the effect of the intake of assayed model MRPs on digestibility of unaltered nitrogen was moderate.

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