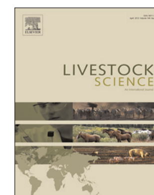
Livestock Science 178 (2015) 255–262

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci



Effects of feruloyl esterase, non-starch polysaccharide degrading enzymes, phytase, and their combinations on *in vitro* degradation of rice bran and nutrient digestibility of rice bran based diets in adult cockerels

Q. Liu *, D.Y. Zhou, L. Chen, R.Q. Dong, S. Zhuang *

Department of Animal Science and Technology, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China

ARTICLE INFO

Article history:
Received 6 February 2015
Received in revised form
7 June 2015
Accepted 9 June 2015

Keywords:
Rice bran
Feruloyl esterase
Non-starch polysaccharide degrading enzymes
Phytase
Digestibility coefficient
Adult cockerels

ABSTRACT

The objective of this research was to evaluate the feasibility of improving the nutritive value of rice bran used as poultry feed ingredient by the combination of feruloyl esterase (FAE), non-starch polysaccharide degrading enzyme cocktail (non-starch polysaccharide enzyme containing xylanase, cellulase, and β-galactosidase, NSPase), and phytase. After analyzing the phenolic acid profile of defatted rice bran (DFRB), an *in vitro* degradation experiment, and an *in vivo* metabolism assay were conducted. The effects of FAE, NSPase, and phytase were assessed in a $2 \times 2 \times 2$ factorial *in vitro* experiment using DFRB as substrate. Feruloyl esterase, NSPase, and their combination increased the release of ferulic acid from DFRB ($P < 0.001$). Non-starch polysaccharide degrading enzyme cocktail increased reducing sugar production ($P < 0.001$). Phytase addition increased the dry matter disappearance ($P < 0.001$), but resulted in a reduction in the levels of ferulic acid and reducing sugar released when combined with FAE and NSPase, respectively. In *in vivo* experiment, 40 adult cockerels (BW$=2.2 \pm 0.1$ kg) were randomly divided into 5 treatments with 8 individual cockerels per treatment. Cockerels in 1 treatment were force-fed 1 of full-fat rice bran based diets [without enzyme supplementation (RB), with NSPase (RBN), NSPase+FAE (RBNF), NSPase+phytase (RBNP), or NSPase+FAE+phytase (RBNFP) addition, respectively]. Enzyme additions improved the nutrient digestibility of rice bran. The greatest enhancements in digestibility coefficients of dry matter ($P=0.036$), gross energy ($P < 0.001$), ether extract ($P < 0.001$), and neutral detergent fiber ($P=0.01$) were found in the RBNF and RBNFP diets. The apparent metabolizable energy of full-fat rice bran based diets increased from 12.05 (RB diet) to 13.05 (RBNF diet), and, then, to 13.13 MJ/kg (RBNFP diet). In conclusion, combining FAE with NSPase and phytase improved both *in vitro* dry matter degradation and ferulic acid release, and *in vivo* nutrient digestibility of rice bran based diets. It showed a high potential in improving the use of rice bran as poultry feed.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Rice bran is the major byproduct when unhusked rice is processed to produce polished rice, which mainly consists of pericarp, aleurone and subaleurone fractions, as well as part of germ, endosperm and husk residual of rice seed. The annual unhusked rice production of China is more than 200 million tons (National Bureau of Statistics of China, 2014), and the corresponding output of rice bran is about 20 million tons, of which about 70% is used as animal feed. Despite the fact that rice bran is rich in most macronutrients, i.e., ether extract, 13–18%, crude protein, 11–15%, nitrogen-free extract, 42–46%, and minerals, 6.5–9% (Chinese Feed Database, 2014), the overall digestibility is not as high as expected when comparing to other grain byproducts, e.g., wheat bran. The animal performance was decreased with increasing amount of rice bran in diets (Warren and Farrell, 1990, 1991).

It has been considered that anti-nutritional factors, such as undigestible polysaccharides and phytate are the main obstacles that impede the nutrient utilization of rice bran. Various strategies have been explored to improve the nutritive value of rice bran, including extrusion, infra irradiation, alkaline or acid hydrolysis, cell wall degrading enzymes and phytase addition etc. However, the results are limited and inconsistent (Deniz et al., 2007; Farrell and Martin, 1998; Martin and Farrell, 1998; Mujahid et al., 2004).

From a chemical composition point of view, rice bran has greater phenolic acid content than other grain byproducts, especially ferulic acid (Laokuldilok et al., 2011; Zhang et al., 2010). Ferulic acid and its dimeric forms are covalently linked to arabinosyl residues of cell wall polysaccharides and lignin (Grabber,

* Corresponding authors. Fax: +86 2584395380.
E-mail addresses: liuayang@njau.edu.cn (Q. Liu), zhuangsu@njau.edu.cn (S. Zhuang).

http://dx.doi.org/10.1016/j.livsci.2015.06.007
1871-1413/© 2015 Elsevier B.V. All rights reserved.

2005). As a result, this structure increases the rigidity of cell wall and impedes the release of nutrients inside of plant cell and restricts the hydrolysis of cell wall components by enzyme preparations used in animal feed.

Feruloyl esterase (FAE, EC 3.1.1.73) is a subclass of the carboxylic esterase that catalyzes the hydrolysis of ferulic acid ester linkages in lignin–carbohydrate complexes. Feruloyl esterase has been widely used in the bioenergy, food and pharmaceutical industry (Damásio et al., 2013; Fazary and Ju, 2008), and demonstrated a synergism with other cell wall degrading enzymes, especially xylanase in hydrolyzing cell wall structures (Faulds et al., 2003, 2006). Yu et al. (2005) developed an optimized multi-enzyme cocktail containing FAE, xylanase, cellulase, endo-glucanase [I, II], and β-glucanase, which improved both *in vitro* enzymatic dry matter disappearance (DMD), and *in vitro* rumen degradability of oat hull dramatically. This mechanism implies the potential of FAE in improving the nutritive value of rice bran in combination with other non-starch polysaccharide (NSP) degrading enzymes. Unfortunately, limited information is available on the effectiveness of FAE and its combination with other enzymes when applied in rice bran based diets.

To explore the potential of application of FAE and its interaction with other feed enzymes in animal diets containing high level of rice bran, an *in vitro* degrading experiment was carried out using defatted rice bran (DFRB) as substrate. The effectiveness of the selected enzyme combinations on *in vivo* nutrient digestibility of a rice bran based diet were verified by force-fed cockerels.

2. Materials and methods

2.1. Sample preparation

Full-fat rice bran was obtained from a commercial source (Zhicheng Feed Co., Ltd., Anhui, China). After removing the remaining broken rice and impurities, samples were vacuum dried at 65 °C to constant weight. In order to eliminate the possible interference of ether extract in analysis of phenolic acid by HPLC, full-fat rice bran was extracted by the Soxhlet-extraction method. The defatted rice bran was ground to a particle size of less than 0.5 mm using a mill (FW100, Taisite Instrument Co., Ltd., Tianjin, China) and was used in phenolic acid analysis and in the *in vitro* experiment.

2.2. Enzymes and activity assays

Xylanase (X2753), cellulase (22178), β-galactosidase (G5160), phytase (P9792), and pepsin (P7000) were obtained commercially (Sigma-Aldrich Co., LLC., Shanghai, China). Feruloyl esterase (Depol 740L, Biocatalysts Ltd., Wales, UK), heat-stable α-amylase (Termamyl 120LS), and amyloglucosidase (AMG 300L, Novozyme Corp., Copenhagen, Denmark) were provided free of charge.

The activity of phytase was measured using method (2000.12) of AOAC (2005). The activity of β-galactosidase was assayed according to the method of Ratto and Poutanen (1988). The activities of cellulase and xylanase were assayed in a 0.2 M phosphate–citric acid buffer at 40 °C, pH 4.8 using carboxymethyl cellulose and oat xylan as substrate, respectively. The amounts of glucose and xylose produced were quantified by the 3,5-dinitrosalicylic acid reagent method (Miller, 1959). One unit of enzyme activity was defined as the amount of enzyme releasing 1 μM glucose or xylose per hour. The method for determination of FAE activity was adapted from Juge et al. (2001). One unit of FAE activity was defined as the amount of enzyme releasing 1 μM ferulic acid per minute.

All assays were performed in triplicate, with blanks to correct for background in enzyme and substrate samples. The activities of FAE, xylanase, cellulase, β-galactosidase, and phytase were 55 U/mL and 2500, 1150, 11,400, and 1000 U/g, respectively.

2.3. Determination of phenolic acid composition

Nine phenolic acid standard chemicals (Sigma-Aldrich Co., LLC., Shanghai, China) were used to identify and quantify the phenolic acids of DFRB, i.e., protocatechuic acid (03930590), hydroxybenzoic acid (PHR 1048), caffeic acid (60018), syringic acid (63627), coumaric acid (55823), ferulic acid (52229), salicylic acid (1609501), cinnamic acid (97013), and vanillic acid (68654). After vacuum drying at 50 °C for 20 h, 10 mg of each standard was weighed and dissolved in double distilled water. The working standard solutions contained 0.0, 0.2, 0.5, 2.0, 5.0, and 10.0 μg/mL of each phenolic acid, respectively. Twenty microliter of these solutions was used for HPLC analysis.

The method to measure the phenolic acid composition of DFRB was adapted from Liao et al. (2007). Briefly, 10.0 g DFRB was extracted by 100 mL 50% ethanol–water solution containing 1% (W/V) NaOH in 85 °C water bath for 6 h (50 mg sodium sulfite was added as reductant). The slurry was centrifuged at 300*g* (6 J MI, Beckman Coulter, Inc., CA, US) for 20 min at room temperature, the sediment was washed with 30 mL distilled water 2 times. The supernatants were collected into 200 mL volumetric flask and adjusted to pH 2.0 with 4 M HCl. After being diluted 5 times with 95% ethanol and filtered through 0.22 μm microfilter, 20 μL of filtrate was analyzed by HPLC.

Standard and sample measurements were performed on Shimadzu LC 10Avp system (Shimadzu Corp., Kyoto, Japan) equipped with reversed phase column (Shimadzu C18 4.6 × 250 mm,[2], 5 μm) and UV detector (L2420). The column oven was set at 30 °C. The mobile phase consists of acetonitrile (A) and 0.5% acetic acid (B). The elution program was as follow: 0–9 min, the gradient of solvent A increased from 10% to 12%; 9-10 min, decreased the gradient of solution A to 6%, and kept at 6% for 9 min; 19-35 min, increased the gradient of solvent A from 6% to 30%, and kept at 30% to the end of the process (50 min). The flow rate was set constantly at 1.0 mL/min. The wavelength of the detector was set to 254 nm in 0–14.5 min, then adjusted to 265 nm in 14.5–25 min, and 296 nm in 25.0–50 min. Before each injection, the system was equilibrated with 10% solution A and 90% solution B for 5 min. Analytical results of the phenolic acid content were expressed on a dry matter (DM) basis. All the analytical procedures were conducted 3 times independently.

2.4. The in vitro hydrolysis of defatted rice bran by enzyme combinations

To evaluate the interactions of FAE with other cell wall degradation enzymes in degradation of rice bran, FAE, NSP degrading enzyme cocktail (NSP degrading enzyme containing xylanase, cellulase, and β-galactosidase, NSPase), and phytase were assessed in a 2 × 2 × 2 factorial *in vitro* experiment using DFRB as substrate, the activities of enzyme supplementation are given in Table 2. The DFRB (5 g) in a 50 mL 20 mM phosphate buffer (pH 6.0) was successively pretreated with α-amylase (50 μL) at 90 °C for 2 h, amyloglucosidase (50 μL) at 55 °C for 4 h. After adjusting the pH value of the slurry to 2.0 using 4 M HCl, the bran suspension was further treated with pepsin (20 mg) at 40 °C for 4 h to mimic the digestive scenario in the poultry gastrointestinal tract in a shaking water bath (125 rpm). The pH of the suspension was then adjusted to 6.0 using 4 M NaOH and 2.0 mL of the enzyme solution was added at different combinations at 40 °C for 4 h, respectively.

At the end of the hydrolysis process, the slurry was centrifuged at 300*g* for 10 min at room temperature, and washed with equal volume of distilled water 3 times, all supernatant was collected for

Download English Version:

https://daneshyari.com/en/article/2447085

Download Persian Version:

https://daneshyari.com/article/2447085

Daneshyari.com