Enzymology properties of two different xylanases and their impacts on growth performance and intestinal microflora of weaned piglets

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A B S T R A C T
The enzyme xylanase is more and more widely used in feed production, but different xylanase have different properties, mechanism and application effects. To provide a theoretical basis for choosing more suitable xylanase in feed production, we selected bacterial xylanase (BX), labeled enzyme A, and tri-choderma xylanase (TX), labeled enzyme B, and studied the enzymology properties and application effects on growth performance and gut flora in weaned piglets. The results showed that the activity levels of both appear parabolic along with increasing pH or temperature, but the amplitude of enzyme activity changing curves and the pH/temperature of optimal activity level are different, where enzyme A has the optimal activity level at 50 °C with a pH value of 5.0. The optimal activity level of enzyme B was achieved at 70 °C with a pH around 6.0. Enzyme B suffered very little activity loss with moisture level at 16% and temperature from around 80 °C to 90 °C. Enzyme A suffered a big drop in activity level when processed with high temperature from around 80 °C to 90 °C, and it was even completely inactivated at 90 °C. Enzyme A has very low activity level after being processed in acid environment, but enzyme B has minor changes in activity level with respect to changes in acid level, indicating significantly different enzymatic properties between the two different sources of xylanases. In feeding experiment, the control group, was fed the basal diet, and the BX group and TX group were fed basal diets supplemented with 0.01% bacterial and fungal xylanases, respectively. The results showed that ADG of the BX group and TX group increased by 3.25% (P < 0.05) and 8.22% (P < 0.05), respectively, and the feed conversion ratio decreased by 6.74% and 7.86% (P < 0.05), respectively compared with the control group; TX group had significantly higher (P < 0.05) ADG compared with BX group; BX group and TX group had significantly lower ileum Escherichia coli level than the control group, which were reduced by up to 12.98% (P < 0.05) and 11.68% (P < 0.05), respectively, but the ileal lactic acid bacteria levels were significantly increased by 16.21% (P < 0.01) and 27.02% (P < 0.01), respectively. There were no significant differences (P > 0.05) between BX group and TX group in terms of lactic acid bacteria E. coli level. We concluded that fungal xylanase (enzyme B) has better performances in improving weaned piglet growth and in increasing ileal lactic acid bacteria level compared with bacterial xylanase (enzyme A).

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1. Introduction

Xylanase is the general term of the enzyme catalyzed hydrolysis the hemicellulose xylan into xylooligosaccharides or wood oligosaccharides (Prade, 1996). Xylanase is quite widely distributed in nature present, in the bacteria of the marine and terrestrial, marine algae, fungi and ruminants, etc, but in monogastric animals relatively. Currently, among more than 20 kinds of bacteria strains, 16 species of fungi, 3 kinds of yeast and 8 kinds of actinomycetes were isolated from the corresponding xylanase (Beg et al., 2001). As the core enzymes in feed enzymes applications, the improvement and
development of feed enzyme post-processing technique has a broader role in the pH range, higher thermal stability, which is the important direction in the future. The application of xylanase in feed industry can be divided into bacterial xylanase and fungal xylanase according to different sources. The activity of xylanase from different sources has a large variation. In particular, as the active protein, they are sensitive to temperature, pH, ion concentration etc., and the activity of xylanase is also the assurance of decomposition in vitro and in vivo, thus having a further comparative study of the application and effect in the two different sources of xylanase makes important sense on guiding the feed product development of enzymes and the application in feed production. Thus, to figure out the optimal activity range of xylanase becomes necessary. There is not an uniform method for the determination of xylanase's activity. This study used 3,5-Dinitrosalicylic acid (DNS) method and gave a comparative analysis on the change of xylanase from different sources when they in different temperatures and different pH. Xylanase supplementation in wheat-based diet could alleviate the anti-nutritional effects of arabinoxylans by limiting the physical entrapment of starch and could increase the available metabolizable energy (Lafond et al., 2015). This study was to investigate the difference of different sources of xylanase enzyme properties, taking the Landrace × Large Whites × Duroc, as test animals to compare bacterial and fungal xylanase's different effects on the growth performance of pigs and the influence on intestinal microbial flora, at last, to provide evidence for xylanase's application in pig feed production.

2. Materials and methods

2.1. Enzymatic characteristics analysis

2.1.1. The source of enzymes

Two different sources and bacterial species of xylanase samples were provided by the Laboratory of Ningxia Yizheng Biotechnology Co., Ltd, labeled enzyme A (bacterial origin) and enzyme B (fungal origin), and the activities were 26,000 and 84,000 (measured values using the following methods).

2.1.2. Reagent preparation

1) Enzyme activity definition: under the condition of 55 °C, pH 5.3, the quantity of enzyme needed to make 1 min of hydrolysis of oat spelt xylan substrate (oat spelt xylan of 1% concentration) to product 1 μmol reducing sugar (calculation by xylose), which is treated as a unity of activity.

2) The substrate (1% oat spelt xylan) configuration: put 1.0000 g xylan into 10 mL 0.5 mol NaOH solution, and magnetically stirred 30 min, then added 40 mL sodium acetate—acetic acid buffer of pH 5.3, next, adjusted with 1 mol acetic acid at pH 5.3, then buffered volume to 100 mL and reserved it in 4 °C to use, which was effective within a week.

3) The DNS reagent configuration: put 10 g DNS into 400 mL water, raised the temperature by water bath to 45 °C, then gradually added 150 mL NaOH solution (16.0 g NaOH plus 150 mL distilled water), stirred constantly till the solution became clear, next gradually injected 300 g of potassium sodium tartrate and stirred to clear. Added distilled water, buffered volume to 1,000 mL, filtered using a porous glass filter and stored in a brown bottle at room temperature. After 7 days, it could be used for 6 months.

4) Buffer configuration of 0.05 mol sodium acetate—acetic acid buffer solution: 4.1 g of sodium acetate was dissolved in approximately 900 mL distilled water, with 1 mol of acetic acid to adjust the pH to 5.3, then diluted with distilled water to 1,000 mL (effective storage at room temperature of the month).

5) Xylose standard curve: took six 50 mL graduated tubes, added a buffer of 0, 0.2, 0.4, 0.6, 0.8, or 1.0 mL, supplemented with distilled water to 2.0 mL and added 2.0 mL DNS reagent, then boiled in water bath for 10 min, cooled and added 10 mL distilled water with a pipette, colorimetric xylose content of the abcissa at 540 mm, the absorbance for the vertical axis, all of this as the standard curve. Each batch of freshly prepared DNS was needed to prepare a new curve.

2.2. Enzyme activity assaying

Drew 200 mL test enzyme solution into a test tube, added 1.8 mL 1% xylan substrate, then put enzyme solution with the enzyme blank together into 55 °C water bath accurately reflected for 10 min, added the test enzyme solution human 2 mL DNS reagent boiled bath for 10 min, cooled water and added 10 mL pipette, turned down the enzyme blank solution to zero, read OD 540nm value, obtained the content of xylose from the xylose standard curve and calculated unit of enzyme activity.

Effects of different conditions on the xylanase activity were as follows. 1) The effect of temperature on the xylanase enzyme activity. 2) Xylanase temperature response curve: respectively measured xylanase activity at a reaction temperature of 40, 50, 60, 70, 80, or 90 °C. Activity was measured during each test sample from each of 3 parallel tests, finally took averaged of 3 parallel data times (the same as the following tests in parallel processing mode). 3) Xylanase temperature performance are as follows. Treatment 1: the enzyme solution was separately tested in a water bath temperature of 40, 50, 60, 70, 80, or 90 °C for 2 min (the temperature was maintained for a time), then detected the enzyme activity under standard conditions. Treatment 2: after the enzyme sample was adjusted to a moisture content of 16% at a bath temperature of 40, 50, 60 70, 80 or 90 °C for 10 min (the temperature was maintained for a time), under the standard conditions to detect activity. 4) Effect of xylanase enzyme activity on pH values. 5) For the xylanase-response curves of pH, the pH value of the buffer and the substrate were configured to 3.5, 4.5, 5.3, 6.5, 7.5, and then xylanase activity was measured using above method. 6) For the acid properties of xylanase, the same samples were tested at pH conditions under 3.5, 4.5, 5.3, 6.5 for each 30 min, and then followed the above-mentioned method for detecting enzyme activity.

2.3. Feeding trials materials and methods

Test was held on pig farm in Hunan Zhenghong technology Co., Ltd. We selected 120 two-line crossbred (Landrace × Yorkshire) weaned pigs of the similar weight, age, health. Using the single factor experimental design, pigs were randomly divided into 3 groups with 5 replicates and 8 pigs per replicate.

Three days of pre-trial and 28 days of formal trial. Enzyme preparation was provided by Hunan Hong Ying Xiang Biological Engineering Co., Ltd., activity was 10,000 U/g. Enzyme activity unit was defined as, on the condition of 50 °C, pH 5.5, one U was the amount of enzyme which release 1 μmol of reducing sugar per minute.

2.4. Diet composition and nutrient levels

The corn—soybean meal was the based diet. The basal diet satisfied the nutrient requirement of piglets according to NRC (2012) standards. Basal diet composition and nutrient levels are showed in Table 1. Pigs in the group 1 were fed the basal diet (Control); the 2 group were fed the basic diet supplemented with
0.01% of bacterial xylanase (BX group), the group 3 were fed the basal diet supplemented with 0.01% fungal xylanase (TX group).

2.5. Feeding and management

Piglets lived in fecal leakage wood floor pens with 8 pigs per pen, ate dry test diets 4 times a day, and had free access water.

2.6. Sample collection and measurement

When the test started and ended, we respectively weighed each pig’s weight, recorded feed consumption and calculated the ADFI, ADG, F:G. On the final day of the test, we randomly select one pig per replicate and by aseptic sampling, immediately put ileum and cecum digesta 5 to 10 g into pre-prepared reagent, accurately weighed 1 g of stomach, duodenum and ileum digesta, then filled with 99 mL sterile saline into the flask, after full dilution oscillations, took 10-fold dilution and imbibed 0.1 mL inoculums on the medium. Escherichia coli, took 10-fold dilution and imbibed 0.1 mL inoculums on the eosin ethylene blue agar as medium colony was counted after 37 °C for 48 h, and lactic acid bacteria to LBS flasks, after full dilution oscillations, took 10-fold dilution and imbibed 0.1 mL inoculums on the medium.

2.7. Statistical analysis

For testing data, we used single factor analysis of variance and the Duncan’s multiple comparison of the statistical software SAS 26.0. All statistical results were expressed by Means ± SE, P > 0.05 for the difference was not significant, P < 0.05 for the difference was significant, P < 0.01 was considered extremely significant.

3. Results

3.1. Effect of temperature on xylanase activity

3.1.1. Temperature response curve of different sources of xylanase

Under the standard conditions, the detection of xylanase activity was 100% of the control (the same below), and xylanase activity changed with increasing reaction temperature as shown in Fig. 1.

As can be seen from Fig. 1, xylanase activity from two different sources were tested. The curves were parabolas with differences in the magnitude. The optimum temperature for enzyme A was about 60 °C, and the optimum reaction temperature for enzyme B was about 70 °C. This illustrated that the amplitudes of xylanase activity changes were not same for the same change of temperature. The optimum reaction temperatures of xylanases from different source were not the same. Wherein, enzyme B had a higher optimum reaction temperature and a higher viability than enzyme A.

3.1.2. Temperature resistant performance of xylanases from different sources

According to the experimental design for determination of the way and time after treatment, xylanase activity changes with the temperature, which are shown in Figs. 2 and 3.

Results from Figs. 2 and 3 showed that the xylanases in a liquid state and in a certain moisture content state were treated at different temperatures, the amplitude of enzyme activity varies greatly. As can be seen from Fig. 2, two xylanase activities in the liquid state at the temperature of 50 °C after 3 min treatment were with almost no loss. With the increase of the treatment temperature, the difference in the loss of enzyme activity varied greatly. Especially at the same temperature point treatment of 60 °C, energy loss of enzyme B was far less than the enzyme A; at points 80 and 90 °C, the two xylanase activities were almost lost completely. The results in Fig. 3 indicated that xylanases at a moisture content of 16% were processed from 40 to 70 °C, and the activity losses were basically the same, but with increasing treatment temperature, the activity loss of enzyme B was small, but the loss of enzyme A was very significant, and enzyme A at 90 °C was almost all deactivated. This showed that in the liquid state, xylanase

![Fig. 1. The temperature responsive curve of xylanase from different sources. A, bacterial origin; B, fungal origin.](image1)

![Fig. 2. The temperature responsive curve of xylanase from different sources in liquid state. A, bacterial origin; B, fungal origin.](image2)
which was treated in a high temperature over 80 °C was very difficult to survive, but maintaining a certain moisture content, i.e., 16% moisture, the loss of activity was very small for enzyme B. Enzyme activity losses of the xylanases from two different sources varied greatly.

3.2. Effect of pH value on xylanase activity

3.2.1. Response curve of xylanase from different sources at different pH values

The xylanase activity detected under standard conditions was 100% of the control (the same below), the change of the xylanase enzyme activity at different pH values was measured in the situation shown in Fig. 4.

As can be seen from Fig. 4, the enzyme activity of 2 xylanases changed with increasing pH value in a parabola type, but the change rate of xylanases were different. The optimum pH value of xylanase A was about 6.0, the optimum pH value of the enzyme B was about 5.0; in the pH value range from 3.5 to 5.0, the dynamic change of amplitude of Enzyme A was large, and the variation width of the enzyme B was smaller than enzyme A; in the range of pH 5.0 to 7.5, the variation width of the enzyme A was smaller than enzyme B, which illustrated that enzyme A was the partial neutral enzyme xylanase, but enzyme B was the partial acidic xylanase.

3.2.2. Acid and alkali resistant performance of different xylanase

Xylanase enzyme activity variation values after the continuous processing of different pH conditions are shown in Fig. 5.

As can be seen from Fig. 5, after treatment of the strong acid (pH value 2.5) and alkaline condition (pH value 6.5) for 30 min, enzyme B still had very high activity, but there was almost no activity for the enzyme A; After the continuous processing from pH value 2.5 to 6.5, the dynamic change of enzyme B was relatively stable, but the enzyme activity A was very low, indicating that acidic environment was not conducive to enzyme A survival.

3.3. The influence of xylanase on the growth performance of piglets

As shown in Table 2, there was no significant difference on average daily feed intake and feed conversion rate in the experimental groups. In contrast with the control group, ADFI decreased 3.56% \((P < 0.05)\) in BX group and increased 1.94% \((P > 0.05)\) in TX group, there was no distinct difference. Compared with the control group, there was a trend of decrement with the decrement of 6.74% and 7.86% on feed conversion rate in BX group and TX group. The results indicated that daily gain of piglets in BX group and TX group was 3.25% \((P > 0.05)\) and 8.22% \((P > 0.05)\) more than the control group, and TX group had significantly higher ADG than BX group.

3.4. The influence of xylanase on the intestinal microflora of piglets

As shown in Table 3, the number of \(E.\ coli\) in ileum and cecum decreased in both BX group and TX group, but lactobacillus increased. Contrasted with the control group, the decrement of BX group was 12.98% \((P < 0.05)\), which was significantly different from that of TX group 11.68% \((P < 0.05)\). In both BX group and TX group, although there was a trend of decrement on the number of \(E.\ coli\), but the difference was not very clear \((P > 0.05)\). The lactobacillus content of BX group was extremely different from that of TX group \((P < 0.01)\), but the numbers of lactobacillus in ileum and cecum in both BX group and TX group were nearly the same.

![Fig. 3. The temperature responsive curve of xylanase from different sources with a content of 16% water. A, bacterial origin; B, fungal origin.](image)

![Fig. 4. Response curve of xylanase from different sources at different pH values. A, bacterial origin; B, fungal origin.](image)

![Fig. 5. Response curve of xylanase from different sources at acid treatment. A, bacterial origin; B, fungal origin.](image)

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Table 2

| Item | Group 1 (Control) | Group 2 (BX group) | Group 3 (TX group) |
|------|------------------|--------------------|-------------------|
| Initial BW, kg | 10.36 ± 0.35 | 10.32 ± 0.32 | 10.31 ± 0.32 |
| Final BW, kg | 25.07 ± 0.59 | 25.43 ± 0.66 | 25.93 ± 0.58 |
| ADG, g | 523 ± 13.0 | 540 ± 17.1 | 566 ± 11.4 |
| ADPI, g | 928 ± 31.9 | 895 ± 48.8 | 946 ± 48.0 |
| FCR | 1.78 ± 0.08 | 1.66 ± 0.07 | 1.64 ± 0.04 |

BX = bacterial xylanase; TX = tri-bacterial xylanase.

\(^{ab}\) Within a row, means without a common superscript differ \((P < 0.05)\).

Table 3

| Item | Group 1 (Control) | Group 2 (BX group) | Group 3 (TX group) |
|------|------------------|--------------------|-------------------|
| \(E.\ coli\) | | | |
| Ileum | 7.7 ± 0.1 | \(6.7 ± 0.2^{b}\) | \(6.8 ± 0.1^{b}\) |
| Appendix | 6.5 ± 0.2 | 6.3 ± 0.2 | 6.1 ± 0.1 |
| \(Lactobacillus\) | | | |
| Ileum | 7.4 ± 0.2 | \(8.6 ± 0.2^{b}\) | \(9.4 ± 0.2^{b}\) |
| Appendix | 7.8 ± 0.2 | 8.4 ± 0.2 | 8.3 ± 0.2 |

BX = bacterial xylanase; TX = Tri-bacterial xylanase.

\(^{ab}\) Within a row, means without a common superscript differ \((P < 0.05)\).
(P > 0.05). The result indicated that xylanase had no significant effect on the number of E. coli and lactobacillus in piglets’ intestinal.

4. Discussion

4.1. The effect of temperature on xylanase activity

Xylanase in different temperature and different kinds of xylanase in the same temperature displayed different activity largely. There is not an unified approach to determine xylanase’s activity. Thus, it is unscientific valuation of xylanase according to enzyme survival. Enzyme survival is just a quality standard of a product. This study showed that enzyme activity trend was a parabola at different temperature for different sources of xylanase, and only the changes in amplitude and the highest temperature points of activity were different. Heat resistance of enzyme has always been the focus in feed enzyme researches. It is a severe test to the activity of feed enzymes with the combined effect of high temperature, high humidity and quenching in the extruded feed pelleting process. The test dealt with xylanase in two different ways, under the liquid state of extreme high temperature treatment, the loss of enzyme activity was a lot, and enzyme A almost was all dead in 90 °C treatment for 2 min; but enzymes B still had a high activity in 90 °C high temperature for 10 min while keeping the moisture of enzymes in 16%. This suggests that the inactivation of enzyme in liquid state is not equal to inactivation in feed processing conditions. When fodders are processed to particles, there is a certain moisture content. Adjusting the moisture of enzyme is to simulate the moisture of material in the container when processing. Under the condition of 16% moisture, to deal with enzymes in high temperature, the loss of enzyme activity is less, and the loss of enzyme activity are of big difference for xylanase from different sources, which is consistent with the research results of Petterson, etc. Along with more xylanase research, enzymes strains and their related enzymes thermodynamic characteristics have been reported. Following the developing of researches about xylanases, xylanases produced by all kinds of bacterial strains and its thermodynamic characteristics have been reported in succession. To use E. coli as recipient bacterium can be expressed for the heat-resisting xylanases (Shendye and Rao, 1993; Lapidot et al., 1996).

The heat stability for the application of enzyme preparation in the feed industry is an important feature. By analyzing the molecular structure of xylanases, Kumar et al. (2000) discovered the heat stability related to the disulfide bond and aromatic amino acid. The heat stability for the actual production of xylanases is a constraint, but now only the XynA of Thermomospora fusca belongs to the 11th tribe in the discovered heat-resisting xylanases, and the others belonging to the 10th tribe. The thermostabilizing domain is responsible for the stability of xylanases, but relevant researches are still not enough in-depth. Now researchers have discovered that there are 22 kinds of xylanases containing the thermostabilizing domain, 16 of which are in N terminal, 5 of which are in C terminal and only one is located to middle area and N terminal (Ken et al., 1988; Sunna and Antranikian, 1997).

4.2. The effect of pH on the xylanase activity

The pH is one of the main factors that affects xylanase activity. It can affect the three-dimensional conformation of enzyme protein, even making it denatured and inactivated. This study showed that the enzyme activity varies with the changes of pH and its reaction curve is parabolic for different sources of xylanase. Enzyme A almost complete lost its enzyme activity when it was under the environment of pH 2.5 for 30 min, and enzyme B activity was almost not lost. Choosing feed enzymes should consider the performance of the tolerance of acid and alkali. The pH in animal’s digestive tract is dynamic, and the pH of pigs from the duodenum to the jejunum of the small intestine is 6.4, where is a main place that xylanase play a role. The activity of xylanase may be low in the stomach. This leads to put forward higher requirements of acid and alkali tolerance for feed enzymes. Xylanase from different sources vary widely in tolerance of acid alkali, which is the main factor cause different effect when enzymes are used. Composition and properties of xylanase from different sources is different, pH stable range and optimum pH range are also different. In general, the xylanase of fungal origin have a maximum dynamic multi under acidic conditions, its optimal pH is between 4.0 and 6.0, and it belongs to acidic xylanase. It is more suitable for bacteria and actinomycetes xylanase to act under neutral or basic conditions, and the pH stability is higher than fungi xylanase. Xylanase which is mostly stable between 6.0 and 8.0 belongs to neutral or alkaline xylanase. A variety of extreme microorganism is able to produce xylanase that similar to their growth environments characteristics. Activity changes of xylanase showed that under the condition of pH 2.5 each maintained 15 min and 120 min then rose to 5.5. Xylanase activity was decreased from 12% to 75% and from 12% to 48%. Under low pH for a long time will cause some losses of enzyme activity (Thacker et al., 1992).

In actual application, there are many factors impacting on the activity of xylanases. In this study we only discussed the impact of pH and temperature on the activity of xylanases and discovered the characterizations of xylanases from different sources have very large difference. How to choose enzyme that is suitable for feed production and animal gut is a key factor for the application of enzyme preparation. According to the goal, the choice of enzyme preparation could refer to the following advice. Firstly, to meet feed processing condition and to choose the high temperature resisting enzyme; secondly, to choose the enzyme which has strong capacity of decomposition corresponding to the substrate in feed ingredients instead of the enzyme having high standard enzyme activity; thirdly, to choose the enzyme which has strong resistance for the decomposition of protease; fourthly, to choose the enzyme suitable for animal gastrointestinal environment; fifthly, to choose the enzyme suitable for storage.

4.3. The influence of xylanase on the growth performance of piglets

Xylan, as one of the important substances, increases the viscosity of the intestinal chyme. It can be degraded by the xylanase into small and very small viscous product, thereby reducing the viscosity of chyme and improving the growth performance of animal (Yin et al., 2000, 2001). It is generally believed that it is not high of weaning digestibility about non-starch polysaccharides (NSP). Adding xylanase in high NSP feed can destroy the cell walls of plant feed, which increases the concentration of various nutrients in feed. The present experiment showed that fungal xylanase is better than bacterial xylanase in terms of improving the effect of ADG.

4.4. The influence of xylanase on the intestinal microflora of piglets

Healthy balance of intestinal microflora is important for ensuring animal health and relevant animal’s gastrointestinal microbial type and quantity of closely related species (Kittelmann and Janssen, 2011). E. coli is generally considered as the potential of intestinal bacteria, and lactobacillus and bifidobase bacteria are beneficial intestinal flora. These two types of animal gut microflora which maintain ecological balance have a very important role. Most basal diets, due to high NSP content, resulted in animal digestive chyme viscosity increases, interfered with digestion and
absorption of nutrients, thus exacerbated gastrointestinal segment microbial fermentation activity. Studies showed that basal diets added xylanase can reduce digesta viscosity, speed up chyme emptying rate, reduce the hindgut microbial fermentation activity (Simon, 1998; Vahjen et al., 1998). In this experiment, xylanase reduced the amount of E. coli in ileum and cecum, increased the number of ileum and cecum lactic acid bacteria. Xylanase from different sources can improve weaning daily gain, and the feed conversion ratio tends to decrease; its possible pathway is that xylanase reduces the number of E. coli in piglets intestinal, increases the number of intestinal lactobacilli, improves nutrition digestion and absorption of substances to improve piglet health, promotes the improvement of growth performance. Fungal xylanase is better than bacterial xylanase in improving growth performance and increasing the amount of lactic acid bacteria ileum.

5. Conclusion

We studied bacterial xylanase and trichoderma xylanase on enzymology properties and effects on growth performance and gut flora of weaned piglets, and concluded that fungal xylanase has better performances in improving weaned piglet growth and ileal lactic acid bacteria level than bacterial xylanase.

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