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Effect of dietary fiber/starch balance on the cecal proteome of growing rabbits

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ABSTRACT

Dietary fiber/starch imbalance can lead to diarrhea in rabbits. However, the underlying molecular mechanisms are largely unknown, and this is the first study on the proteome profile of the cecal tissue of diarrheic rabbits. In this study, one group of weanling rabbits was fed a high fiber/starch diet (control group), and another group was fed a low fiber/starch diet (diarrhea group). We applied 2-D gel electrophoresis, coupled with histological and biochemical analyses, to study dynamic changes in the proteome of the cecal tissue from healthy and diarrheic growing rabbits. We identified 29 protein spots showing differential abundance between the two groups. We identified the proteins and found that they participated in key biological processes, including absorption, digestion and transport; cell structure and motility; inflammatory response; glucose and energy metabolism; mineral and vitamin metabolism; stress response and cell redox homeostasis. Moreover, the results of our proteomics analysis revealed continuous impairment and compensatory intestinal repair in the rabbits with diarrhea. Therefore, these findings shed light on the main mechanisms and the main proteins involved in intestinal degradation in diarrhea. These findings have important implications for understanding the mechanism of cecal damage and repair in diarrhea in rabbits caused by dietary factors.

Biological significance
The different cecal protein expressions were compared between healthy and diarrhea in growing rabbit model fed different fiber/starch diets. Diarrhea caused by feeding high-starch diet induced mucosal injury and inflammation. In this work we identify protein spots by MALDI-TOF MS and classify by biological function. The finding that the expression of all other proteins belonging to seven functional groups was significantly increased in the diarrheic rabbits, except for CMBL, suggests that many intestinal functional proteins may be related to up-regulation, such as cell absorption, digestion, transport, structure, motility, metabolism (including energy mineral and vitamin), inflammatory response, stress response, and redox homeostasis in diarrhea.

1. Introduction

Digestive health is important for an animal to maintain intestinal equilibrium after nutrient intake. The digestive system of the rabbit is adapted to an herbivorous diet: this includes

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specific adaptations, from the teeth to the enlarged ceco-colic segment containing active microbiota, and the separation of cecal digestive particles that allows for cecotrophy. Starch and fiber are important components of the rabbit diet. However, an excess of dietary fiber limits energy intake and growth performance [1], and many experiments have found an association between excessive fiber and starch intake and the incidence of diarrhea in growing rabbits [2,3].

A decrease in the amount of dietary fiber or starch (<30% neutral detergent fiber (NDF), <15% acid detergent fiber (ADF), >20% starch) could lead to both lower ileal flow of dry matter and bacterial biomass production in the cecum of young rabbits, which are often associated with lower fermentative activity (higher pH), a modified fermentation pattern (higher proportion of butyrates) and lower fibrolytic activity of bacteria [4–8]. Thus, the intestinal microbial system is largely affected by diet. Intestinal microorganisms invade the epithelium and multiply intracellularly, damaging the surface epithelium and causing inflammation. Diarrhea is caused by both epithelial damage (exudation and decreased absorptive capacity) and the action of inflammatory mediators.

The intestinal barrier is composed of a thick secreted mucus layer, a layer of epithelial cells and underlying nonepithelial mucosal cells, chiefly leukocytes with a range of regulatory and effector functions. The intestinal barrier plays a key role in the protection of rabbits and other animals against pathogens because it prevents the colonization and translocation of bacteria and toxins. However, diarrhea causes changes in the microecological environment of the intestines: Argenzio reported that the intestines show abnormal permeability, movement, ion transfer and microbial digestion in the diarrheal state [9]. Although much is known about the effect of fiber/starch on gut morphology, digestion efficiency, growth performance and gut microbiology [4–7], little information is available about changes in the protein profiles of intestinal epithelial cells in response to excessive fiber/starch intake. Therefore, in this study, the proteome profiles in healthy and diarrheic cecal tissue of growing rabbits fed diets with different levels of fiber/starch were investigated using two-dimensional electrophoresis (2-DE), and the differences were analyzed by mass spectrometry to identify the proteins that play a role in intestinal damage and repair. This is the first such study on the proteome profile of the cecal tissue of diarrheic rabbits.

2. Material and methods

2.1. Experimental design, animal model and tissue collection

After a 7-day acclimatization period, 100 New Zealand weanlings (35 days of age, 1030 ± 55 g in weight) of both sexes (half/treatment) were divided into two treatment groups (n = 50 in each group) assigned two experimental diets according to the average live weight (the high fiber [HF] and low fiber [LF] groups). Two rabbits were housed per cage (size of each cage: 60 × 40 × 40 cm^3), and they had free access to food and water. The animals were placed in a closed building with a semi-controlled environment during the experimental period, and room temperature was maintained at 15–25 °C. The ingredients and chemical composition of the diets are shown in Table 1. Food was provided as pellets of 4 mm diameter, and no antibiotics were added to the feeds or the drinking water during the whole experiment.

On day 62, five rabbits with diarrhea (from the LF group) and five rabbits without diarrhea (from the HF group) were weighed and then slaughtered. The whole cecum was rapidly removed and placed in saline. After the length and weight of the whole cecum were measured, an approximately 10-cm segment of the middle portion of the cecum was taken for further experiments. A 2-cm portion of the cecum was then fixed in 2.5% glutaraldehyde at room temperature for observation under a scanning electron microscope. The remaining cecal segment was immediately placed in liquid nitrogen and stored at −80 °C for proteomic and western blot analyses.

The animal experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Shandong Agricultural University and performed in accordance with the "Guidelines for Experimental Animals" of the Ministry of Science and Technology (Beijing, China).

2.2. Histological analysis of the cecal mucosa

Gut tissues were fixed with 2.5% glutaraldehyde (Sigma, St. Louis, MO) in 0.1 M phosphate buffer (pH 7.4) for 2 h at room temperature. They were then washed three times for 30 min with the same buffer, placed in 1% osmium tetroxide (Sigma, St. Louis, MO) for 1 h, and then washed again three times for

Table 1 – Composition and nutrient levels of the experimental diet (g/kg).

Items	Experimental diets	
	HF	LF
Ingredients (g/kg)		
Corn	200	350
Soybean meal	180	180
Wheat bran	180	130
Peanut vine	410	310
Calcium carbonate	15	15
Sodium chloride	5	5
Vitamin–mineral premix[a]	10	10
Analyzed composition (g/kg of DM)		
Digestible energy (DE) (MJ/kg DM)	10.2	11.1
Dry matter	878.4	875.4
Acid detergent fiber (ADF)	157.1	110.0
Acid detergent lignin (ADL)	45.7	35.5
Neutral detergent fiber (NDF)	305.7	249.5
Crude fiber	127.9	86.9
Starch	163.8	250.0
Ash	95.8	77.7
Crude protein	168.1	162.8
Calcium	6.0	5.8
Phosphorus	7.0	6.9
NDF/Starch	1.9	1.0

Digestible energy (DE) was calculated using the gross energy (GE) of the feed and the coefficients of total tract apparent digestibility (CTTAD) of energy.

[a] The premix provides the following vitamins/minerals per kilogram of diet: VA, 8000 IU; VD 31,000 IU; VE, 50 mg; Lys, 1.5 g; Met, 1.5 g; Cu, 50 mg; Fe, 100 mg; Mn, 30 mg; Mg, 150 mg; I, 0.1 mg; Se, 0.1 mg.