Effects of Low Protein on Performance and Intestinal Microflora of Piglets

Ma Peiling

Dingxi Livestock Husbandry and Veterinary Bureau, Gansu Province, 743000, China

Keywords: low protein; growth performance; intestinal flora; intestinal mucosal morphology; weaned piglets

Abstract: The purpose of this experiment was to study the effects of low protein (LF) supplementation on growth performance, intestinal flora and intestinal mucosal morphology of weaned piglets. Ninety-six 21-day-old Du Chang Da crossbred weaned piglets with similar body weight were randomly divided into four groups: basal diet (control group), basal diet + 250 mg/kg low protein, basal diet + 500 mg/kg low protein and basal diet + 750 mg/kg low protein, with four replicates in each group and six piglets in each replicate. The trial period is 21 days. The results showed that compared with the control group and 750 mg/kg low protein group, dietary supplementation of 250 and 500 mg/kg low protein could significantly increase the average daily gain of piglets ($P < 0.05$); compared with the control group, different levels of low protein could significantly reduce the number of E. coli in cecum ($P < 0.05$), and the addition of 500 mg/kg low protein could significantly increase the number of Lactobacillus in cecum and colon ($P < 0.05$), and significantly reduce the number of colonic bacteria ($P < 0.05$). The number of Escherichia coli ($P < 0.05$) and the ratio of villus height to crypt depth in duodenum, jejunum and ileum were significantly increased by adding 250 and 500 mg/kg low protein ($P < 0.01$). The results showed that the addition of low protein in diet could stimulate the growth of beneficial bacteria and reduce the proliferation of harmful bacteria in intestinal tract, thus improve intestinal function and improve the growth performance of piglets. The suitable amount of low protein was 250 mg/kg under the experimental conditions.

1. Introduction

Antibiotics have been used as growth promoters for livestock and poultry for more than 50 years. They have played an important role in animal husbandry and greatly promoted the development of animal husbandry. However, in recent years, with the improvement of people's living standards and health awareness and the negative impact of the abuse of antibiotics in feed, food safety issues have been highly valued. Therefore, the research and development of green, non-residue antibiotic substitutes has become one of the hotspots in the field of animal nutrition. Low protein (LF) is a natural substance extracted from milk. It is safe and reliable. It has the characteristics of antimicrobial, bactericidal, no side effects and no residue. At present, the research on low protein is concentrated on mice, humans and fish. The fish mainly focus on the innate immunity of fish. The research on pig is mainly about the influence of pig's non-specific immunity, and the effect on the intestinal microorganism and intestinal development of pigs is rarely reported. Therefore, the purpose of this study was to investigate the effects of dietary low protein supplementation on growth performance, intestinal flora and intestinal mucosal morphology of early-weaned piglets, and to improve intestinal health of early-weaned piglets and develop their genetic growth potential.

2. Materials and methods

2.1 Test Material

Low protein was purchased in Nanjing Tianchun Trading Co., Ltd. Its production process is directly extracted from fresh milk, purified, ultrafiltered and dried. The product is light pink dry powder with protein content (> 95%) and iron saturation (> 90%).

  DOI: 10.25236/ibmc.2019.034

2.2 Basic Diet

The basic diet was formulated according to the nutrient requirement of 5*10 kg piglets in NRC (1998) of the United States. The composition and nutrient level of the basic diet were shown in Table 1.

Table 1 Composition and nutrient levels of the basal diet (air-dry basis) %

Items	Content
Ingredients	
Corn	59.2
Fish meal	3
Soybean meal	24
Wheat middlings	3
CaHPO4	2
Limestone	1
Whey powder	3
NaCl	0.3
Emulsified fatty powder	3
Choline choride	0.1
Lysme	0.34
Methionine1)	0.06
Premix	1
Total	100
Nutrient levels2)	14.46
DE／(MJ／kg)	
CP	19.5
Lysine	1.35
Ca	0.8
P	0.66

1) The premix provided the following per kg of the diet：VA2 200 IU,VD3 200IU,VE 16mg,VK lmg, choline 200mg, pantothenic acid 6mg,VB2 2mg, folic acid 0.3mg, nicotinic acid 25mg,VBl 1.6mg,VB6 6mg, biotin 0.08mg,VBl20.01mg,Cu(as copper sulfate)6mg,Fe(as ferrous sul—fate)100mg,Zn(as zinc sulfate)100mg,Mn20mg,I 0.14mg,Se 0.3mg。

2) DE was acalculat-ed value．The other nutrient levels were measured values．

2.3 Test Design

Ninety-six 21-day-old crossbred weaned piglets with similar body weight were randomly divided into four groups: basal diet (control group), basal diet + 250 mg/kg low protein, basal diet + 500 mg/kg low protein and basal diet + 750 mg/kg low protein. Each group had four replicates, six piglets per replicate. The preliminary period is 7 days and the positive period is 21 days.

2.4 Feeding Management

Feeding experiment was carried out in the crossing pig farm of Changsha Muyuan Ecological Breeding Co., Ltd. The enclosure was disinfected one week before the experiment. Piglets were fed in enclosed pens at 08:00, 14:30 and 18:00 a day, respectively. The piglets were fed three times a day for free feeding and drinking water. 2 times a day, 1 times per 3D spray disinfection, piglet immunity, insect repellent and other feeding management were carried out according to the routine procedure of pig farms.

2.5 Measuring Indicators

2.5.1 Growth Performance

At the beginning and end of the experiment, the average daily gain (ADG), average daily feed

intake (ADFI) and feed-to-weight ratio (FI) of piglets were calculated according to the following formula:

Average daily gain (kg/d) = (final weight-initial weight) / test days; average daily feed intake (kg/d) = (feed weight-residual weight) / test days;

Feed-to-weight ratio = total feed intake/total weight gain.

2.5.2 Number of intestinal flora

At the end of the experiment, one piglet was randomly selected from each repetition and slaughtered, dissected, and the colon and cecum segments were intercepted respectively. The two ends were tightened by surgical line, and immediately sent to the laboratory for the determination of the number of E. coli and lactobacillus. The number of bacteria in LG intestinal contents was expressed by logarithm [lg (CFU/g)].

2.5.3 Intestinal mucosal morphology

At the end of the experiment, a piglet with close to average weight and good health was randomly selected from each replicate for slaughter. About 1 cm tissue samples were cut from duodenum, jejunum and middle ileum, fixed with 10% neutral formalin, routinely dehydrated with alcohol and embedded in paraffin sections. The sections were 4-6 m thick and stained with hematoxylin-eosin (HE). Using DT2000 general image analysis and processing system, three discontinuous slices were observed for each sample. Ten typical visual fields were selected for each sample. The villus height and recess depth of small intestine were measured and their average values were taken.

2.6 Data Statistical Analysis

The original data were preliminarily processed by Excel, and then analyzed by one-way ANOVA program in SPSS13.0 statistical software. Duncan's method was used for multiple comparisons, $P < 0.05$ was significant difference, $P < 0.01$ was extremely significant difference. The results are expressed as standard deviation of average soil.

3. Results

3.1 Effect of Low Protein on Growth Performance of Weaned Piglets

Table 2 shows that adding different levels of low protein in the diet can promote the growth of piglets. Compared with the control group, 250 and 500 mg/kg of low protein could significantly increase the average daily gain of weaned piglets ($P < 0.05$); compared with the control group, adding low protein could increase the average H intake and reduce the feed-Weight ratio of weaned piglets, but the difference was not significant ($P > 0.05$).

Table 2 Effects of lactoferrin supplementation on growth performance of weaned piglets

Items	Control group	Lactoferrin supplemental level/ (mg/kg)		
		250	500	750
Initial weight/kg	7.89±0.21	7.90±0.52	7.88±0.27	7.88±0.20
Final weight/kg	13.56±0.19a	14.23±0.28ab	14.45±0.35b	13.79±0.16ab
ADG/(kg/d)	0.27±0.02 a	0.30±0.03b	0.31±0.04b	0.28±0.04 a
ADFl/(kg/d)	0.44±0.01	0.46±0.01	0.46±0.01	0.45±0.01
FIG	1.62±0.05	1.51±0.03	1.48±0.04	1.59±0.05

In the same row, values with different small letter superscripts mean significant difference ($P<0.05$), and with different capital letter superscripts mean extremely significant difference ($P<0.01$).The same as below.

Table 3 Effects of lactoferrin supplementation on intestinal microflora of weaned piglets lg(CFU/g)

Items	Control group	Lactoferrin supplemental level/ (mg/kg)		
		250	500	750
Cecum				
Escherichia coli	7.69±0.28Bb	6.77±0.20ABa	6.51±0.22Aa	6.84±0,24ABa
Lactobacillus	7.53±0.25a	8.23±0.23ab	8.55±0.21b	8.23±0.22ab
Colon				
Escherichia coli	8.03±0.06b	7.55±0.21ab	7.28±0.2a	7.62±0.2ab
Lactobacillus	7.54±0.23Aa	8.15±0.04ABb	8.61±,029Bb	8.06±0.07ABab

3.2 Effect of Low Protein on Intestinal Mucosa Morphology in Weaned Piglets

From Table 4, compared with the control group, the addition of 250 and 500 mg/kg low protein significantly increased villus height ($P < 0.01$), 750 mg/kg low protein significantly increased villus height ($P < 0.05$), and the ratio of villus height to recess depth ($P < 0.0I$) was significantly increased in each experimental group compared with the control group. In jejunum, the ratio of villus height and villus height to crypt depth was significantly increased in 250 and 500 mg/kg low protein groups compared with the control group ($P < 0.0I$). There was no significant difference between 750 mg/kg low protein group and the control group ($P > 0.05$). From the ileum, the villus height of each experimental group was significantly higher than that of the control group ($P < 0.05$), in which ($P < 0.01$), the ratio of villus height to crypt depth of each experimental group was extremely higher than that of the control group ($P < 0.01$), and the ratio of villus height to crypt depth of 250 and 500 mg/kg low protein group was significantly higher than that of the control group ($P < 0.01$).

Table4 Effects of lactoferrin supplementation on intestinal mucosal morphology of weaned piglets

Items	Control group	Lactoferrin supplemental level/ (mg/kg)		
		250	500	750
Duodenum				
Villus height/μm	185.31±13.14Aa	238.72±9.93Bc	41.45±8.33Bc	205.28±6.88ABb
Crypt depth/μm	133.58±4.72b	121.63±3.51b	117.93±8.43a	123.61±5.87b
V/C	1.49±0.48Aa	1.96±0.29Cc	2.05±0.22Cc	1.71±0.34Bb
Jejunum				
Villus height/μm	155.13±14.18Aa	196.68±15.92Bb	204.18±8.78Bb	182.11±9.18ABab
Crypt depth/μm	101.93±3.19c	92.78±8.81Bc	90.25±4.76ab	100.27±1.77bc
V/C	1.52±0.10Aa	2.12±0.57Bb	2.27±0.45Bb	1.82±0.36ABab
Ileum				
Villus height/μm	170.51±9.21Aa	205.52±14.5Bb	206.35±8.89Bb	194.93±13.23ABb
Crypt depth/μm	117.50±4.15Bb	110.25±3.73ABa	96.12±5.73Aa	110.55±3.31ABa
V/C	1.45±0.25Aa	1.87±0.46Bb	2.15±0.54Cc	1.77±0.72Bb

4. Discussion

4.1 Effect of Low Protein on Growth Performance of Weaned Piglets

After weaning piglets are stressed by nutrition, environment, psychology and other aspects, which leads to the decline of body function and resistance, thus causing intestinal flora imbalance of piglets, and then causing diarrhea to varying degrees, leading to piglet death in severe cases. Low protein can reduce diarrhea rate and improve growth performance of weanling piglets through antimicrobial, antiviral, immune regulation, promoting intestinal iron absorption, stimulating intestinal Bifidobacterium growth, and protecting intestinal mucosa.

Wu Xilin*s research showed that adding low protein in diet could increase daily gain, improve feed conversion rate and reduce diarrhea rate of piglets. The average daily gain of piglets with 100, 230 and 400 mg/kg low protein was significantly higher than that of control group and 50 mg/kg low protein group ($P < 0.05$). There was no significant difference in feed-to-weight ratio among the

experimental groups (P > 0.05), but the lowest was in the group with 230 mg/kg. In this experiment, adding 250 and 500 mg/kg low protein in diet could significantly increase average daily gain (P < 0.05), increase average daily feed intake and reduce feed-Weight ratio (P > 0.05), which was consistent with the results of Wu Xilin's research.

4.2 Effect of Low Protein on Intestinal Microflora in Weaned Piglets

A large number of studies have shown that low protein can play an indirect role in regulating intestinal flora of animals by reducing or limiting the growth of specific microorganisms through bacteriostasis and bactericidal action. Teraguchi et al. studied the effect of bovine low protein on intestinal bacteria in SPF mice. The results showed that the growth of C. coli in the digestive tract of rats was inhibited by feeding cow milk containing 0.5%~2.0% low protein, and the inhibition effect was not related to the ability of low protein iron binding. Liu Hongyun et al. reported that the number of bifidobacteria and Lactobacillus in intestine of breast-fed infants was significantly higher than that of infants fed with formula milk powder, which was directly related to the low protein content in normal breast milk. Hellweg et al. added different levels of low protein to the diet of adult Beagle dogs, which were 0, 120 and 1800 mg/kg, respectively. The results showed that the number of Escherichia coli in feces of the groups with low protein had a decreasing trend. Wang et al. added 1.0g/kg low protein to the diet of weaned piglets. After feeding for 1 month, compared with the control group, the number of E. coli and Salmonella was reduced the most (P < 0.05), and the number of Lactobacillus and Bifidobacteria in colon was increased (P < 0.05). Similar results were obtained in this experiment. Adding low protein to the diet of weaned piglets could significantly reduce the number of enteric Escherichia coli and increase the number of Lactobacillus (P < 0.05). The reason is that low protein belongs to broad-spectrum antimicrobial agents, which not only inhibit Gram-negative bacteria such as E. coli, Shigella and Salmonella, but also Gram-positive bacteria such as Listeria monocytogenes, Staphylococcus aureus and Helicobacter pylori in stomach. However, the microorganisms (such as Lactobacillus) that do not need much iron are basically not inhibited.

4.3 Effect of Low Protein on Intestinal Mucosa Morphology in Weaned Piglets

The small intestine is the main part of nutrient absorption and transport in weaned piglets. The normal morphology and structure of small intestinal mucosa is the key to ensure the digestive and absorption function of piglets. The ratio of villus height to recess depth and the ratio of villus height to recess depth are the most direct indicators reflecting the morphological structure and function of small intestinal mucosa.

A large number of experiments have proved that low protein can increase the height of small intestinal villi and reduce the depth of crypt. When Zhangxiang added 250 and 1000 mg/d low protein to calf milk substitute, the villus height of anterior jejunum, middle jejunum and posterior jejunum was significantly higher than that of control group (p < 05), and the ratio of villus height to crypt depth of duodenum, anterior jejunum and middle jejunum was significantly higher than that of control group (P < 0.05). Humphrey et al. added rice expressing lactoferric molar gene to broiler diet and compared it with the group added antibiotics. The results showed that the duodenal villus height of low protein group was significantly higher than that of low protein group.

Antibiotic group (P < 0.05). The results showed that the villus height and crypt depth in intestinal slices increased significantly (P < 0.05) and decreased significantly after dietary low protein supplementation. The reason is that low protein can enhance the dissolution and absorption of iron in intestinal tract, avoid its direct stimulation to intestinal tract, and then reduce intestinal damage. At the same time, low protein can stimulate the growth of intestinal cells, increase the number of*beneficial*bacteria in intestine, such as bifidobacteria and lactobacillus, reduce the reproduction of harmful?Bacteria such as E.coli, thus ensuring the healthy development of intestinal tract, enhancing the digestion and absorption of nutrients in intestine, reducing diarrhea rate and improving growth performance of piglets.

5. Conclusion

Low protein can significantly promote the proliferation of Lactobacillus and other beneficial bacteria in intestine, inhibit the proliferation of Escherichia coli, improve the morphology and structure of intestinal mucosa, and improve intestinal function, thereby improving the growth performance of weaned piglets.

Under the experimental conditions, the suitable amount of low protein was 250 mg/kg.

References

[1] The effect of low protein diet on performance, nitrogen metabolism and blood biochemical indexes of finishing pigs [J].Zeng Yanxia, Wang Jing, Ji Haifeng, Wang Sixin, Liu Hui, Zhang Dongyan, Wang Yamin, Zhaochen, Journal of Livestock Ecology*2017(06)

[2] Effect of low protein diet on metabolites and microflora in cecum of fattening pigs [J].Luo Zhen, Cheng Yanfen, Zhu Weiyun, Animal Husbandry and Veterinary Medicine*2015(10)

[3] The pig gut microbial diversity: Understanding the pig gut microbial ecology through the next generation high throughput sequencing [J]. Hyeun Bum Kim,Richard E. Isaacson. Veterinary Microbiology. 2015 (3-4)

[4] Microencapsulated lysine and low-protein diets: Effects on performance, carcass characteristics and nitrogen excretion in heavy growing–finishing pigs [J]. A. Prandini,S. Sigolo,M. Morlacchini,E. Grilli,L. Fiorentini. Journal of Animal Science. 2013 (9)