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Original research article

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

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ABSTRACT

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 trichoderma 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 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).

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

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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-Dinotrosalicylic 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 spelled 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 abscissa 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 μL 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