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Bioavailability of Corn Gluten Meal Hydrolysates and Their Effects on the Immune System

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Abstract

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The bioavailability of food is central to human nutrition, health and wellbeing. Here, we tested the bioavailability of hydrolysed corn gluten meal using a protein efficiency ratio method, and then analysed differences in bodyweight, weight of organs, routine blood tests and histological sections. The results indicate that the average protein intake of the hydrolysed corn gluten meal (HCGM) group was higher than that of the crude corn gluten meal (CCGM) group, and was associated with an increase in average bodyweight. The corrected protein efficiency ratios (PERs) of the HCGM and CCGM groups were 0.374 and 0.217, respectively; the corrected PER of the HCGM group was 1.72 times higher than that of the CCGM group. These results show that hydrolysis increased the bioavailability of corn gluten meal. Furthermore, there was a significant difference in organ weights (salivary gland $P < 0.01$; thymus gland $P < 0.05$; spleen $P < 0.01$) between the HCGM and CCGM groups. Finally, no inflammatory cell infiltrates nor cell necrosis could be found in any of the histological sections. We speculate that hydrolysed protein preparations can improve immunity.

Keywords: bioavailability; corn gluten meal; hydrolysis; immune system change

As the leading cereal crop in the world, corn plays a significant role in human diets. Global production of corn exceeded 1 billion metric tons in 2013 and about 35% of this total was produced in the USA. Corn is a staple food for large populations in Latin America, Africa and Asia, where it is consumed as 'corn on the cob' or corn kernels and used to prepare various kinds of traditional foods (Ai & Jane 2016). Corn is also an important source of protein in human diets, particularly for those populations who consume corn as a staple.

A whole corn kernel is composed of four different parts: endosperm (82–84%, db), germ (10–20%, db), bran (5–6%, db), and tip cap (1%, db). Starch, non-starch polysaccharides, protein and lipids are distributed heterogeneously in the corn kernel (Ai & Jane 2016). After carbohydrates, protein is the 2nd largest component (6–12%, db) in corn kernels. Corn gluten meal (CGM), a by-product of the corn wet-milling process employed for the production of corn starch, contains 600–710 g/kg protein. The major protein fractions of the CGM are zein and glutelin, representing 680 and 280 g/kg of total protein weight, respectively (Zhou *et al.* 2013; Jin *et al.* 2015b). Unfortunately, a large number of proteins in the CGM cannot be absorbed because of their compositions and structures, and in most cases the CGM is discarded. This represents a great loss of

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protein resources which could be used to resolve the problem of protein shortage worldwide caused by increasing population growth and improving economies.

The digestion of food is central to human nutrition, health, and well-being. Sufficient attention should be paid not only to the availability of food and its nutritional composition but also to the bioavailability of the nutrients during digestion. Food has no nutritional value if its nutrients cannot be digested, absorbed and utilised. Changes in population demographics in many developed countries mean that there is an increasing proportion of older persons, and older people need a substantial intake of protein at regular mealtimes during the day to avoid loss of muscle tissue (Paddon-Jones & Rasmussen 2009). Moreover, it is increasingly apparent that it is not only the amounts of nutrients that are released from the food that is important but also the rate of release and uptake (Boland 2016). In the present study, we focused on processing CGM to improve its bioavailability before digestion, and evaluated its true bioavailability *in vivo* using the protein efficiency ratio (PER) method. As well as increases in bioavailability, we observed some interesting effects on the immune system.

MATERIALS AND METHODS

Preparation of corn gluten meal hydrolysates. Corn gluten meal (crude protein content 651 g/kg) was hydrolysed using alcalase (Novozym Biotechnology Co., Ltd., China) with an enzymatic activity of 24 000 U/ml. The other optimised parameters were determined as the following: substrate concentration, S = 50 g/l; enzyme-substrate ratio, E/S = 4800 U/g; temperature, t = 55°C; pH = 9.0. The degree of hydrolysis in percent (DH%) was calculated according to the pH-stat method as follows:

$$DH\ (\%) = V \times N/(\alpha \times M \times h_{tot}) \times 100\%$$

where: V – volume of 0.5 mol/l NaOH consumed (ml); N – concentration of NaOH (mol/l); α – average degree of dissociation of the α-NH_2 groups; M – mass of protein to be hydrolysed (g); h_{tot} – total number of peptide bonds in the protein substrate (9.2 mmol/g for corn protein)

The products were terminated by boiling the mixtures for 10 min followed by centrifugation at 5000 *g* for 15 min after cooling to room temperature. We measured each DH (%) together with the sequence of alcalase, temperature, PH and reaction time in single factor test method. Finally, we gained an optimised DH (%) of 26.5. The hydrolysates produced under the optimised conditions were prepared and stored at –20°C for the next step.

Preparation of experimental diets. The experimental subjects were divided into three groups, the hydrolysed corn gluten meal group (HCGM group), the crude corn gluten meal group (CCGM group) and the casein group. The diets of the HCGM, CCGM and casein groups were produced by Opensource Animal Diets (Changzhou Co., Ltd., China). All diets were formulated by the American Institute of Nutrition Rodent Diets (rodent diet AIN-76A), and had the same amount of protein. The diets were generally composed of 10% protein, 76% carbohydrate, and 5% fat. The ingredients (beside protein) of the diet were corn starch, sucrose, cellulose, corn oil, mineral mix, vitamin mix, and choline bitartrate. These ingredients were purified and were completely free of protein. Proteins were added to the corresponding groups in the form of hydrolysed corn gluten meal, crude corn gluten meal and casein, respectively. To enable discrimination of the different groups by colour, dyes (food-grade red, blue, and white) were added to the diets of the three groups.

Animal experiment. With a view to raising the animal through the entire growth period so as to enable evaluation of the capability for protein uptake and utilisation, Sprague-Dawley rats weighing 40–50 g (1–2 days after weaning) were obtained from the Laboratory Animal Research Centre of Jiangsu University. The rats were weighed and randomly divided into three groups (eight per group). All animals were housed in groups on a 12-h light/dark cycle (lights on at 7:00 AM, lights off at 7:00 PM) under controlled temperature (22 ± 2°C), and humidity (50 ± 10%). The animals were fed a standard diet and had access to water *ad libitum*. The rats were acclimatised to the environment for three days, and then the standard diet was changed to the experiment diet at start of the experiment. The protocol for the study was reviewed and approved by the Animal Use and Care Committees of Jiangsu University. The bodyweight, weight of the diet and water intake were recorded each day.

Bioavailability in vivo. We evaluated protein bioavailability *in vivo* using the protein efficiency ratio method. We recorded animal bodyweight, the weight of the diet and intake on each day, and calculated the

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average bodyweight increase value for the consumption of 1 g protein (protein content 10%) in 28 days. The PER and corrected PER formulas are as follows:

PER = WI/PI; corrected PER = PER × (2.5/casein PER)

where: WI – animal bodyweight increase value; PI – weight of protein intake; casein PER – corrected factor using casein group data and represents the PER value calculated in this experiment

Organ inspection and routine blood tests. On the last day of the experiment, most organs including the heart, liver, kidney, spleen, lung, thymus gland, salivary gland and testis/ovary were inspected and weighed after bodyweight was determined. Subsequently, pathological sections were made for comparative analysis using the haematoxylin-eosin (HE) staining method. For the HE staining procedure, tissue was collected and fixed in 4% paraformaldehyde. The fixed tissue was then rapidly removed, post-fixed in 4% paraformaldehyde at 4°C, and embedded in paraffin. Tissue samples were sectioned (RM2245; Leica Biosystems Nussloch GmbH, Germany) and stained with haematoxylin and eosin. The blood of each individual was collected and tested using routine blood testing equipment (BC-2800VET; Merei Biomedical Electronic Co., Ltd., China).

Statistical analysis. All data from animal experiments are presented as mean ± standard deviation (SD). Statistical analysis of data was performed using an independent t-test for comparisons of means using SPSS 19.0 software (SPSS Institute Inc., USA). Differences were considered statistically significant if $P < 0.05$.

RESULTS AND DISCUSSION

Effect of hydrolysis on bodyweight increase. Figure 1 shows that average animal bodyweight increased in response to feeding with HCGM and CCGM, respectively. The bodyweights of the HCGM group were larger than those of the CCGM group. Figure 2 show the initial animal bodyweight and the final animal bodyweight for the experiment period. The average bodyweight in the HCGM group increased from 45.50 g to 52.10 g, while the average bodyweight of the CCGM group increased from 43.93 g to 47.30 g. The bodyweight increase in the HCGM group was statistically significantly higher than in CCGM group ($P < 0.05$). On the other hand, because the diets contained only a single protein source (the hydrolysed corn gluten meal, the crude corn gluten meal or casein), all rats in the experiment group were malnourished and gained less bodyweight. There were no statistical differences in bodyweight between male rats and female rats in different groups (data not shown).

Effect of hydrolysis on bioavailability in vivo. The protein efficiency ratio is a classic method for evaluating protein bioavailability. Here, we for first time evaluated the bioavailability of hydrolysed protein using this method *in vivo*. Table 1 shows several parameters related to corrected protein efficiency ratio. We found that the average protein intake of the HCGM group was higher than that of the CCGM group, which was associated with an increase in average bodyweight and average dietary intake. The corrected PERs of the HCGM and CCGM groups were 0.374 and 0.217, respectively; thus, the corrected PER of the HCGM group was 1.72 (0.374/0.217 = 1.72) times higher than that of the CCGM group. This strongly suggests that hydrolysis increased the bioavailability of corn gluten meal. The casein group was designed to correct the protein efficiency ratio and to calculate the corrected protein efficiency ratio.

With the rapid increase in the world's population, sustainable food protein productions will pose a serious challenge in the future due to increased consumer demand for protein and the constraints on the amount of agricultural land available. Some researchers have turned their attention to new food resources, such as insects and leaves, and have already reported results with respect to the characterisation of the components of these resources (Yi *et al*. 2013; Tamayo *et al*. 2016). Other researchers, including our

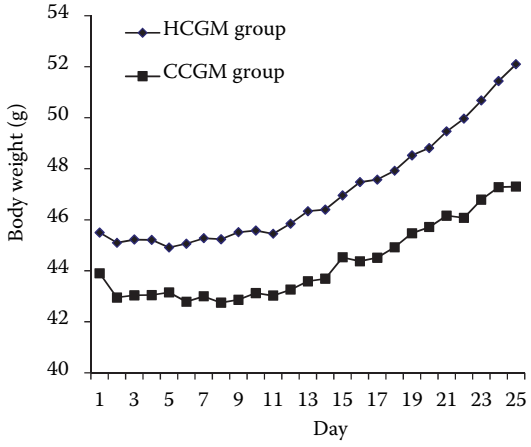

Figure 1. Body weight growth curves of the hydrolysed corn gluten meal (HCGM) and crude corn gluten meal (CCGM) groups

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Table 1. Protein efficiency ratio

	Casein group	HCGM group	CCGM group
Average body weight increase	60.463	6.600	3.400
Average diet intake	792.470	397.410	355.680
Hydrolysis protein content	0.0	0.539	0.0
Average protein intake	79.247	57.777	51.228
Protein efficiency ratio	0.763	0.114	0.066
Corrected protein efficiency ratio	2.500	0.374	0.217

HCGM – hydrolysed corn gluten meal; CCGM – crude corn gluten meal

group, have focused on the use of physical, chemical, and biological methods to improve bioavailability in present food resources (Jin *et al*. 2015a, 2016; Li *et al*. 2016b; Zhou *et al*. 2016). In the present study, we evaluated the protein bioavailability of hydrolysed corn gluten meal using the protein efficiency ratio method for the first time, and the results indicated that the protein efficiency ratio of the HCGM group was 1.72 times higher than that of the CCGM group. Furthermore, there was a significant difference in the bodyweight increase between the HCGM and CCGM groups. These results obviously indicate that the hydrolysed corn meal possesses superior bioavailability *in vivo*. Hydrolysis is a common method of breaking down protein, while ultrasound and microwave methods can also assist in increasing the bioavailability of corn gluten meal or other protein substances (Wang *et al*. 2012; Zhang *et al*. 2015; Li *et al*. 2016a; Nooshkam & Madadlou 2016). The ultrasound method was recommended for its capability to increase bioavailability and because of its non-toxic nature (Anese *et al*. 2013; Filho *et al*. 2015; Kadam *et al*. 2015; Yu *et al*. 2015b). We believe that bioavailability would undergo substantial improvement if these auxiliary methods were applied.

Organ inspection and routine blood tests. After finishing the feed experiment, we dissected each rat and inspected the organs and tissues of all animals. All organs and tissues exhibited normal morphological characteristics and normal weight proportions of whole bodyweight except for the salivary gland, thymus gland and spleen. Figure 3 shows the average weight of the salivary gland, thymus gland, and spleen in the HCGM and CCGM groups. There was a significant difference in organ weight between the HCGM and CCGM groups: salivary gland $P < 0.01$, thymus gland $P < 0.05$, and spleen $P < 0.01$. The colours of organs and tissues were normal except for the liver. Liver colour in the HCGM and CCGM group was weaker compared to normal liver colour. In order to find the reason for the differences in liver colour and organ weight, histologic section and routine blood tests were employed for further comparative analysis. Figure 4 shows the liver, spleen and thymus gland sections. In liver sections, vacuolar degeneration of liver cells around the central

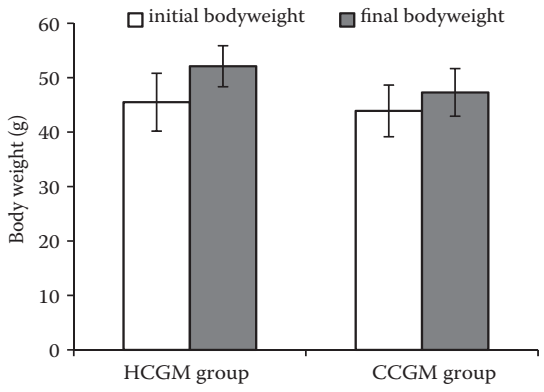

Figure 2. Initial and final body weights in the hydrolysed corn gluten meal (HCGM) and crude corn gluten meal (CCGM) groups

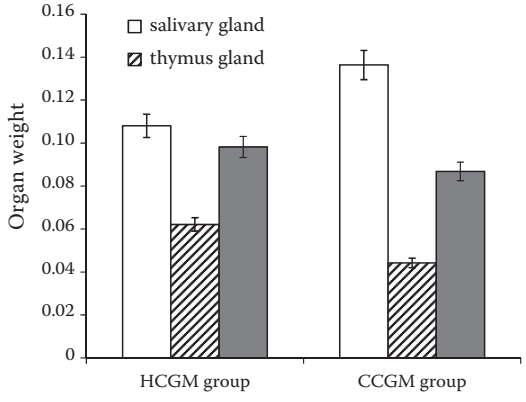

Figure 3. Average organ weights in the hydrolysed corn gluten meal (HCGM) and crude corn gluten meal (CCGM) groups

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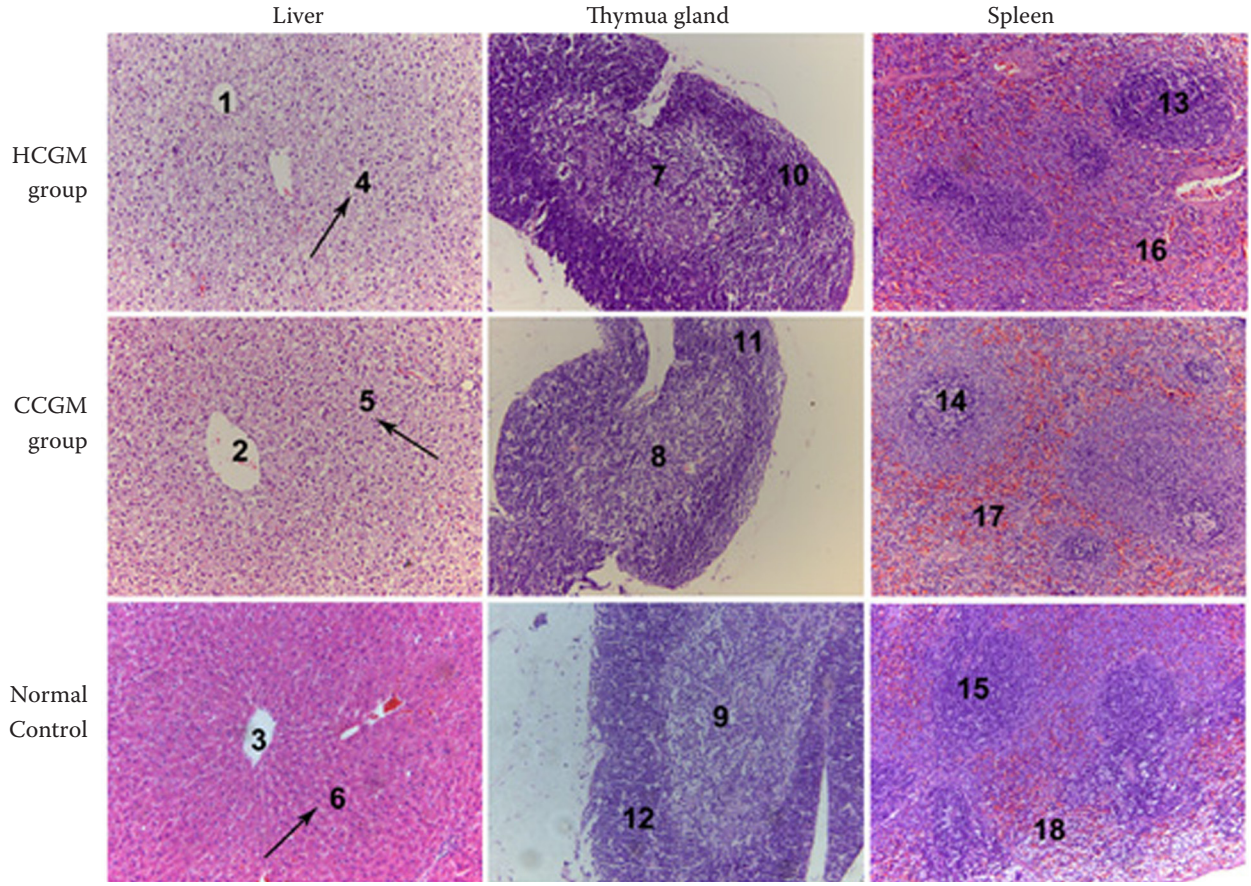

Figure 4. Liver, spleen and thymus gland sections of the hydrolysed corn gluten meal (HCGM) group, crude corn gluten meal (CCGM) group, and normal tissues

The magnification is 100 ×. 1, 2, 3 – central veins of the liver; 4, 5 – vacuolar degeneration of liver cells; 6 – normal liver cell; 7, 8, 9 – the medulla area; 10, 11, 12 – the cortex; 13, 14, 15 – white pulp area; 16, 17, 18 – red pulp area

vein was observed throughout the whole liver in the HCGM and CCGM groups, and liver cells in these two groups were arranged in a disorderly and unsystematic fashion. These results indicated that the colour change in the HCGM and CCGM group might be caused by large amounts of vacuolar degeneration. In thymus gland sections, the cortex and medulla had developed well and the border was clearly defined. In spleen sections, the red pulp and the white pulp had developed well and the border was also clearly defined. The cells of the thymus gland and spleen developed well and their morphologies were normal. Furthermore, no inflammatory cell infiltrates nor cell necrosis could be found in any of the sections. These results excluded the possibility of inflammation.

Routine blood tests were performed after individual samples were collected. We tested WBC, Lymph %, Mon %, Gran %, Lymph #, Mon #, Gran #, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, PLT, MPV, PDW, and the PCT value of each sample and compared the results using the independent sample method in SPSS software (data not shown because of a lack of differences). Routine blood tests indicated that there was no difference between the HCGM and CCGM groups. The results of organ inspection and routine blood tests suggested that the differences in salivary gland, thymus gland, liver, and spleen between the HCGM and CCGM groups might be caused by protein hydrolysates.

We were surprised to discover changes in the immune organs. At first, we found differences in the thymus gland and spleen between the HCGM group and the CCGM group, thymus gland $P < 0.05$ and spleen $P < 0.01$. We suspect that this was caused by inflammation elicited by the experimental procedure. Then, we performed the routine blood tests and made histological sections stained by the HE method. The results indicated that the differences in the thymus gland and spleen were not caused by inflammation. It is well known that the hydrolysis process can pro-

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duce large amounts of small proteins, amino acids and small peptides. These polypeptides improve the development of the immune system and increase the immunological defence capabilities of an animal. Numerous studies have elucidated the relationship between bioactive peptides and the immune system (Norberg *et al*. 2003; Zimmer *et al*. 2003; Scerbo *et al*. 2008; Grasso 2011). Beside the effect on the immune system, many studies from our group have also proven that hydrolysed protein has antihypertensive functions (Qu *et al*. 2012; Qu *et al*. 2015; Yu *et al*. 2015a; Li *et al*. 2016a). A vast number of other studies have indicated that protein hydrolysates exert many kinds of effects on human health. Food protein-derived bioactive substances possess antihypertensive, anticancer, anti-calmodulin, hypocholesterolaemic and multifunctional properties, which play important roles in human health by affecting the digestive, endocrine, cardiovascular, immune and nervous systems (Udenigwe & Aluko 2012; Bhat *et al*. 2015). Putting these results together, we speculate that the hydrolysed corn gluten meal can evoke changes in the animal immune system. Animals were maintained in a state of malnutrition by feed restriction in order to test the protein efficiency ratio over the experimental period. Nutrients may first be employed to meet the requirements of the immune system and only then be redirected to other organs, which might explain differences in the weight of immune organs in animals in different stages of development. In future work, we aim to confirm these findings using molecular methods.

Our results also indicate that the salivary gland weight of the HCGM group is smaller than that of CCGM group, and that the difference is significant. Together with the vacuolar degeneration of liver cells, these results imply that the digestive system might also be affected by hydrolysis. The digestive tract and the interaction between food and the digestive tract have been studied and speculated about since the time of the ancient Greeks. The physicochemistry and biology of digestion of specific food components in the normal human gastrointestinal tract are quite well understood. The salivary gland is an important organ that secretes saliva when food enters the mouth. The saliva controls viscosity and surface tension, making the bolus bind together, and also assisting with swallowing and passage through the gastrointestinal tract. The saliva also contains amylase and lysozyme: amylase digests the starch and oligosaccharides in food and lysozyme possesses antibacterial activity (Boland 2016). The composition of saliva varies greatly from individual to individual, and several different glands are involved in saliva production, including the parotid glands, the submaxillary glands, the sublingual glands, cells on the surface of the floor of the mouth and the tongue. Thus, many factors could affect the secretion of saliva and salivary gland development. To sum up, future work should be undertaken to better explore the implications of these results.

CONCLUSIONS

In the present paper, we have described the first use of the protein efficiency ratio to confirm that the hydrolysis process increases the bioavailability of corn gluten meal. We demonstrated that the bioavailability in the HCGM group was 1.72 higher than that of the CCGH group. We also speculate that, based on our results, hydrolysed protein preparations may improve immunity.

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