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Agriculture and Agricultural Science Procedia 11 (2016) 65 – 70

Agriculture and Agricultural Science Procedia

International Conference on Inventions & Innovations for Sustainable Agriculture 2016, ICIISA 2016

Improvement of Taro Leaves Using Pre-treated Enzyme as Prebiotics in Animal Feed

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Abstract

The objective of this study was to improve quality of taro leaves using pre-treated enzymes as prebiotic in animal feed. This study was assigned in T-test for dependent sample and consists of two *in vitro* experiments. First experiment consists of 2 treatments by different enzyme levels (0 and 1% w/v) with 3 replications. All treatment samples were measured for chemical compositions, reducing sugar content and oligosaccharides. The results showed that chemical compositions were significantly different among treatments ($P<0.01$) except crude protein and cellulose. Enzyme-treated taro leaves had lower fat content (2.96 vs. 4.31%) and hemicelluloses (14.55 vs. 15.18%) as compared to untreated taro leaves ($P<0.01$). Moreover, enzyme-treated taro leaves had higher reducing sugar content than untreated taro leaves ($P<0.01$). They were 29.78 and 6.23 mg/g, respectively. In addition, oligosaccharides analysis by thin layer chromatography (TLC) found that oligosaccharides in enzyme-treated and untreated taro leaves can digest product and releasing oligosaccharides. Second experiment was conducted to examine prebiotic properties. The results found that sugar product from taro leaves using pre-treated enzymes (Hemicell®) can increase growth of *Lactobacillus plantarum* (concentration of hydrolyzed products 400, 800, 1,200 and 1,600 µg/ml) but cannot inhibit the growth of *Escherichia coli*. In conclusion, it was suggested that enzyme-treated taro leaves can be used as prebiotic in animal feed.

Peer-review under responsibility of the Faculty of Animal Sciences and Agricultural Technology, Silpakorn University

Keywords: taro leaves; enzyme; prebiotic

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Peer-review under responsibility of the Faculty of Animal Sciences and Agricultural Technology, Silpakorn University
doi:10.1016/j.aaspro.2016.12.011

1. Introduction

Taro (*Colocasia esculenta*) is from the family of Arecaceae and a single polymorphic species which is grown in tropical regions around the world such as Africa, Asia, the West India and South America. The corms or root is edible and leaves are by-product from harvesting. However, young taro leaves are used as a main vegetable. Taro root is a good source of carbohydrate, vitamin B-complex and potassium but poor source of ascorbic and carotene (Lee, 1999). Protein content of taro is approximately 7% in root and 23% in leaves and they are rich in essential amino acid but is rather low in histidine, methionine, lysine, tryptophan and isoleucine (Jiang, 1999). Taro had high calcium oxalate which is an anti-nutrition and toxic. Boiling and fermentation can reduce calcium oxalate. Very few previous studies used taro leaves as protein replacement in animal feed. Rodriguez et al. (2006) reported that fresh taro leaves can replace soybean meal up to 50% and that it had no adverse effect on growth in young pigs. Similarly, Pheng et al. (2008) also reported that taro leaves silage can replace fish meal up to 75% with no adverse effect on digestibility. Cell wall of taro are mainly cellulose (1,4-β-D glucans) and non-cellulosic polysaccharides (galacturonorhamnans, galactomannan, glucomannan and arabinoxylan) (Jiang,1999). Manno-oligosaccharides (MOS) are mannose based oligosaccharides with β-1,4 linkages (Ozaki et al., 2007) which acts as prebiotic by stimulating non-pathogenic bacteria (*Bifidobacterium* spp. and *Lactobacillus* spp.) and limiting pathogenic bacteria in intestine (Titapoka et al., 2008). Therefore, the objective of this study was to examine the improvement of taro leaves using pre-treated enzyme as prebiotic in animal feed.

2. Materials and Methods

2.1 Preparation of enzyme-treated taro leaves

Taro leaves were harvested and were cut and dried in hot air oven at 60 °C for 24 hours. This experiment consists of two treatments and three replicates per treatment. The taro leaves were treated with commercial enzyme (Hemicell®) at 2 different levels (0 and 1% w/v). The taro leaves samples were incubated at 55 °C for 6 hours. After that the samples were centrifuged at a speed of 10,000 rpm. The supernatant sample was collected for reducing sugar and oligosaccharides analysis. The solid sample was dried in hot air oven at 60 °C for 24 hours and stored at 4 °C prior to proximate analysis.

2.2 Determination of reducing sugar

Approximately 0.5 ml of the supernatant was transferred into sample tube in which 0.5 ml of dinitrosalicylic acid (DNS) reagent was added into each tube. The mixture was boiled for 5 min and left to cool by immersing the sample tube into cold water immediately. The absorbance was read at 540 nm with distilled water as blank (Miller, 1959). Different concentrations of glucose (0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50, 0.55 and 0.60 mg/ml) were prepared using similar procedure described above to develop a standard curve for the above assay.

2.3 Determination of oligosaccharides

Oligosaccharides analysis was performed using thin-layer chromatography (TLC) described by Srinang et al. (2008). The supernatant sample was spotted near the bottom of silica gel plate (20×20 cm). Next, the TLC plate was placed in a shallow pool of a solvent (5 n-butanol: 2 acetic acids: 3 distilled water) in a developing chamber so that only the very bottom of the plate was in contact with the liquid. This liquid will act as the mobile phase which will slowly rise up the TLC plate by capillary action. After thoroughly drying the TLC plate, chromatography was developed by using a freshly prepared solution (1 ml aniline: 1 ml α-diphenylamine: 50 ml acetone: 7.5 ml phosphoric acid), and heated at 100^{o}C until dry. The spot on TLC plate was compared with glucose and cellobiose.