Effects of Dietary Vitamin B12 Supplementation Level on Growth Performance, Intestinal Development, and Cecal Microbiota Structure in 5-15-Week-Old Wulong Geese (Postprint)

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Abstract

This study was conducted to investigate the effects of dietary vitamin B12 supplementation levels on growth performance, intestinal development, and cecal microbiota structure in 5- to 15-week-old Wulong geese, and to determine the appropriate supplementation level of vitamin B12 in diets for this age period. A total of 360 Wulong geese at the end of 4 weeks of age were randomly allocated into 6 groups with 6 replicates per group and 10 geese per replicate (half male and half female). Group I served as the control group and was fed a basal diet without vitamin B12 supplementation (the measured vitamin B12 content in the basal diet was 0), while groups II-VI were experimental groups fed diets supplemented with 0.005 (group II), 0.010 (group III), 0.015 (group IV), 0.020 (group V), and 0.025 mg/kg vitamin B12 (group VI) based on the basal diet. The experimental period lasted 11 weeks. The results showed that: 1) The final body weight (FBW) of groups II-IV was significantly or extremely significantly higher than that of the control group ($P<0.05$ or $P<0.01$), and the average daily gain (ADG) of groups III-IV was significantly or extremely significantly higher than that of the control group ($P<0.05$ or $P<0.01$). 2) The jejunal villus height (VH) of all experimental groups was significantly or extremely significantly higher than that of the control group ($P<0.05$ or $P<0.01$), with group III having the highest VH; the jejunal crypt depth (CD) of all experimental groups was significantly or extremely significantly lower than that of the control group ($P<0.05$ or $P<0.01$), with group III having the lowest CD; the jejunal VH to CD ratio of all experimental groups was significantly or extremely significantly greater than that of the control group ($P<0.05$ or $P<0.01$), with group III having the greatest VH to CD ratio. 3) Dietary supplementation with different levels of vitamin B12 could alter the relative abundance of dominant cecal microbiota at the class, family, and genus levels in meat geese. At the class level, Clostridia, Bacteroidia, and Deltaproteobacteria showed obvious changes; at the family level, Bacteroidaceae, Verrucomicrobiaceae, and Desulfovibrionaceae showed obvious changes; at the genus level, Bacteroides and Desulfovibrio showed obvious changes. In conclusion, appropriate dietary supplementation levels of vitamin B12 could improve the growth performance of 5- to 15-week-old Wulong geese and alter the relative abundance of dominant cecal microbiota; based on FBW and ADG as evaluation indices, the recommended supplementation level of vitamin B12 in diets for 5- to 15-week-old Wulong geese is 0.012 6-0.013 4 mg/kg.

Full Text

Effects of Dietary Vitamin B12 Supplemental Level on Growth Performance, Intestinal Development, and Cecal Microflora Structure in Wulong Geese Aged 5–15 Weeks

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Abstract: This experiment was conducted to investigate the effects of dietary vitamin B12 supplemental level on growth performance, intestinal development, and cecal microflora structure in Wulong geese aged 5–15 weeks, and to determine the optimal supplemental level of vitamin B12 for this age group. A total of 360 Wulong geese at the end of 4 weeks of age were randomly allocated into 6 groups with 6 replicates per group and 10 geese per replicate (half male and half female). Group served as the control group and was fed a basal diet without vitamin B12 supplementation (the measured vitamin B12 content in the basal diet was 0). Groups – were experimental groups fed the basal diet supplemented with 0.005 (Group), 0.010 (Group), 0.015 (Group), 0.020 (Group), and 0.025 mg/kg vitamin B12 (Group), respectively. The trial lasted for 11 weeks. The results showed: 1) The final body weight (FBW) of Groups – was significantly or extremely significantly higher than that of the control group ($P<0.05$ or $P<0.01$), and the average daily gain (ADG) of Groups – was significantly or extremely significantly higher than that of the control group ($P<0.05$ or $P<0.01$). 2) The jejunal villus height (VH) of all experimental groups was significantly or extremely significantly higher than that of the control group ($P<0.05$ or $P<0.01$), with Group showing the highest VH. The jejunal crypt depth (CD) of all experimental groups was significantly or extremely significantly lower than that of the control group ($P<0.05$ or $P<0.01$), with Group showing the lowest CD. The VH/CD ratio (V/C) of all experimental groups was significantly or extremely significantly higher than that of the control group ($P<0.05$ or $P<0.01$), with Group showing the highest V/C ratio. 3) Dietary supplementation with different levels of vitamin B12 altered the relative abundances of dominant cecal microflora at the class, family, and genus levels. At the class level, Clostridia, Bacteroidia, and Deltaproteobacteria showed obvious changes; at the family level, Bacteroidaceae, Ruminococcaceae, and Desulfovibrionaceae showed obvious changes; at the genus level, Bacteroides and Desulfovibrio showed obvious changes. In conclusion, an appropriate supplemental level of dietary vitamin B12 can improve the growth performance and alter the relative abundance of dominant cecal microflora in Wulong geese aged 5–15 weeks. Based on FBW and ADG as evaluation indices, the recommended supplemental level of vitamin B12 in diets for 5–15-week-old Wulong geese is 0.0126–0.0134 mg/kg.

Keywords: vitamin B12; Wulong geese; growth performance; intestinal development; cecum; microflora structure

Introduction

Vitamin B12 is one of the most important water-soluble vitamins in humans and animals, with extensive physiological functions. Its most critical role is participating in intracellular DNA biosynthesis and protein metabolism. Vitamin B12 deficiency can lead to impaired DNA synthesis in erythrocytes, blocked cell division and proliferation, enlarged erythrocyte volume, loose nuclear chromatin, and megaloblastic anemia [1]. In animals, vitamin B12 deficiency manifests as anorexia, poor growth, and anemia [2-3]. The intestinal microbial system, as the most complex microecosystem in the animal gut, contains numerous microorganisms involved in nutrient absorption, distribution, metabolism, and immunity, thereby affecting animal growth and health [4]. The gut microbiota can produce abundant probiotics, and beneficial bacterial flora plays a significant role in improving nutrient utilization and maintaining animal health. Therefore, in-depth research on the effects of vitamin B12 on gut microflora structure, nutrient utilization efficiency, and production performance is of great significance for guiding livestock and poultry diet formulation.

Food-derived vitamin B12 is bound to proteins and released in the digestive tract through the action of gastric acid, pepsin, and trypsin. It then binds with intrinsic factor (IF), a glycoprotein secreted by gastric mucosal cells, and the vitamin B12-IF complex is absorbed in the ileum. Normal intestinal microorganisms, such as *Bifidobacterium* and *Lactobacillus*, can synthesize various B vitamins essential for human growth and development. Huang et al. [5] reported that dietary supplementation with 0.008 mg/kg vitamin B12 significantly increased the body weight of broiler chicks. While numerous studies have investigated vitamin B12 absorption and function in animals, few have examined the relationship between vitamin B12 and intestinal microorganisms, particularly regarding the molecular-level exploration of relationships among vitamin B12, growth performance, intestinal development, and cecal microflora structure in geese. This study therefore investigated the effects of different dietary vitamin B12 levels on growth performance and intestinal development in geese, and analyzed cecal microflora structure using 16S rRNA high-throughput sequencing technology to compare microflora composition and structure. The aim was to explore the relationship between vitamin B12 and gut microflora structure, scientifically determine the vitamin B12 requirement for 5–15-week-old geese, and provide a basis for establishing nutritional requirements for geese.

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1 Materials and Methods

1.1 Experimental Materials

The basal diet was formulated based primarily on the NRC (1994) [6] nutrient requirements for poultry, with composition and nutrient levels shown in Table 1 . High-performance liquid chromatography detected no vitamin B12 in the basal diet. The vitamin B12 preparation used in the experiment (product of Ningxia Jinwei Pharmaceutical Co., Ltd.) contained 1% active substance.

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

1.2 Experimental Design

Experimental geese were provided by Gaomi Yinhe Runyan Goose Industry Co., Ltd., a breeding base of the National Waterfowl Industry Technology System. A total of 360 Wulong geese at the end of 4 weeks of age with no significant differences in initial average body weight (P>0.05) were randomly divided into 6 groups with 6 replicates per group and 10 geese per replicate (half male and half female). Group was the control group fed the basal diet without vitamin B12 supplementation (measured vitamin B12 content was 0 in the basal diet). Groups – were experimental groups fed the basal diet supplemented with 0.005, 0.010, 0.015, 0.020, and 0.025 mg/kg vitamin B12, respectively. The experimental period lasted 11 weeks.

1.3 Management Practices

Before the feeding trial, the goose house and equipment were washed and disinfected with caustic soda solution spray, then fumigated with formaldehyde and potassium permanganate with closed doors and windows for 24 hours. Geese were raised on net beds with ad libitum access to feed and water throughout the experimental period. The brooding house was maintained at normal temperature with natural lighting during the trial.

1.4 Sample Collection and Index Determination

1.4.1 Sample Collection At the end of 15 weeks of age, after fasting weighing, 2 geese were randomly selected from each replicate (half male and half female), totaling 72 geese across 6 groups. After slaughter by jugular vein exsanguination, the abdominal cavity was quickly opened, and the cecum was aseptically removed and rapidly collected in cryovials, snap-frozen in liquid nitrogen, and transferred to a -80°C freezer for later analysis. From the 72 geese that had cecal samples collected, 6 geese per group were selected, and approximately 1 cm of jejunal segment was excised. After washing surface chyme with physiological saline, the samples were immediately fixed in 10% formalin fixative, stored overnight at room temperature, and then placed in a 4°C refrigerator for histological section preparation.

1.4.2 Growth Performance Indices Calculation After the feeding trial, feed was withheld for 6 hours, and geese were then weighed individually after fasting. Body weight and weight gain of experimental geese in each group were recorded to calculate final body weight (FBW), average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G).

1.4.3 Intestinal Histological Section Measurement Methods Fixed tissues were trimmed, washed, dehydrated, cleared, paraffin-embedded, sectioned, and stained with hematoxylin-eosin (HE) to prepare histological sections. Six tissue sections were selected from each group, and jejunal villus height (VH), crypt depth (CD), and the VH/CD ratio (V/C) were measured under a 10×10 microscope.

1.4.4 Extraction of Total Cecal Microbial DNA Each sample was analyzed individually, and total cecal microbial DNA was extracted using a genomic DNA kit from Tiangen Biotech Co., Ltd.

1.4.5 Quantification and Purity Detection of Total Cecal Microbial DNA DNA content was measured using a DNA quantifier, DNA purity was expressed as OD260/OD280, and DNA fragment size was detected by 0.8% agarose gel electrophoresis.

1.4.6 PCR Amplification and Bacterial 16S rDNA Sequencing Analysis Bacterial 16S rRNA (V3+V4) region primers: 5' - ACTCCTACGGGAGGCAGCA-3'(forward primer) and 5'-GGACTACHVGGGTWTCTAAT-3' (reverse primer). The PCR program for sample detection was as follows: pre-denaturation at 95°C for 5 min, followed by 25 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 40 s.

1.5 Statistical Analysis

Data were analyzed using the GLM procedure in SPSS 17.0 software for main effect analysis, followed by ANOVA for variance analysis. When significant differences were detected, LSD method was used for multiple comparisons among groups, with $P<0.05$ as the criterion for significant difference. Optimized sequences were clustered to define operational taxonomic units (OTUs). Based on OTU analysis results, samples were analyzed at various taxonomic levels to obtain community structure diagrams at class, family, and genus levels.

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2 Results

2.1 Effects of Dietary Vitamin B12 Supplemental Level on Growth Performance of Wulong Geese

As shown in Table 2 , dietary supplementation with different levels of vitamin B12 had significant effects on FBW and ADG ($P<0.05$). Specifically, FBW of Groups – was significantly or extremely significantly higher than that of the control group ($P<0.05$ or $P<0.01$), and ADG of Groups – was significantly or extremely significantly higher than that of the control group ($P<0.05$ or $P<0.01$). No significant differences were observed in ADFI and F/G between Groups – and the control group ($P>0.05$). No significant differences were found in FBW, ADG, ADFI, or F/G between Groups and ($P>0.05$), indicating that increasing vitamin B12 supplementation from 0.020 to 0.025 mg/kg had no significant effect on these parameters ($P>0.05$).

Quadratic curve fitting was performed between FBW (YFBW), ADG (YADG) and vitamin B12 supplemental level (X), establishing the following regression equations:

$YFBW = 3,887.357 + 29,202.381X - 1,160,476.19 \times 10\ X^2$ ($R^2 = 0.544$, $PQ < 0.001$)
$YADG = 33.729 + 315.57X - 11,761.255X^2$ ($R^2 = 0.443$, $PQ < 0.001$)

Based on these regression equations, the maximum FBW was achieved at a dietary vitamin B12 supplemental level of 0.0126 mg/kg, and the maximum ADG was achieved at 0.0134 mg/kg. From a comprehensive benefit perspective, the recommended supplemental level of vitamin B12 in diets for 5–15-week-old Wulong geese is 0.0126–0.0134 mg/kg.

Table 2 Effects of dietary vitamin B12 supplemental level on growth performance of Wulong geese

2.2 Effects of Dietary Vitamin B12 Supplemental Level on Jejunal Development of Wulong Geese

2.2.1 Effects on Jejunal VH, CD, and V/C As shown in Table 3 , jejunal VH increased initially and then decreased with increasing vitamin B12 supplemental level. VH of Groups and was extremely significantly higher than that of the control group ($P<0.01$), while VH of Groups , , and was significantly higher than that of the control group ($P<0.05$), with Group showing the highest VH. Jejunal CD showed no obvious change pattern, but CD of all experimental groups was lower than that of the control group, with Group showing significant difference ($P<0.05$) and Groups – showing extremely significant differences ($P<0.01$) compared to the control group, and Group showing the lowest CD. The V/C ratio showed no obvious change pattern, but V/C of all experimental groups was significantly or extremely significantly higher than that of the control group ($P<0.05$ or $P<0.01$), with Group showing the highest V/C ratio. These results indicate that appropriate dietary vitamin B12 supplementation can promote jejunal development in Wulong geese.

Table 3 Effects of dietary vitamin B12 supplemental level on VH, CD, and V/C in jejunum of Wulong geese

2.2.2 Jejunal Villus Histological Sections As shown in Figure 1 [Figure 1: see original paper], the jejunal VH of the control group was lower than that of all experimental groups, with deeper CD. Groups and showed high and relatively orderly arranged VH with shallow CD, indicating that appropriate dietary vitamin B12 supplementation had positive effects on jejunal villus morphology in Wulong geese.

Figure 1 Jejunal tissue sections of geese (100×)

2.3 Effects of Dietary Vitamin B12 Supplemental Level on Cecal Microflora Structure of Wulong Geese

2.3.1 Alpha Diversity Index Analysis A total of 960,455 paired reads were obtained from sample sequencing in this experiment. After merging and filtering the paired-end reads, 640,817 clean tags were generated, with an average of 80,038 clean tags per sample. Alpha diversity reflects species richness and diversity in samples. Based on the alpha diversity index statistics for each group (Table 4), Group showed the highest OTU, ACE index, and Chao1 index, indicating that cecal microflora species richness in Group was higher than in the other five groups. Group showed the smallest Shannon index and highest Simpson index, indicating that Group had the highest community diversity and most uniform individual distribution. The coverage index reflecting OTU sequencing depth was greater than 0.996 in all groups, showing a high probability of species detection in the samples. These results demonstrate that dietary supplementation with different levels of vitamin B12 can alter cecal microflora species richness and diversity in Wulong geese.

Table 4 Alpha diversity indices

2.3.2 Species Accumulation Curve Analysis Species accumulation curves reflect the relationship between sample number and annotated species number. As shown in Figure 2 [Figure 2: see original paper], the accumulation curve composed of red boxes rose sharply and then flattened, indicating that species number increased with sample number but did not continue to increase when samples reached a certain quantity. The common quantity curve composed of green boxes declined sharply, indicating that newly discovered common species among samples gradually decreased until the curve flattened and common species became saturated. These results demonstrate that sampling in this experiment was sufficient for data analysis.

Figure 2 Cumulative curve chart of species at genus level

2.3.3 Principal Coordinates Analysis (PCoA) Figure 3 [Figure 3: see original paper] shows the PCoA plot based on four distance matrices obtained from beta diversity analysis using R language tools. Samples with closer coordinates on the plot have greater similarity. Group and Group had the closest coordinate distance, indicating high similarity between them, while Group and Group had the farthest coordinate distance, indicating low similarity. These results demonstrate that dietary vitamin B12 supplemental level affected cecal microflora similarity in Wulong geese.

Z1: Group ; Z2: Group ; Z3: Group ; Z4: Group ; Z5: Group ; Z6: Group . The same as below.

Figure 3 PCoA chart

2.5 Dominant Microflora Analysis

Figure 4 [Figure 4: see original paper] shows the effects of dietary vitamin B12 supplemental level on relative abundances of cecal microflora at the class level. At 97% similarity at the class level, relative abundances of cecal microbial classes were calculated, with Bacteroidia, Clostridia, and Deltaproteobacteria being the dominant classes. Inter-group comparisons of the top 5 dominant classes in Wulong goose cecum are shown in Table 5 . Group showed the highest relative abundance of Bacteroidia at 53.40%, significantly or extremely significantly higher than other groups ($P<0.05$ or $P<0.01$). Group showed the highest relative abundance of Clostridia at 27.60%, significantly or extremely significantly higher than other groups ($P<0.05$ or $P<0.01$). Group showed the highest relative abundance of Deltaproteobacteria at 19.80%, significantly or extremely significantly higher than other groups ($P<0.05$ or $P<0.01$). Melainabacteria showed the highest relative abundance in Group at 6.14%, and Deferribacteres showed the highest relative abundance in Group at 12.45%. These results demonstrate that dietary supplementation with different levels of vitamin B12 altered relative abundances of cecal microflora at the class level, with obvious changes in Clostridia, Bacteroidia, and Deltaproteobacteria, indicating that vitamin B12 can change the relative abundance of dominant cecal microflora at the class level.

Figure 4 Distribution histogram of species at class level

Table 5 Relative abundances of dominant microorganisms at class level of each group (%)

Figure 5 [Figure 5: see original paper] shows the effects of dietary vitamin B12 supplemental level on relative abundances of cecal microflora at the family level. At the family level, Bacteroidaceae, Ruminococcaceae, and Desulfovibrionaceae showed relatively high abundances. Inter-group comparisons of the top 5 dominant families in Wulong goose cecum are shown in Table 6 . Group showed a Bacteroidaceae relative abundance of 31.12%, with no significant differences from Groups , , and (P>0.05) but significantly or extremely significantly higher than other groups (P<0.05 or P<0.01). Group showed the highest Ruminococcaceae relative abundance at 17.60%, significantly higher than Group (P<0.05). Group showed the highest Desulfovibrionaceae relative abundance at 19.78%, significantly or extremely significantly higher than other groups (P<0.05 or P<0.01). Group showed the highest Prevotellaceae relative abundance at 7.43%, significantly higher than Groups and (P<0.05). Group showed the highest relative abundance of unknown bacteria at 7.33%, with no significant difference from Group (P>0.05) but significantly or extremely significantly higher than other groups (P<0.05 or P<0.01). These results demonstrate that dietary supplementation with different levels of vitamin B12 altered relative abundances of cecal microflora at the family level, with obvious changes in Bacteroidaceae, Ruminococcaceae, and Desulfovibrionaceae, indicating that vitamin B12 can change the relative abundance of dominant cecal microflora at the family level.

Figure 5 Distribution histogram of species at family level

Table 6 Relative abundances of dominant microorganisms at family level of each group (%)

Figure 6 [Figure 6: see original paper] shows the effects of dietary vitamin B12 supplemental level on relative abundances of cecal microflora at the genus level. At the genus level, Bacteroides, unknown bacteria, and Desulfovibrio showed relatively high abundances. Inter-group comparisons of the top 5 dominant genera in Wulong goose cecum are shown in Table 7 . Group showed the highest Bacteroides relative abundance at 31.12%, significantly higher than Group (P<0.05) and extremely significantly higher than Group (P<0.01). Group showed the highest relative abundance of unknown bacteria at 27.49%, significantly or extremely significantly higher than other groups (P<0.05 or P<0.01). Group showed the highest Desulfovibrio relative abundance at 19.75%, significantly or extremely significantly higher than other groups (P<0.05 or P<0.01). Group showed the highest Alistipes relative abundance at 4.70%, significantly higher than Groups and (P<0.05). Group showed the highest Mucispirillum relative abundance at 12.45%, significantly higher than other groups (P<0.05). These results demonstrate that dietary supplementation with different levels of vitamin B12 altered relative abundances of cecal microflora at the genus level, with obvious changes in Bacteroides and Desulfovibrio, indicating that vitamin B12 can change the relative abundance of dominant cecal microflora at the genus level.

Figure 6 Distribution histogram of species at genus level

Table 7 Relative abundances of dominant microorganisms at genus level of each group (%)

3 Discussion

3.1 Effects of Dietary Vitamin B12 Supplemental Level on Growth Performance of Geese

Early studies have shown that vitamin B12 deficiency reduces feed intake, body weight gain, and feed conversion efficiency in broiler chickens [7]. High vitamin B12 supplemental levels decreased feed intake in Peking ducks during summer but had no significant effect during winter [8]. Vitamin B12 exists only in animal feed and is easily deficient in production. Normal vitamin B12 content in animals does not necessarily indicate adequate vitamin B12 status [9], and chickens cannot synthesize vitamin B12. Dietary vitamin B12 mainly originates from animal feed, while plant feed contains virtually no vitamin B12. Animals are more prone to vitamin B12 deficiency when plant-based feed predominates [10]. Vitamin B12 intake is positively correlated with serum vitamin B12 content [11-12]. Aged livestock and poultry are prone to vitamin B12 deficiency, with approximately 60% of cases resulting from malabsorption of food-bound cobalamin due to gastrointestinal causes [13]. Huang et al. [5] reported that dietary supplementation with 0.008 mg/kg vitamin B12 significantly increased body weight in broiler chicks under both high- and low-energy conditions. Appropriate dietary vitamin B12 supplementation can improve feed conversion efficiency and promote livestock growth, while deficiency leads to loss of appetite, growth stagnation, simple anemia, and severe neurological symptoms [14]. The present experiment indicated that maximum FBW and ADG were achieved at dietary vitamin B12 supplemental levels of 0.0126 and 0.0134 mg/kg, respectively.

3.2 Effects of Dietary Vitamin B12 Supplemental Level on Intestinal Development of Geese

The integrity of intestinal morphological structure is a prerequisite for normal functional performance, mainly including changes in intestinal villus structure, mucosal layer, and muscle layer thickness. Animals primarily rely on circular folds on the intestinal wall, intestinal villi, and columnar epithelial cells on villi to absorb and digest nutrients [15]. Studies have found that VH affects the absorption area of the small intestine for nutrients, and higher VH is more conducive to nutrient absorption [16]. The present experiment showed that jejunal VH initially increased and then decreased with increasing vitamin B12 supplemental level, with VH in all vitamin B12-supplemented groups higher than that in the control group, CD lower than that in the control group, and V/C ratio higher than that in the control group. These results indicate that appropriate dietary vitamin B12 supplementation is beneficial for promoting intestinal development in geese. The main reasons are: 1) Vitamin B12, as a cofactor of methyltransferase, participates in the synthesis of methionine, thymine, etc. For example, it converts methyltetrahydrofolate to tetrahydrofolate while transferring the methyl group to methyl receptors (such as homocysteine), converting them into methyl derivatives (such as methionine). 2) Vitamin B12 can activate amino acids and promote nucleic acid biosynthesis, thereby promoting protein synthesis. 3) Vitamin B12 can increase folic acid utilization and promote carbohydrate, fat, and protein metabolism. 4) Vitamin B12 also promotes fatty acid metabolism, enabling appropriate utilization of fats and carbohydrates by the body.

3.3 Effects of Dietary Vitamin B12 Supplemental Level on Cecal Microflora Structure of Geese

The microecology in animals is an extremely complex system, and its included microflora is closely related to host immunity, nutrition, and chronic infection [17]. The intestinal microecological system contains the most abundant microflora in the animal body, and this system has a profound impact on maintaining intestinal microecological balance.

PCoA analysis in this experiment showed that Groups and had the closest coordinate distance and greatest species similarity, while Groups and had the farthest coordinate distance and lowest species similarity. At 97% similarity at the class level, Bacteroidia, Clostridia, and Deltaproteobacteria were identified as the dominant classes in goose cecum. This experiment analyzed intergroup distribution differences in relative abundances of dominant microflora from three aspects: class, family, and genus. At the class level, Group showed the highest relative abundance of Bacteroidia, Group showed the highest relative abundance of Clostridia, and Group showed the highest relative abundance of Deltaproteobacteria. At the family level, Group showed the highest relative abundances of Bacteroidaceae and Desulfovibrionaceae, while Group showed the highest relative abundance of Ruminococcaceae. At the genus level, Group showed the highest relative abundances of Bacteroides and Desulfovibrio.

Regarding the probiotic effects of Bacteroides, numerous studies have been conducted by domestic and foreign scholars from different perspectives. Bacteroides can promote host polysaccharide degradation to improve nutrient utilization efficiency [18], accelerate intestinal mucosal angiogenesis [19], and promote immune system development, playing pivotal roles in improving host immunity [20] and maintaining intestinal microecological balance [21-22], particularly in polysaccharide utilization. Compared with the control group, all vitamin B12-supplemented groups showed changes in cecal microflora richness, with Bacteroides showing the most obvious changes. This indicates that dietary supplementation with different levels of vitamin B12 altered cecal microflora structure in 15-week-old geese, increased the dominance of intestinal Bacteroides, thereby improving the body' s ability to degrade polysaccharides and increasing the utilization efficiency of protein, carbohydrates, and fats to enhance growth performance. Additionally, since vitamin B12 accelerates intestinal mucosal angiogenesis and promotes intestinal development, it expands the intestinal nutrient absorption area. The author believes this is the main reason why appropriate dietary vitamin B12 supplementation can improve growth performance in geese.

There are various types of intestinal Bacteroides, some of which have both beneficial and harmful characteristics. Only when the intestinal microecology is in a balanced state can the body develop better and maintain health. The results of this experiment indicate that dietary supplementation with different levels of vitamin B12 altered cecal microflora structure in geese. The reason is that different supplemental levels of vitamin B12 had varying effects on intestinal development and nutrient digestion/absorption, leading to changes in the reproduction environment for different microorganisms and consequently altering microflora structure.

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4 Conclusion

1. Appropriate dietary vitamin B12 supplementation can increase ADG. Too low vitamin B12 supplemental level reduces growth performance, while too high a level has no biological significance.
2. When dietary vitamin B12 supplemental level is 0.015 mg/kg, geese show the highest jejunal VH and lowest CD.
3. Dietary supplementation with different levels of vitamin B12 can alter relative abundances of dominant cecal microflora at class, family, and genus levels. At the class level, Clostridia, Bacteroidia, and Deltaproteobacteria showed obvious changes; at the family level, Clostridiales, Ruminococcaceae, and Desulfovibrionaceae showed obvious changes; at the genus level, Bacteroides and Desulfovibrio showed obvious changes.
4. Based on FBW and ADG as evaluation indices, the recommended supplemental level of vitamin B12 in diets for 5–15-week-old geese is 0.0126–0.0134 mg/kg.

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Note: Figure translations are in progress. See original paper for figures.