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Development of a microbial fermentation process for detoxification of gossypol in cottonseed meal

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Abstract

The objective was to study effects of selected fungi, carbohydrate sources, urea, heat treatment and minerals on the reduction of free gossypol (FG) levels during solid substrate fermentation of cottonseed meal (CSM), and to determine the crude protein (CP) and amino acid (AA) content as well as the *in vitro* digestible CP and AA of CSM substrate fermented under optimal conditions. Six groups of autoclaved CSM substrate were incubated for 48 h after inoculation with either of the fungi *Candida capsuligena* ZD-1, *Candida tropicalis* ZD-3, *Saccharomyces cerevisae* ZD-5, *Aspergillus terricola* ZD-6, *Aspergillus oryzae* ZD-7, or *Aspergillus niger* ZD-8. One non-inoculated group was the control. Levels of initial and final FG and CP were assayed. Results indicated that microbial fermentation could greatly decrease FG levels in CSM, but it differed among species of microorganisms with *C. tropicalis* ZD-3 the most effective. The CSM substrate was supplemented with carbohydrate sources, urea and minerals, and one group of non-autoclaved CSM substrate was used to investigate effects of heat treatment. The treated CSM substrates were inoculated with *C. tropicalis* ZD-3 and incubated for 24 or 48 h. Results showed that CSM substrate supplemented with starch and sucrose enhanced detoxification of gossypol, but heat treatment and minerals were also

Abbreviations: CSM, cottonseed meal; CP, crude protein; AA, amino acids; FG, free gossypol; DM, dry matter

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effective in reducing FG levels during solid substrate fermentation of CSM. The detoxification effect with minerals was the best among treatments.

Keywords: Fungi; Free gossypol; Detoxification; Fermentation; Cottonseed meal

1. Introduction

The use of cottonseed meal (CSM) as an animal feed is limited by the presence of gossypol ($C_{30}H_{30}O_8$), a toxic polyphenolic pigment produced in the seeds of the cotton plant. Feeding diets containing gossypol can cause negative effects on growth, reproductive performance, as well as intestinal and internal organ abnormalities (Berardi and Goldblatt, 1980; Robinson et al., 2001; Francis et al., 2001). Feeding gossypol to laboratory rodents has resulted in irregular estrous cycles and reduced pregnancy rates (Hahn et al., 1981; Lagerlof and Tone, 1985; Bender et al., 1988). In addition, Eisele (1986) reported that feeding sows and gilts diets containing 0.2 and 1.36 g/kg of free gossypol (FG) reduced conception rates by 72 and 77%, respectively; and of those sows and gilts that did conceive, many aborted, produced stillborn piglets and/or had reduced litter sizes. Inhibition of ovarian steroidogenesis by gossypol in the monogastric female has been implicated as a possible cause for these detrimental effects on estrous cyclicity, as well as establishment and maintenance of pregnancy.

Negative effects of gossypol on animal health have long been recognized, and toxic effects of gossypol are much greater in non-ruminants *versus* ruminants due to binding of FG to soluble proteins in the rumen (Willard et al., 1995). Thus, if FG was transformed into bound gossypol (BG), it would not harm the animals because BG cannot be absorbed from digestive tract. Cottonseeds are commonly processed into oil and meal, which may contain high concentrations of gossypol, and further processing is necessary to reduce it to acceptable levels. Development of glandless cottonseed by plant breeders to overcome this problem has been limited due to the lower crop yields and increased susceptibility of the crop to insects and diseases. Consequently glandless cottonseed accounts for less than 0.5% of the total world cottonseed crop.

A number of methods have been developed to remove gossypol from cottonseed including solvent extraction of free gossypol (Damaty and Hudson, 1975; Canella and Sodini, 1977; Cherry and Gray, 1981; Rahma and Narasingo Rao, 1984), chemical treatment with ferrous sulfate (Tabatabai et al., 2002; Barraza et al., 1991) or calcium hydroxide (Nagalakshmi et al., 2002, 2003), microbial fermentation (Wu and Chen, 1989; Shi et al., 1998). All of these methods play roles in detoxification of CSM, but the reduction of gossypol using solvents suffers from the difficulty of totally removing residual solvents that may be potentially harmful to the animals that consume them. Ferrous sulfate can cause feed to turn black, whereas calcium hydroxide often reduces the biological activity of vitamins and lowers detoxification efficiency. Microbial fermentation offers promise as a detoxification method, but there are few reports on the use of this strategy to improve the feeding value of CSM (Wu and Chen, 1989; Shi et al., 1998).