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EFFECTS ON INTESTINAL HEALTH OF WEANED-REX RABBITS OF DIETS WITH DIFFERENT NDF LEVELS

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ABSTRACT

To study the effect of dietary NDF on intestinal health of weaned-rex rabbits, 56 rabbits fed on diets with NDF at levels of 29%, 33%, 37% and 41% from 35 days old to 68 days old. Intestinal samples collected from them at 68 days were to determine *MUC1* and *MUC2* expressions, intestinal IgA, cecal bacterium populations. The results were as below. (1) The abnormal expression probability of *MUC1* was the highest (30%) at the NDF level of 37% and the lowest (10%) at the NDF level of 33%, and the abnormal expression probability of *MUC2* was the highest (23%) at the NDF level of 37% and the lowest (8%) at the NDF level of 33%. (2) The expressions of the genes of *FASN* and *MUC2* were highly correlated (P < 0.01) and the linear relation between them was $y = 0.50 + 2.06 \times (R^2 = 0.44)$. (3) The IgA content in duodenum and jejunum increased as the NDF levels increased (P < 0.05). (4) *Bacteroides* population first increased and then decreased as the NDF levels increased (P < 0.05). *Lactobacillus* and *Escherichia coli* population decreased as the NDF levels increased (P < 0.05). Lactobacillus and *Escherichia coli* population decreased as the NDF levels increased (P < 0.05). The NDF level of 33% was more favorable for keeping intestine of weaned-rex rabbits healthy.

Key words: weaned-rex rabbits, NDF, *MUC*, IgA, cecal bacteria.

INTRODUCTION

Dietary fiber fractions in animal feeding are important because of their influence on digestion passage rate and mucosal functionality, and their role as substrates for gut microbiota related to performance and digestive health (Gidenne 2015). There have been researches done on optimum NDF levels for dairy cow, sheep and slaughter rabbit, but no deeper research done on optimum NDF levels for weaned-rex rabbit.

Both MUC1 and MUC2 as main functional proteins of mucosal barrier play an important role in protecting intestinal mucosa. Dietary fiber has effects on mucin synthesis and secretion crucial for enhancing intestinal immunity. So far, mucin researches have mainly involved human beings. Rabbits are as models in researching on human eye diseases. There was no research work done on influence of the mucin genes of *MUC1* and *MUC2* on intestinal health using weaned-rex rabbits.

This research was to study effects of NDF at different levels on intestinal health of weaned-rex rabbits.

MATERIALS AND METHODS

Animals and experimental design

The test diets were prepared to have four NDF levels of 29%, 33%, 37% and 41%. Separately raised in different cages, 56 thirty-five-day-old weaned-rex rabbits were randomly divided into four 14-rabbits groups and fed on the prepared diets. The pretesting and testing lasted 5 days and 28 days, respectively.

Chemical determination

Expressions of *MUC1*, *MUC2* and *FASN* in duodenum, jejunum, ileum and cecum were determined through qPCR. The IgA content in small intestine was determined through DAB method. Cecal bacterium (General bacteria, *Bacteroides, Lactobacillus, Escherichia coli*) at four NDF levels was extracted using Takara bacterial genomic DNA extraction kit and then determined through qPCR.

Data processing

The expressions of the genes of *MUC1* and *MUC2* above 10 and 8 were defined as their abnormal expressions, respectively. In each bowel segment, the abnormal expression probability of the gene was percentage of abnormal expression sample numbers to the sample numbers in each bowel segment concerned. All data were analyzed by Microsoft excel 2010 and IBM-SPSS 19.0. Differences among four NDF levels were tested using Duncan's multiple comparison.

RESULTS AND DISCUSSION

Cecal probability of *MUC1* and *MUC2* abnormal expressions at the four NDF levels were both 0%, but ileac probability of *MUC1* and *MUC2* abnormal expressions were relatively high. This was probably because nutrient absorption and fiber fermentation in the cecum restrained pathogens, thereby presenting positive effects on intestinal micro-ecological balance. The relative greater population of bacterium of ileac content (McGuckin *et al.* 2011) resulted in the upregulation of *MUC1* and *MUC2* expressions (Figure 1 and Figure 2).

The abnormal expression probability of MUC1 was the highest (30%) at the NDF level of 37% and the lowest (10%) at the NDF level of 33%, and the abnormal expression probability of MUC2 was the highest (23%) at the NDF level of 37% and the lowest (8%) at the NDF level of 33%. The intestine, especially ileum, was easier to infect, because of its modified internal environments by the lower fiber levels through nerves, immune and microorganisms, which were unfavorable for the growth of weaned-rex rabbits. At the higher levels, dietary fiber stimulated intestine wall, enhanced intestinal peristalsis and increased physical mucus loss, thereby accelerating the regeneration of MUC1 and MUC2. In fact, dietary NDF at a too low or high level was not so good for the growth of weaned-rex rabbits (Figure 1 and Figure 2).



Figure 1: Probability of *MUC1* abnormal expression

Figure 2: Probability of MUC2 abnormal expression.

This study found that expressions of the genes of *FASN* and *MUC2* were highly correlated, which meant *MUC2* expression needed to be supported by *FASN*. *FASN* related to the development of tumor tissue (Migita *et al.* 2009). Lesion proliferation of intestinal epithelial cells were probably induced by high expression of *FASN*, which depleted immune reserve along with high expressions of *MUC1* and *MUC2* thus made it difficult to treat severe intestinal diseases. The study found that maintaining the integrity of the intestinal mucous barrier, decreasing the probability of bacterial infection and

rendering *FASN* to express at a low level were favorable for reducing the risk of severe intestinal disease (Figure 3).



Figure 3: Correlation between the expression of *FASN* and *MUC2*

Nodule aggregation closest to ileum may be the reason why the IgA content was the highest in ileum compared with those in duodenum and jejunum. However, ileac IgA content at the four levels did not differ significantly and the reason for this was probably that IgA production reached the maximum level. The IgA content in duodenum, jejunum and ileum increased as the NDF levels increased, which meant that NDF higher levels were favorable for increasing the IgA content and then making the intestine more capable of fending

off pathogens and enhancing intestinal mucus immune. Duodenal and ileac IgA content at the NDF level of 33% did not differ significantly than those at the NDF levels of 37% and 41%. Therefore, the NDF level of 33% was a relatively proper choice (Table 1).

Table 1: Intestinal IgA at the different NDF levels

| Item | | NDF | SEM | D values | | |
|----------|-------------------|--------------------|--------------------|-------------------|-------|----------|
| | 29% | 33% | 37% | 41% | SEIVI | 1 values |
| Duodenum | 25.7 ^b | 30.5 ^{ab} | 35.8 ^a | 38.8 ^a | 1.79 | 0.042 |
| Jejunum | 29.6 ^b | 33.9 ^{ab} | 36.3 ^{ab} | 39.2 ^a | 1.39 | 0.032 |
| Ileum | 36.8 | 45.0 | 42.0 | 45.8 | 2.17 | 0.070 |

Means with different letters on the same row differed significantly (P < 0.05).

Dietary fibers regulated intestinal microflora selectively, stimulating beneficial microorganisms and inhibiting harmful ones (Zhong *et al.* 2009). *Bacteroides* content and *Lactobacillus* content were relatively high at the NDF level of 33%. *Lactobacillus* content increased as the NDF levels increased, which was not consistent with Gu *et al.* (2008). The main reason was that the higher NDF levels resulted from using the rice hull powder containing higher content of lignin in this study. Fermentable fiber feeding increased the content of *Lactobacillus*, but lignin was unfermentable fiber (Brownlee 2011). *Escherichia coli* population decreased as the NDF levels increased probably because the increased NDF levels decreased excessive fermentation and shortened the passage time in hindgut. Therefore, NDF performed relatively better at the level of 33% than at the other levels.

Table 2: Cecal bacterium populations at the different NDF levels

| Itom | | SEM | D values | | | |
|------------------|--------------------|-------------------|--------------------|--------------------|------|----------|
| Item | 29% | 33% | 37% | 41% | SEM | 1 values |
| General bacteria | 1.91 | 2.53 | 2.26 | 1.64 | 0.28 | 0.719 |
| Bacteroides | 11.78 ^b | 20.76^{a} | 15.72 ^b | 14.91 ^b | 2.21 | 0.034 |
| Lactobacillus | 2.52^{a} | 2.12^{ab} | 1.99 ^b | 1.48 ^b | 0.06 | 0.041 |
| Escherichia coli | 2.37 ^a | 1.98 ^a | 1.35 ^{ab} | 0.82^{b} | 0.08 | 0.048 |

Means with different letters on the same row differed significantly (P < 0.05).

CONCLUSIONS

From the above analyses, the NDF level of 33% was more favorable for keeping the intestine of weaned-rex rabbits healthy.

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