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Research note: Effects of *Laminaria japonica* polysaccharides on serum digestive enzyme activity in weaned piglets

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ABSTRACT

The study investigated how varying levels of *Laminaria japonica* polysaccharides (LJP) in weaned piglet feed influenced the activity of digestive enzymes in the serum. 120 healthy piglets of at 21 days were selected for the experiment and randomized to four treatment groups, each comprising five replicates of six piglets. In the experiment, LJP was added at 0 (control), 100, 200 and 400 milligrams per kilogram (mg/kg) to the basal feed of four treatment groups for 21-day. A significant decrease in serum protease activity was observed in the 200 mg/kg LJP group when compared to the untreated group ($P < 0.05$). Adding LJP to the feed had a trend of increased serum lipase activity with increasing LJP concentrations, but these increases were not statistically significant ($P > 0.05$). Furthermore, dietary supplementation with 400 mg/kg LJP to the feed was observed to result in the highest serum amylase activity. However, the observed changes were not considered to be of statistical significance ($P > 0.05$). In conclusion, LJP has a positive effect on nutrient metabolism in serum.
Keywords: Digestive enzymes, Lminaria japonica polysaccharides, Weaned piglets

INTRODUCTION

Piglets may suffer from weaning stress following sudden maternal separation. Various external stressors, including changes in feed, physical environment, and social environment exacerbate this stress. These factors have the potential to lead to a reduction in feed intake, feed utilization rate, and digestive enzyme activity in the piglets' digestive tracts. Consequently, nutrient metabolism ability is affected, growth rate is reduced, and in severe cases, it can result in death (Tang *et al.*, 2022). Generally, *Laminaria japonica* polysaccharides (LJP), principally comprising alginates, fucoidans and laminarins, are a category of complex sulfated polysaccharides mainly derived from kelp. LJP exhibits a range of physiological functions including anti-inflammatory, antioxidant (Lin *et al.*, 2020), and immune-enhancing (Li, X. Y. *et al.*, 2020). Research has demonstrated that kelp extract strengthened intestinal barrier function, shield the gut, and improved animals' ability to digest nutrients (Ai *et al.*, 2022). Wang *et al.* (2023) demonstrated that LJP (which is primarily composed of fucoidan) markedly increased the activity of amylase in the faeces of piglets, and had the potential to increase lipase activity in faeces, which was beneficial for enhancing the metabolism of piglets. Liu *et al.* (2024) also demonstrated through a series of experiments that both kelp powder and fucoidan could help to maintain the balance of intestinal flora and anti-inflammatory effect in the large yellow croakers, which could effectively enhance its digestive ability. In conclusion, LJP has a beneficial impact on the healthy growth of animals and can enhance their digestive capabilities. The majority of studies examining the impact of LJP on animal digestive function have concentrated on the intestinal tract. Conversely, there is a paucity of research that has investigated the impacts of LJP on nutrient metabolism in the serum of weaned piglets. Therefore, the study examined the influence of dietary supplementation with LJP on the serum activity of digestive enzymes in piglets to provide a scientific basis for alleviating the stress response in weaned piglets.

MATERIALS AND METHODS

Piglets, Diets, Management, and Experimental Groups

The experiment was carried out at the standardized commercial swine production facility Shicheng Shanxia Pig Farm (Ganzhou, Jiangxi, China). For the study, 120 21-day-old Berkshire × Licha Black crossbred piglets with a mean body weight of 6.13 ± 0.21 kg were chosen at random. The piglets were randomized to one of four treatment groups, each group consisting of five replicates of six piglets. The piglets were fed

Table 1. Basal feed ingredients and calculated nutrient composition (as fed, %)

Items	Composition
Ingredients	
Expanded corn	57.50
Expanded soybean	12.00
Soybean meal	8.00
Whey powder	8.00
Lactose	8.00
Fish meal	3.00
Limestone	0.70
Calcium hydrogen phosphate	1.50
Salt	0.30
Premix[1]	1.00
Nutrient content, %	
ME/ ($MJ \cdot kg^{-1}$)	14.30
Crude protein	20.50
Calcium	0.94
Phosphorus	0.75
Lysine	1.45
Methionine	0.45

[1] The premix provides the following per kilogram of feed: VA l5000 U, VD_3 2000 U, VE 30.00 IU, VK 3.75 mg, VI mg, VB_2 7.00mg, VB_6 2.4 mg, VB_{12} 0.03 mg, nicotinic acid 40.00 mg, calcium pantothenate 25.00 mg, Folic acid 2: mg, biotin 0.25 mg, cholinechloride 600 mg, Cu 20 mg, Fe 120 mg, Zn l60 mg, Mn 100 mg, I 0.54 mg, Se 0.27 mg

an unsupplemented control feed and three experimental feeds supplemented with LJP at 100, 200, and 400 mg/kg. Feed and water were provided to piglets without restriction. The contents and nutritional values of the basal feeds during the period of 21 days under standard feeding management are shown in Table 1.

Sample Collection

Upon the conclusion of the trial, one piglet was chosen at random for each replicate (n=20) and a blood sample was taken via the piglet's jugular vein into a heparinised tube. The collected blood was kept stationary for 2 h, centrifuged at 3000 r/min for 10 min to isolate serum.

Determination of Digestive Enzyme Activities in Serum

The activities of the digestive enzymes in the serum were quantified with commercially available enzyme test kits as per the instructions supplied by the manufacturers. The serum protease activity kit was provided by Shanghai Yuanxin Biotechnology Co., Ltd. and the total protease activity was determined by enzyme-linked immunosorbent assay using a double-antibody one-step sandwich assay**.** Commercial kits from the NanJing JianCheng Bioengineering Institute were utilized to quantify serum lipase content (turbidimetric method) and α-amylase activity (iodine-starch colorimetry)**.**

Statistical Analysis

IBM SPSS Statistics 27.0 was employed for statistical analyses. One-way analysis of variance (ANOVA) was performed on the dataset, with significant differences further examined by Tukey's honestly significant difference (HSD) post-hoc test ($\alpha = 0.05$). Additionally, the linear and quadratic effects between means were examined using orthogonal contrast analysis to assess the impact of LJP on piglets.

RESULTS AND DISCUSSION

Table 2 summarizes the effects of dietary LJP supplementation on serum digestive enzyme activities in weaned piglets. Protease activity was significantly reduced ($P < 0.05$) when 200 mg/kg LJP was added to the feed compared with this control group. Dietary LJP supplementation did not markedly change serum lipase activity compared to the control group, but serum lipase activity tended to increase with increasing LJP concentration ($P > 0.05$). The 400 mg/kg LJP group exhibited elevated amylase activity relative to controls, though this difference was not statistically significant (P > 0.05).

Proteases, lipases, and amylases are mainly secreted by the pancreas into the intestines, with a small amount actively transported into the bloodstream. In pathological conditions, these enzymes leak into the bloodstream in large quantities due to tissue damage. Protease facilitates polypeptide degradation into amino acids, reflecting protein metabolic homeostasis and nutritional status in vivo. Protease is responsible for the hydrolysis of protein polypeptides into amino acid fragments, which reflect protein metabolic homeostasis and nutritional status in vivo. In this study, the addition of 100~400 mg/kg of LJP to the feed was found to reduce the serum protease activity of piglets to varying degrees. The serum protease activity was the lowest when LJP was added at 200 mg/kg, indicating that the incorporation of LJP into the feed could decrease the serum protease activity in piglets. Abdel-Mawla *et al.* (2023) found that the addition of LJP to the feed of Thinlip Grey Mullet resulted in significantly higher protease activity in the intestines of Mullets than in that of the zero-addition group after 60 d. Furthermore, a substantial body of research has corroborated the notion that moderate intake of plant polysaccharides can augment the levels of intestinal proteases, thereby facilitating the digestion and absorption of proteins in animal feeds, and maintaining the integrity of the intestinal barrier (Feng *et al.*, 2023; Liyanage *et al.*, 2023). Therefore, one of the factors contributing to the decline in serum protease activity may be that LJP, by enhancing the catalytic function of intestinal digestive enzymes, facilitates a more comprehensive breakdown of macromolecules and mitigates the irritation caused by undigested substances in the intestine. Consequently, this enhances the repair of tight junctions and improves the mechanical barrier function of the animal's intestinal tract, thereby restricting the entry of digestive enzymes into the bloodstream (Zou *et al.*, 2021). Zhou (2022) found that canine serum protease activity was markedly reduced after adding Seaweed polysaccharides to the feed of dogs fed for one week, and this finding aligns well with our study. Rattigan *et al.* (2020) and Wang *et al.* (2019) confirmed LJP's essential contribution to maintaining intestinal health, which in turn promotes more proteins to be thoroughly digested and absorbed into amino acids in the intestines, with only a minute proportion of small peptides being absorbed into the bloodstream. Fu *et al.* (2023) also found that LJP improved the protein metabolism ability of weaned piglets. Consequently, a potential underlying factor contributing to the observed decline in serum protease activity in piglets is the capacity of LJP to enhance the intestinal protein digestion capacity of the piglet. This enhanced digestion leads to a more complete breakdown of proteins into amino acids within the intestinal tract. Thus, this results in a reduction in the entry of small peptides into the bloodstream, thereby decreasing the demand for high levels of protease in the bloodstream.

Lipases are water-soluble enzymes with a variety of catalytic capabilities that hydrolyze water-insoluble lipid molecules and play a major role in lipid digestion. In this experiment, LJP had a tendency to increase serum digestive enzyme activity, but the difference was not statistically significant compared to the control. Bile acids are essential for fat and cholesterol digestion and absorption and are able to enhance lipid metabolism by increasing the contact area between lipase and substrate, maintaining TG homeostasis, and promoting lipid metabolism-related gene expression (Li *et al.*, 2023). Sulfated polysaccharides such as LJP can bind bile acids and interfere with bile acid function through various actions such as electrostatic adsorption and hydrophobic wrapping (Hofmann *et al.*, 2008 and Yuan *et al.*, 2020). Li, N. *et al.* (2020) and He *et al.* (2023) assessed the effects of polysaccharides extracted from seaweeds on mice on a high-fat feed, and both found that the polysaccharides ameliorated intestinal damage, reduced fat deposition, and could have hypolipidemic and anti-obesity effects. The addition of polysaccharides derived from the Fortunella margarita (Lour.) Swingle to the feed has been demonstrated to significantly enhance serum lipase activity in rats with hyperlipidaemia, a finding that aligns with the outcomes of present research (Zeng *et al.*, 2019). LJP may elevate serum digestive enzyme levels by interfering with the emulsification and digestion of fats in the intestines through binding to bile acids. This mechanism triggers a compensatory response in the body, resulting in increased levels of pancreatic lipase secretion and elevated serum lipase activity.

Amylase, a principal enzyme involved in the digestion of carbohydrates in mammals, is mainly synthesized and secreted by pancreatic alveolar cells and salivary gland cells, and a small amount of amylase is actively secreted into

Table 2. Effect of dietary LJP on digestive enzyme activity in the serum of weaned piglets

Items	LJP, mg/kg				SEM	P-value		
	0	100	200	400		ANOVA	Linear[1]	Quadratic[1]
Total protease activity,U/L	284[a]	276[a]	257[b]	269[ab]	4.61	0.006	0.028	0.007
Lipase activity,U/L	41.30	53.21	54.18	57.98	7.17	0.461	0.175	0.485
Amylase activity,U/L	10.92	8.61	10.32	11.96	1.35	0.418	0.357	0.287

[a,b] Means listed in the same row with different superscripts are significantly different ($p \leq 0.05$).
[1] Utilization of orthogonal polynomial contrast was employed to ascertain the influence of different dietary LJP concentrations.

the bloodstream through the basement membranes of the alveolar cells or passively infiltrated into the bloodstream due to tissue damage. After polysaccharides enter the intestine, the intestinal flora degrade them into monosaccharides or oligosaccharides, which are then further degraded and fermented to produce short-chain fatty acids (SCFAs). Cui *et al.* (2021) have shown that Laminarin can significantly increase short-chain fatty acid content in animals. Butyrate is one of the important functional metabolites produced by LJP (Morrison *et al.*, 2016). Wu *et al.* (2024) found that supplementation with sodium butyrate promoted the exocrine development of the pancreas, increased the size of zymogen granules, and improved amylase activity. In this experiment, the highest serum amylase activity in piglets was observed when 400 mg/kg LJP was added to the feed, which may be due to the fact that LJP promoted the secretion of amylase from the pancreas, and ultimately the amount of amylase that was absorbed into the blood increased, and there was no tendency to increase amylase in the other experimental groups, which may be attributed to the low amount of LJP added to the feeds.

LJP has been demonstrated to enhance the intestinal barrier function of weaned piglets, regulate the microecological balance, optimize the intestinal environment, improve the digestion and absorption of nutrients, and thereby improve their growth performance (Rattigan *et al.*, 2020). The impact of LJP on the growth performance of piglets possesses direct practical guiding significance for the evaluation of its practical application. McDonnell *et al.* (2010) added 300 mg/kg laminarin to the diet of 24-day-old weaned piglets for 21 days. Compared with the control group, the average daily gain (ADG) of the experimental group increased from 288 g to 319 g ($P < 0.05$), the average daily feed intake (ADFI) increased from 436 g to 471 g ($P > 0.05$), and the feed-to-gain ratio (G:F ratio) increased from 0.654 to 0.675 ($P < 0.05$). Heim *et al.* (2014) added 300 mg/kg laminarin to the weaning diet of 24-day-old piglets for 32 days. Compared with the control group, the ADG of the experimental group increased from 280 g to 353 g ($P < 0.05$), the ADFI increased from 513 g to 556 g ($P > 0.05$), and the G:F ratio increased from 0.514 to 0.608 ($P < 0.05$). Walsh *et al.* (2012) added 300 mg/kg laminarin to the diet of 24-day-old weaned piglets for 35 days. Compared with the control group, the ADG of the experimental group increased from 340 g to 390 g ($P < 0.05$), the ADFI increased from 591 g to 629 g ($P > 0.05$), and the G:F ratio increased from 0.574 to 0.622 ($P > 0.05$). In conclusion, the addition of LJP to the diets of weaned piglets has a positive effect on the growth performance of piglets, and one of the reasons for this may be related to the change of digestive enzyme activities in the intestinal tract, which in turn indirectly affects the activity of digestive enzymes in the serum.

CONCLUSION

This study demonstrates that adding 200 mg/kg of LJP to the feed significantly decreased the serum protease activity. Additionally, the supplementation of 400 mg/kg LJP in the feed showed a trend towards enhanced activity of serum lipase and amylase, but these increases were not statistically significant. Further investigation is required to determine the impact of LJP on serum digestive enzyme activity and the associated underlying mechanisms.

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ETHICAL STATEMENT

The study was approved by the Institutional Review Board of the Animal Care and Use Committee of the Jiangxi Science and Technology Normal University (protocol code JSTNU-20230315).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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