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Effects of dietary fiber and starch levels on the non-specific immune response of growing rabbits

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ABSTRACT

The effects of dietary fiber and starch on the non-specific immune response were studied using four different experimental diets (I, II, III, and IV) on 200 growing rabbits. The following parameters were assessed: the number of membranous epithelial (M) cells in the appendix, total SIgA titers in the gut, total IgG levels in the serum and the CD4+/CD8+ T cell ratio in the peripheral blood; as these measures were taken to determine the effect of diet with age, samples were obtained at 52, 62, 72 and 82 days of age. The number of M cells was increased with dietary fiber enhancement and starch reduction at 52 and 62 days of age ($P<0.001$). However, at 72 and 82 days of age, there was no difference ($P>0.05$) in M cell number among the four diets. Using immunohistochemistry, the high fiber/low starch diet (Diet I) resulted in an increase in M cell size and number when compared with the other diets. The only differences($P<0.001$) in SIgA titers from gut tissues were detected between animals that received a high fiber (Diet I) and a low fiber (Diet IV) diet at an early stage (52 d and 62 d). Serum IgG titers were only affected ($P=0.05$) at 82 days of age. The ratio of CD4+/CD8+ T cells in the peripheral blood was not affected. The study revealed that increased levels of fiber might improve mucosal functionality, but only during the early stage of growth of rabbits. The effect of dietary fiber and starch levels on intestinal immunity was greater than their effect on humoral immunity in the peripheral blood.

1. Introduction

Infectious diseases of the digestive system currently account for 70% of all diseases in rabbits (Carabaño et al., 2008). Antibiotics are frequently used to prevent or to treat such illness. Unfortunately, the long term and extensive use of antibiotics has lead to the appearance of worrying bacterial drug resistance and stressed the problem of food residues. So, treatment in rabbit farming has stimulated the search for alternative solutions. Among those, nutritional management has become a priority. The dietary component that is most related to digestive troubles in rabbits is fiber. The rabbit is a monogastric, herbivorous animal, and because rabbits are adapted to ingest plant cell walls, fiber is the main constituent of their diet. A sufficient supply of dietary fiber is essential to prevent digestive troubles in growing rabbits, and the appropriate dietary levels range from $160 \leq ADF < 185$, $320 \leq NDF < 350$, $NDF \geq 55$ g/kg (Gidenne, 2010). It has been demonstrated that high levels of fiber lead to decreased incidences of digestive troubles (Gidenne, 2003). Starch is the main energetic component in diets intended for growing or fattening rabbits. Gidenne et al. (2010) proposed that the dietary starch level could be restricted more than the current level (150–155 g starch kg^{-1} dry matter (DM)) or even removed. Gidenne and Carcía (2006) demonstrated that the fiber level, not the starch level, played a role in the occurrence of digestive problems. Although many studies have evaluated the respective effects of fiber and starch on the incidence of diarrhea in

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growing rabbits, the experiments mainly assessed dietary fiber levels (also linked to starch change) and their effects on the performance, mortality, diarrhea and intestinal morphology of rabbits (Gidenne et al., 2007; Perez et al., 2000). However, the effect of different levels of dietary fiber with increasing starch content on the immune system of growing rabbits has not been determined. Somatic diversification occurs in gut-associated lymphoid tissue (GALT), and by about 1–2 months of age nearly all Ig VDJ genes are somatically diversified. Diversification process may not be developmentally regulated, but may require interaction with exogenous factors derived from the gut (Lanning et al., 2000). Therefore, the aim of this work was to investigate the effects of dietary fiber and starch levels on the local intestinal immune environment and the peripheral humoral non-specific immune responses of growing rabbits with age.

2. Material and methods

2.1. Ethics statement

All animal experiments were 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). All surgery was performed according to recommendations proposed by the, and all efforts were made to minimize suffering.

2.2. Animals and housing

Two hundred weanling rabbits of both sexes (half/treatment, 35 days of age, 1030 ± 55 g in weight) were used for experiments. The beginning of the total experiment was at 42 days of age after an adaptation period of 7 days. The rabbits were divided into fifty healthy rabbits per treatment group and assigned to the four experimental diets by average live weight. The experimental rabbits were caged per two (size of cage: $60 \times 40 \times 40$ cm^3) and had ad libitum access to food and water. The animals were located in an environmentally semi-controlled closed building during the experimental period, and room temperature was maintained at 15–25 °C.

2.3. Chemical analysis of experimental diets

The diets were formulated according to the values of the De Blas and Mateos (1998), and the food was administered in pellet form (Table 1). The diameter of the pellets was 4 mm, and no antibiotics were added to the food or drinking water during the experiment. The four different levels of fiber and starch experimental diets (I, II, III, IV) were formulated by including peanut seedling as the main of fiber. By decreasing the amount of peanut seedling and adding corn, Diets I, II, III, and IV contained starch/ADF ratios were 0.87, 1.04, 1.27, and 2.27, respectively. The diets were analyzed following the recommendation of the Association of Official Analytical Chemists (National Standards Recommend Method, China). Starch concentrations were measured by a polarimeter (TFSR, 1999). Crude fiber was determined using the acid–base method, and NDF and ADF were determined using the detergent method of Van Soest et al. (1991).

Table 1
Ingredient composition of the diets.

Item	Experimental diets			
	I	II	III	IV
Ingredients (g/kg)				
Corn	180	200	240	350
Soybean meal	180	180	180	180
Wheat bran	150	180	190	130
Peanut seedling	460	410	360	310
Calcium carbonate	15	15	15	15
Sodium chloride	5	5	5	5
Vitamin–mineral premix[a]	10	10	10	10
Analyzed composition (g/kg of DM)				
Dry matter	877	878	875	875
Digestible energy (MJ/kg DM)	9.8	10.2	10.5	11.1
Acid detergent fiber (ADF)	167	157	151	110
Acid detergent lignin (ADL)	49	46	42	36
Neutral detergent fiber (NDF)	336	306	273	250
Crude fiber	138	128	125	87
Starch	145	164	192	250
Ash	101	96	88	78
Crude protein	174	174	183	184
Calcium	12.5	11.4	11.3	13.2
Phosphorus	6.7	7.2	7.1	6.9
Starch/ADF	0.9	1.0	1.3	2.3

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

[a] The premix provides the following 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.

2.4. Analysis of intestinal M cells by immunohistochemistry

Five rabbits per group were euthanized at 52, 62, 72 and 82 days of age to harvest the appendix. The tissue samples were rinsed in physiological saline to eliminate intestinal contents and fixed in paraformaldehyde solution for one week. The tissue samples were embedded in paraffin through a series of steps that included incubations in graded alcohols, methyl benzoate and benzole. Sections of 5 μm in thickness were cut from the paraffin blocks and used to observe the general structure. Sections were placed on poly-L-lysine slides, and immunohistochemical analysis was performed using the PV-9002 Plink-2 plus Polymer HRP Detection System for Mouse Primary Antibody (ZSGB-BIO, Beijing, China) according to the manufacturer's instructions. In brief, the paraffin sections were deparaffinized in xylene twice for 30 min followed by a series of washes in ethanol, including absolute ethanol, 95%, 90%, 85%, 80% and 70% ethanol, and distilled water for 10 min. The samples were boiled in 99 °C citrate buffer (pH 6.0) for 20 min in a water bath to restore antigenic properties. The sections were then cooled to room temperature and kept in 3% hydrogen peroxide (H_2O_2) in methanol for 30 min to eliminate endogenous peroxide. The sections were incubated with a monoclonal mouse anti-vimentin antibody (1:100, Clone V9, ZSGB-BIO, Beijing, China) at 4 °C overnight and