Effects of Yeast Polysaccharides on Gastrointestinal Development and Digestive Enzyme Activity in Suckling Calves: Postprint

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Abstract

This experiment aimed to investigate the effects of dietary supplementation with different doses of yeast polysaccharides on gastrointestinal development and digestive enzyme activities in suckling calves. Fifty-six healthy Chinese Holstein calves with similar birth weights were selected and randomly divided into 4 groups with 14 calves per group. Group (control group) was fed a basal diet, while groups , , and were fed the basal diet supplemented with 1, 2, and 3 g/(head · d) of yeast polysaccharides, respectively. The experimental period lasted 60 days. The results showed: 1) The activities of rumen amylase, pepsin, lipase, and carboxymethyl cellulase in calves of groups , , and were higher than those in group , with group being significantly higher than group ($P<0.05$); the activities of duodenal amylase, trypsin, lipase, and carboxymethyl cellulase in calves of group were significantly higher than those in group ($P<0.05$); the activities of jejunal amylase ($P<0.05$) and trypsin ($P<0.01$) in calves of group were significantly or extremely significantly higher than those in group , and the activities of jejunal lipase and carboxymethyl cellulase in groups , , and were significantly higher than those in group ($P<0.05$). 2) The papilla length, width, and mucosal thickness in the rumen of calves in group were significantly higher than those in group ($P<0.05$); the villus height in the duodenum and mid-jejunum was significantly higher than that in group ($P<0.05$), the crypt depth was significantly lower than that in group ($P<0.05$), and the villus height/crypt depth ratio (V/C) was significantly higher than that in the other groups ($P<0.05$). These results indicate that supplementation with yeast polysaccharides can promote gastrointestinal morphological development and improve gastrointestinal digestive enzyme activities in calves. Under the conditions of this experiment, the appropriate supplementation level of yeast polysaccharides in the diet of suckling calves was 2 g/(head · d).

Full Text

Effects of Yeast Polysaccharide on Gastrointestinal Development and Digestive Enzyme Activity of Sucking Calves

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Abstract

This experiment was conducted to investigate the effects of dietary supplementation with different doses of yeast polysaccharide on gastrointestinal development and digestive enzyme activity in suckling calves. Fifty-six healthy Chinese Holstein calves with similar birth weights were selected and randomly allocated into four groups of 14 calves each. Group I (control) received a basal diet, while groups II, III, and IV received the basal diet supplemented with 1, 2, and 3 g/(head · d) of yeast polysaccharide, respectively. The experimental period lasted 60 days. The results showed that: (1) Calves in groups II, III, and IV exhibited higher activities of ruminal amylase, pepsin, lipase, and carboxymethylcellulase compared to group I, with group III being significantly higher than group I ($P<0.05$). Group III also showed significantly higher activities of duodenal amylase, trypsin, lipase, and carboxymethylcellulase compared to group I ($P<0.05$). In the jejunum, group III demonstrated significantly higher amylase ($P<0.05$) and trypsin ($P<0.01$) activities than group I, while groups II, III, and IV all showed significantly higher lipase and carboxymethylcellulase activities ($P<0.05$). (2) Group III exhibited significantly greater ruminal papilla length, width, and mucosal thickness compared to group I ($P<0.05$). The duodenal and mid-jejunal villus heights in group III were significantly higher than those in group I ($P<0.05$), while crypt depth was significantly lower ($P<0.05$), resulting in a villus height/crypt depth (V/C) ratio that was significantly higher than in all other groups ($P<0.05$).

In conclusion, yeast polysaccharide supplementation promotes gastrointestinal morphological development and enhances digestive enzyme activity in calves. Under the conditions of this experiment, the optimal supplementation level of yeast polysaccharide in the diet of suckling calves is 2 g/(head · d).

Key words: yeast polysaccharide; suckling calves; gastrointestinal development; digestive enzyme activity

Introduction

The gastrointestinal tract is the primary site for nutrient digestion and absorption in animals. The underdeveloped digestive function of the gastrointestinal tract in newborn calves directly affects their nutrient utilization and overall growth. Yeast polysaccharide (YPS) is a water-soluble polysaccharide extracted from yeast cell walls, comprising -glucan and mannan oligosaccharides (MOS) [1]. It has been shown to promote early gastrointestinal development, enhance digestive capacity, and improve nutrient absorption in animals. Therefore, investigating the effects and mechanisms of yeast polysaccharide on gastrointestinal development in suckling calves is of significant importance for promoting calf growth.

Previous studies have reported that yeast polysaccharide, as a green feed additive, can improve animal growth performance [2-5]. In concurrent research from our laboratory, we found that calves supplemented with 1, 2, and 3 g/(head · d) of yeast polysaccharide exhibited higher average daily gain (ADG) and dry matter intake (DMI) than the control group, with the 2 g/(head · d) group showing the highest ADG and DMI. This may be attributed to the combined effects of mannan oligosaccharides and -glucan in yeast polysaccharide, which increase feeding frequency and promote intestinal digestion and absorption capacity, thereby enhancing feed intake and daily gain [6]. Supplementation with 75 mg/kg yeast -glucan in calf diets has been shown to significantly improve the digestibility of dry matter (DM), crude protein (CP), ether extract (EE), and phosphorus (P) [7]. Similarly, dietary supplementation with yeast cell wall in beef cattle improved the apparent digestibility of acid detergent fiber (ADF) and total phosphorus (TP) [4]. Our concurrent research also demonstrated that supplementation with 1, 2, and 3 g/(head · d) yeast polysaccharide significantly improved the apparent digestibility of DM, CP, EE, neutral detergent fiber (NDF), and ADF in calves, indicating that yeast polysaccharide promotes nutrient digestion and metabolism in suckling calves [6].

While numerous studies have reported that yeast polysaccharide promotes intestinal development and increases digestive enzyme secretion in chickens [8], pigs [9], and fish [10], research on its effects on gastrointestinal development and enzyme activity in suckling calves remains limited. Therefore, this experiment aimed to investigate the effects of different dietary doses of yeast polysaccharide on gastrointestinal development and digestive enzyme activity in suckling calves, providing a theoretical basis for its application in calf feeding.

Materials and Methods

1.1 Experimental Materials and Design Yeast polysaccharide (Fubang brand) was produced by Angel Yeast Co., Ltd. (Hubei, China). The product composition was: 20.0% -glucan 30.0%, 20.0% -mannan peptide 30.0%, peptides and protein 30.0%, chitin 2.0%, with purity >50%.

Fifty-six healthy Chinese Holstein calves with similar birth weights [(45.00±5.29) kg] were selected and randomly divided into four groups of 14 calves each, with no significant differences in average birth weight among groups (P>0.05). Yeast polysaccharide was supplemented at 0 (Group I), 1 (Group II), 2 (Group III), and 3 g/(head · d) (Group IV). The experimental period lasted 60 days.

1.2 Feeding Management All calves received 4 L of colostrum within 1 h after birth and were then housed in individual calf hutches with ad libitum access to water. During the experiment, each calf was fed 4 kg of milk daily. From days 1-6, yeast polysaccharide was added to the milk; from days 7-60, it was added to the calf starter. Starter feed was introduced on day 7, and alfalfa hay was offered ad libitum from day 45. The composition and nutrient levels of the starter feed are shown in Table 1 , while the nutrient levels of fresh milk and alfalfa are presented in Table 2 .

1.3 Sample Collection 1.3.1 Collection of Rumen and Small Intestinal Chyme Samples

Four calves from each group with body weights close to the group average and in good health were selected and euthanized by jugular exsanguination at 60 days of age. The abdomen was immediately opened, and the rumen and small intestine were removed and rapidly immersed in physiological saline. The junction between the duodenum and abomasum was ligated and severed.

Rumen chyme samples were first collected in 10 mL centrifuge tubes and immediately placed in liquid nitrogen for subsequent enzyme activity analysis. After removing mesenteric and external fat, the small intestine was placed naturally in a porcelain tray cooled with ice. Chyme samples (10 g) were collected from the duodenum and mid-jejunum into EP tubes, rapidly frozen in liquid nitrogen, and stored for enzyme activity determination.

1.3.2 Collection of Gastrointestinal Tissue Samples

Tissue samples (10 cm) were collected from the anterior dorsal blind sac of the rumen, duodenum, and mid-jejunum. After flushing residual contents with physiological saline and ligation, samples were immediately immersed in Bouin' s fixative for histological sectioning.

1.4 Laboratory Analyses 1.4.1 Determination of Enzyme Activity in Chyme

Activities of amylase, lipase, and carboxymethylcellulase in ruminal and intestinal chyme, as well as pepsin in ruminal chyme and trypsin in intestinal chyme, were determined using enzyme-linked immunosorbent assay (ELISA) kits (Zhongsheng Beikong Biological Co., Ltd., batch number: 201710) following the manufacturer' s instructions. A microplate reader (Beijing Pulang New Technology Co., Ltd., model: DNM-9602) was used for measurements.

1.4.2 Preparation and Measurement of Tissue Sections

Tissue sections were prepared using the paraffin sectioning method [12]. Observation and measurement were performed under a microscope (ToupCam) at 10×4 magnification [Toup View (×86)]. Image J software was used to measure ruminal papilla length, width, and mucosal thickness, as well as intestinal villus height, crypt depth, and mucosal thickness. For each sample, three nonconsecutive sections were examined, with three fields of view per section and 2-6 measurements taken per field.

1.5 Statistical Analysis Experimental data were analyzed using one-way ANOVA in SPSS 19.0 software, with Duncan' s multiple comparison test applied where appropriate. Results are expressed as mean ± standard deviation, with $P<0.05$ considered statistically significant and $P<0.01$ considered highly significant.

Results

2.1.1 Effects of Yeast Polysaccharide on Rumen Chyme Enzyme Activity in Suckling Calves As shown in Table 3 , ruminal amylase activity in groups II, III, and IV increased by 4.36% ($P>0.05$), 10.48% ($P<0.05$), and 1.88% ($P>0.05$) compared to group I, respectively. Ruminal pepsin activity in groups III and IV was significantly higher than in group I ($P<0.05$), showing increases of 20.04% and 38.00%, respectively. Ruminal lipase activity in groups III and IV increased by 20.12% and 34.75% ($P<0.05$) compared to group I. Ruminal carboxymethylcellulase activity in groups III and IV was significantly higher than in group I ($P<0.05$), with increases of 9.61% and 8.45%, respectively.

2.1.2 Effects of Yeast Polysaccharide on Duodenal Chyme Enzyme Activity in Suckling Calves As presented in Table 4 , duodenal amylase activity in groups III and IV increased by 37.98% and 23.95% ($P<0.05$) compared to group I. Duodenal trypsin activity in groups II and III increased by 26.17% and 42.77% ($P<0.01$) compared to group I. Duodenal lipase activity in groups II, III, and IV was significantly higher than in group I ($P<0.05$), with increases of 19.92%, 42.52%, and 69.33%, respectively. Duodenal carboxymethylcellulase activity in groups II, III, and IV increased by 9.47% ($P<0.05$), 26.43% ($P<0.01$), and 7.71% ($P<0.05$) compared to group I.

2.1.3 Effects of Yeast Polysaccharide on Mid-Jejunal Chyme Enzyme Activity in Suckling Calves As shown in Table 5 , mid-jejunal amylase activity in group III increased by 18.70% ($P<0.05$) compared to group I. Mid-jejunal trypsin activity in groups II, III, and IV increased by 2.17% ($P>0.05$), 7.14% ($P<0.01$), and 1.79% ($P>0.05$) compared to group I. Mid-jejunal lipase activity in groups II, III, and IV increased by 30.34%, 65.55%, and 47.57% ($P<0.05$) compared to group I. Mid-jejunal carboxymethylcellulase activity was higher in groups II, III, and IV than in group I, with group III showing a 16.88% increase ($P<0.05$).

2.2 Effects of Yeast Polysaccharide on Gastrointestinal Morphological Structure in Suckling Calves As shown in Table 6 and Figure 1 [Figure 1: see original paper], ruminal papilla length in groups II, III, and IV increased by 11.58% ($P<0.01$), 27.55% ($P<0.01$), and 15.86% ($P<0.01$) compared to group I. Papilla width increased by 17.72% ($P<0.05$), 37.23% ($P<0.05$), and 25.60% ($P<0.05$), respectively. Ruminal mucosal thickness increased by 8.08% ($P<0.05$), 30.93% ($P<0.01$), and 26.38% ($P<0.01$), respectively.

Duodenal villus height in groups II, III, and IV increased by 9.74% ($P<0.01$), 23.91% ($P<0.01$), and 14.61% ($P<0.01$) compared to group I. Crypt depth decreased by 7.06% ($P>0.05$), 19.42% ($P<0.05$), and 26.56% ($P<0.05$), respectively. The villus height/crypt depth (V/C) ratio was significantly higher than in group I ($P<0.05$), with group IV showing the highest value, though the difference between groups III and IV was not significant ($P>0.05$).

In the mid-jejunum, villus height in group III increased by 24.56% ($P<0.05$) compared to group I, while crypt depth decreased by 19.49% ($P<0.05$), resulting in a significantly higher V/C ratio ($P<0.05$).

Discussion

3.1 Effects of Yeast Polysaccharide on Gastrointestinal Chyme Enzyme Activity in Suckling Calves Changes in digestive enzyme activity directly reflect alterations in feeding performance, and the level of enzyme activity profoundly influences the degree of nutrient absorption and utilization. Sun Hongxin [7] reported that amylase activity was highest in the jejunum of lambs. Wang Baoshan [13] also demonstrated that amylase activity varied across different segments of the small intestine in Small-tailed Han sheep, with the highest activity observed in the jejunum, significantly higher than in the duodenum. Wu et al. [14] found that feeding konjac mannan oligosaccharide and mannan oligosaccharide to fish enhanced intestinal protease, amylase, and lipase activities. Xing Guanglin [15] observed that dietary mannan oligosaccharide supplementation in broilers increased duodenal digestive enzyme activity, with 0.1% mannan oligosaccharide significantly elevating duodenal amylase activity compared to the control group. Yang Min et al. [16] reported that dietary

supplementation with 0.20%-0.50% mannan oligosaccharide increased intestinal trypsin, amylase, and lipase activities in European eels, indicating that appropriate concentrations of mannan oligosaccharide can promote digestive enzyme secretion and growth. Tan Chonggui et al. [17] found that supplementing Litopenaeus vannamei diets with 0.2% -glucan and 0.4% mannan oligosaccharide significantly increased pepsin and hepatopancreatic lipase activities. Qin Zhibiao [18] reported that -glucan significantly affected pepsin, trypsin, amylase, lipase, and cellulase activities in Nile tilapia, effectively enhancing digestive enzyme activity and nutrient absorption while supporting normal growth. However, Gao Jin [19] found that yeast cell wall polysaccharide had no significant effect on intestinal trypsin and amylase activities in large yellow croaker larvae.

Carboxymethylcellulase can swell cellulose and carboxymethylcellulose, randomly cleaving cellulose polymers to produce cellodextrins, cellobiose, and glucose, playing a crucial role in cellulose digestion [20]. In the present study, dietary supplementation with 2 g/(head · d) yeast polysaccharide enhanced both ruminal and intestinal enzyme activities. Yeast polysaccharide promoted the proliferation of cellulolytic bacteria and cellulase production, improving the enzymatic hydrolysis of starch, fat, and protein, and stimulating gastrointestinal morphological development, thereby enhancing overall ruminal fermentation function.

3.2 Effects of Yeast Polysaccharide on Gastrointestinal Morphological Structure in Suckling Calves Ruminal function development results from stimulation by volatile fatty acids produced through fermentation. Lesmeister et al. [21] identified papilla height, width, and mucosal thickness as important indicators for evaluating ruminal development. Zhou Yi et al. [22] found that ruminal papilla length, width, and mucosal thickness increased with dietary yeast -glucan levels. Similar results were obtained in this study, where 2 g/(head · d) yeast polysaccharide significantly increased ruminal papilla length, width, and mucosal thickness, possibly due to increased volatile fatty acid production in the rumen, which promoted early establishment of ruminal function and accelerated ruminal development.

The small intestine plays a vital role in nutrient digestion and absorption during the pre-ruminant stage. Villus height, crypt depth, mucosal thickness, and the V/C ratio are key indicators of digestive and absorptive function [23]. Villus height correlates significantly with cell number, and greater villus height indicates more mature epithelial cells [24]. Crypt depth reflects cell maturation rate; deeper crypts indicate reduced nutrient absorption capacity, whereas shallower crypts suggest enhanced digestive function [25]. The V/C ratio comprehensively reflects small intestinal function status, with higher ratios indicating improved mucosal condition and enhanced digestive capacity [26].

Li Yuxin et al. [27] found that dietary supplementation with *Pichia pastoris* mannan oligosaccharide in piglets significantly increased jejunal villus height, decreased crypt depth, and elevated the V/C ratio. Huang Junwen et al. [28]

reported that mannan oligosaccharide significantly increased small intestinal villus height and V/C ratio in piglets. De Los Santos et al. [29] observed that dietary yeast-derived mannan oligosaccharide supplementation in turkey poults resulted in consistently higher villus heights and crypt depths in the duodenum and jejunum compared to the control group, indicating accelerated gastrointestinal maturation. Wen Ruozhu [30] found that dietary mannan oligosaccharide significantly increased duodenal V/C ratio in broilers, with a similar trend in jejunal V/C ratio. Ferket et al. [31] reported that mannan oligosaccharide supplementation did not increase small intestinal villus height in broilers but significantly reduced crypt depth and increased V/C ratio. Muthusamy et al. [32] found that yeast cell wall supplementation significantly increased jejunal villus height in broilers. Additionally, dietary mannan oligosaccharide supplementation in half-smooth tongue sole larvae resulted in significantly higher microvillus height and fold height compared to the control group [33].

In this study, calves receiving 2 g/(head · d) yeast polysaccharide showed higher duodenal and mid-jejunal villus heights and V/C ratios than other groups, suggesting that increased villus surface area promoted epithelial cell maturation, enhanced nutrient absorption, and increased digestive enzyme secretion at villus tips, thereby improving digestibility [34]. The reduced crypt depth further indicates that yeast polysaccharide enhanced small intestinal digestive and absorptive capacity.

Conclusion

Dietary supplementation with yeast polysaccharide promotes gastrointestinal morphological development and enhances digestive enzyme activity in calves. Under the conditions of this experiment, the optimal supplementation level of yeast polysaccharide for suckling calves is 2 g/(head · d).

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