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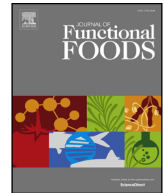

Microbiome and butyrate production are altered in the gut of rats fed a glycated fish protein diet

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ABSTRACT

The glycated fish protein (GP) was fed to rats to firstly evaluate their impacts on gut health. The rats were fed fish protein (FP) diet, heated fish protein (HP) diet, GP diet or AIN-93G (SD) diet for two weeks. Compared with FP diet, GP diet significantly reduced body weight gains in rats ($p < 0.05$), showed the weaker intensity of protein fermentation in large intestine of rats, and also considerably increased the butyrate level, which was closely related to the formation of fructoselysine via the glycation of FP. The cecal bacterial communities in rats fed GP diet indicated a great abundance of *Allobaculum*, *Akkermansia*, *Turicibacter* and *Lactobacillus animalis*, while a low abundance of *Escherichia-Shigella*, *Fusobacterium* and *Erysipelatoclostridium*. The abundance of *Collinsella*, *Allobaculum* and Ruminococcaceae_UCG-014 was significantly positively correlated with the production of butyrate. Overall, glycated protein enriched in Amadori products may have a potential beneficial effect on gut health.

1. Introduction

The human intestine contains tens of trillions of microbial cells that belong to at least 1000 different species, which play an important role in host health (Tremaroli & Bäckhed, 2012). The gut microbiota is affected by numerous factors, such as age, geography, genetics, lifestyle and diet (Sommer & Backhed, 2013). Diet is a major factor influencing the composition and metabolic activity of gut microbiota (De Filippo et al., 2010).

It is estimated that around 6–18 g nitrogen-containing compounds, mostly from dietary origin but also a small amount from endogenous origins, reach the human large intestine daily (Cummings & MacFarlane, 1991). The intake level and digestibility of the protein itself are two main factors that dictate the amount of dietary proteins escaping digestion into the large intestine (Yao, Muir, & Gibson, 2016). In the large intestine, these undigested dietary and endogenous proteins offer nitrogen sources for the hindgut microbes. Protein fermentation by gut bacteria is able to produce a wide range of metabolites, including short-chain fatty acids (SCFAs), branched-chain fatty acids (BCFAs), ammonia, amines, phenols and sulfur-containing metabolites (Portune et al., 2016). The profiles of these metabolites depend not only on the amount and digestibility of available proteins, but also on the available carbohydrate-nitrogen ratio (Nakata, Kyoui, Takahashi, Kimura, & Kuda, 2017). Recently, more attention has been paid to the effects of dietary protein source and level on the composition of gut microbiota by high-throughput sequencing technologies (Zhu et al. 2015; Mu, Yang, Luo, Guan, & Zhu, 2016).

The glycation, a complex series of non-enzymatic reactions between the ε-amino group in lysine-residues of proteins and the carbonyl group in reducing saccharides, is the most frequent reaction during thermal food processing, cooking and storage (Zhang et al., 2014), and produce the so-called Maillard reaction products (MRPs), including early stage products such as Amadori products, advanced reaction products (AGEs) such as Nε-carboxymethyllysine (CML), and terminal complex macromolecules called melanoidins (Morales, Somoza, & Fogliano, 2012; Delgado-Andrade & Fogliano, 2018). Interestingly, the glycation via Maillard reaction rich in Amadori products is considered as one of the most promising chemical modifications to improve protein functionalities for food uses (Liu, Ru, & Ding, 2012; de Oliveira, Coimbra, de Oliveira, Zuñiga, & Rojas, 2016). However, the glycation might result in some negative effects on proteins including a low susceptibility to proteases and a decline of nutritional value of proteins (de Oliveira et al., 2016; Świątecka, Małgorzata, Aleksander, Henryk, & Elżbieta, 2010), and more proteins tend to enter colon to be fermented by microorganism.

The interactions between gut microbiota and MRPs have aroused

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widespread interest. The Amadori product, especially fructoselysine (FL), can be converted into butyrate by a bacterium Intestinimonas AF211 in pure culture system (Bui et al., 2015). The individual MRPs, including FL, CML and pyrraline, were degraded by gut microbiota *in vitro* (Hellwig et al., 2015). Dietary MRPs were reported to modulate the gut microbiota *in vivo*, and the effects were inferred to be closely related to chemical structure and dietary amounts of different MRPs (Seiquer, Rubio, Peinado, Delgado-Andrade, & Navarro, 2014). Delgado-Andrade et al. (2017) concluded that dietary MRPs from bread crust changed both SCFA production and the microbiota composition in healthy adult rats. Furthermore, dietary bread melanoidins have been shown prebiotic effect in different studies (Helou et al., 2015; Borrelli & Fogliano, 2005). To the best of our knowledge, the effects of glycated proteins rich in Amadori products on the composition and metabolism of gut microbiota *in vivo* are still unknown.

Our previous study has found that the glycated fish protein, which was rich in Amadori products and lower browning intensity, may benefit the stability of bacterial community structure in an *in vitro* fermentation (Han et al., 2017). The objective of the present study was to further examine how dietary glycated fish proteins from a fish protein–glucose model system affect the composition of gut microbiome in rats, and their cecal and fecal metabolites including SCFAs, BCFAs and ammonia levels. The correlations between metabolites and microbiota were also studied, aiming to understand the impact of the glycation of proteins on gut health. Our hypothesis is that dietary glycated proteins reaching the large intestine may alter the fermentation pattern of gut microbiota.

2. Materials and methods

2.1. Materials and reagents

Fresh grass carp (*Ctenopharyngodon idella*) was purchased from Taidong supermarket (Qingdao, China). All chemicals and reagents used were of analytical grade or higher.

2.2. Preparation and purification of glycated fish protein

The fish protein from grass carp was prepared according to the method of Han et al. (2017) with a slight modification. Briefly, the muscles of fish were mixed with 10 times of volume of ice-cold distilled water and homogenized for 2 min by a homogenizer. The homogenate was extracted for 60 min and centrifuged using a Xiangyi centrifuge (GL-21 M, Xiangyi Instrument Co., Ltd, Changsha, China) at 11,378*g* at 4 °C for 15 min. The supernatant was removed and precipitate was washed another two times according to the above steps. The obtained precipitate was lyophilized and prepared as fish protein (FP).

On the basis of method of Han et al. (2017), fish protein and glucose were mixed at the ratio of 1:1 (w/w), then lyophilized and incubated at 60 °C and 63% relative humidity (saturated KI solution) for 9 h to obtain glycated fish protein. In order to remove free glucose, the prepared glycated fish protein were dispersed in distilled water (w/v = 1:20), stirred for 30 min after 2 min of homogenization, and then the homogenate was centrifuged at 11,378*g* at 4 °C for 15 min. The supernatant was removed and precipitate was washed another twice according to the above steps. The final precipitate was lyophilized and prepared glycated fish protein (GP) for feeding of rats. Control experiment with heated fish protein without glucose (HP) was also conducted.

2.3. Animals and diets

Thirty-two Sprague-Dawley rats (55–62 g) were obtained from experimental animal center of Shandong Lukang Pharmaceutical Co., Ltd. (Shandong, China), and housed in a controlled temperature (23 °C ± 2 °C) and humidity (55% ± 10%) condition with a 12 h light/dark cycle. Before experiments, the rats were acclimated to the environment with an AIN-93G standard diet for a week. After acclimatization, rats were weighed and randomly assigned to four groups with eight rats each. Each group of rats was housed in subgroups of two rats per cage, providing with AIN-93G standard diet (SD group), fish protein diet (FP group), heated fish protein diet (HP group) and glycated fish protein diet (GP group), respectively. The experimental diets were produced by Trophic Animal Feed High-Tech Co., Ltd. (Nantong, China) through replaced the casein in AIN-93G standard diet and the detailed protein composition was presented in Supplementary Table S1. All rats were given *ad libitum* access to their food and water. Food intake was recorded daily and body weight was monitored every two days. Fresh feces were collected after rats were fed for 13 and 14 days, and the feces samples were immediately frozen in liquid nitrogen and stored at -80 °C for further analysis. After 14 days of feeding, the animals were sacrificed. The cecum tissue and content were collected and weighted. The distal colon tissue which was 2–7 cm of distance from anus was collected. The collected samples were immediately frozen in liquid nitrogen and stored at −80 °C until analysis. All management and experimental procedures were approved by the Animal Care and Ethics Committee at Ocean University of China (Approval No. AE20161220-26).

2.4. Composition or characteristic of glycated fish proteins and the corresponding diets

The protein, moisture, ash content and browning intensity of GP were measured by the AOAC official methods (AOAC, 2005). The amino acid composition of GP and the four diets was analyzed by acid hydrolysis according to the method of Gressler et al. (2010), and the bound sugar content of GP was determined by anthrone sulfuric acid method (Zhao et al., 2017). The furosine content of glycated proteins and four diets were determined according to the methods described by Han et al. (2017). The analysis of CML and Nε-carboxyethyllysine (CEL) of GP and four diets were according to the methods described by Sun et al. (2015).

2.5. Determination of SCFAs, BCFAs and ammonia in cecal contents and feces

The cecal contents and feces samples were dispersed in distilled water (250 mg/mL), and vortex-mixed (Heidolph Multi Reax vibrating shaker, Germany) for 30 min. After the mixture was centrifuged at 16,000*g* for 15 min, the supernatant was collected for further analysis. SCFAs (acetate, propionate and butyrate) and BCFAs (iso-butyrate and iso-valerate) concentrations were determined on the basis of method described by Tian et al. (2016) with some modifications. Two hundred and fifty milligram of samples were mixed with 1 mL distilled water and then shaken at 1900 rmp (Heidolph Multi Reax vibrating shaker, Germany) for 30 min. The mixture was centrifuged at 16,000*g* for 5 min, and the supernatant was collected. Five hundred microliters of supernatant or standard solutions (0.05–0.3 mg/mL of each standard) were mixed with 0.5 mL of 0.2 M HCl containing 0.15 mg/mL 2-ethylbutyric acid (internal standard), then 200 μL of 0.15 M oxalic acid was added. The mixture was vortex-mixed for 5 min, and centrifuged at 16,000*g* for 5 min after 30 min of standing. The obtained supernatant was analyzed using gas chromatography (GC; Agilent GC 6890, Palo Alto, CA, USA), which equipped with a flame ionization detector (FID) and a HP-INNOWax column (30 m × 0.320 mm × 0.25 μm). Nitrogen was supplied as carrier gas at a flow rate of 2.5 mL/min, and the injection volume was 1 μL with a splitless injection. The inlet temperature was 250 °C, and the temperature program was 100 °C of initial oven temperature held for 0.5 min followed by an increase to 160 °C at a rate of 5 °C /min. The ammonia concentration was determined by the phenol-hypochlorite method (Broderick & Kang, 1980).

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