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# Effects of soybean meal replacement with fermented alfalfa meal on the growth performance, serum antioxidant functions, digestive enzyme activities, and cecal microflora of geese

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**Abstract**

Fermented forages are important feedstuffs. *Bacillus subtilis* inoculants are often used to improve the value of forage legume fermentation. The present work was conducted to study the effects of replacing soybean meal with solid-state fermented alfalfa meal (FAM) with *B. subtilis* ACCC 01746 on growth performance, serum antioxidant and digestive enzyme activities, and cecal microflora in goose. 300 healthy geese with similar body weights were randomly assigned to six treatment groups with five replicates of 10 geese (five males and five females) each. Geese were fed *ad libitum* for 35 days. Results showed that the geese fed with 4 and 8% FAM exhibited no significant effects on their final body weights (FBW) and average day gain (ADG) ($P$>0.05), whereas 12% or higher FAM caused poor growth of the geese compared with control diet (linear (L): $P$<0.05). The average daily feed intake (ADFI) (quadratic (Q): $P$<0.05) and feed conversion ratio (FCR) (L: $P$<0.05) with 8% or higher supplementation level were higher than those of the control group. The activities of antioxidant enzyme in serum increased, and the level of malondialdehyde (MDA) significantly decreased with increasing dietary FAM level (L: $P$<0.05). However, no significant differences were observed at 8% or lower supplementation level for glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) ($P$>0.05) and at 4% for catalase (CAT) supplementation level compared with the control group. All diets containing FAM increased digestive enzyme activities in geese. However, geese fed diets with 12% FAM supplementation showed the highest trypsin activities in pancreas (Q: $P$<0.05). Supplementation with 12% or higher FAM significantly increased amylase activities in pancreas (L: $P$<0.05) and duodenum (L: $P$<0.05) compared with the control group. Significant differences were not observed in total anaerobic bacteria between geese fed with FAM and control diets on day 35 ($P$>0.05). The numbers of *Bifidobacterium* and *Lactobacillus* in the cecum of geese fed with FAM significantly increased (L: $P$<0.05), but no significant effects were found with 4 and 8% FAM supplementation levels compared with the control ($P$>0.05). By contrast, the coliform counts of cecum decreased with increasing inclusion of FAM, but these counts were significantly reduced in geese fed diets with 12% or higher FAM supplementation level (L: $P$<0.05). Collectively, our results indicated that supplementation of the basal geese diet with 8% FAM had no apparent adverse effects on growth performance, serum antioxidant enzyme activities, and digestive parameters and beneficial microbiota.

**Keywords:** fermented alfalfa meal, geese, growth performance, serum antioxidant enzyme, digestive enzyme, cecal microflora

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## 1. Introduction

Among forage legumes, alfalfa is considered one of the best

due to its high protein and mineral contents (NRC 1998). Alfalfa improves nutrient absorption efficiency, enhances animal productivity (Sibbald 1979), and generates higher yields (Yan *et al.* 2010). Other researchers have also reported that incorporating moderate amounts of alfalfa meal (AM) in the diets of experimental animals improves growth performance (Shi *et al.* 2011) and reduces physiological stress (Landers *et al.* 2005). However, Kass *et al.* (1980) reported that poor palatability of AM reduces feed intake of swine and thus influences their growth performance. The negative effects of AM are probably related to its high fiber content (Cheeke *et al.* 1983; Thacker and Haq 2008). Renteria-Flores *et al.* (2008) reported that feed intake decreases as the fiber level of alfalfa meal increases in swine diet. However, the negative aspects of AM can be changed by adjusting its supplementation levels in experimental animal rations or by fermenting to degrade fiber and improve the palatability of AM.

Solid-state fermentation (SSF) is a common method to improve the functional and nutritional properties of AM (Pandey *et al.* 2000). A commonly used microorganism for this purpose is *Bacillus subtilis* (Liu 2006), which is speculated as the main factor that affects the quality of fermented alfalfa meal (FAM) (Cao *et al.* 2002). *B. subtilis* is a non-pathogenic organism that can produce enzymes, such as protease, lipases, and amylase, and is a suitable probiotic for application in poultries (Tannock 2001; Boguhn *et al.* 2006; Chen *et al.* 2009). Chiang *et al.* (2010) reported that the *B. subtilis*-fermented ingredients have a positive influence on digestive enzyme activity and bacterial ecology in broiler chicks. In addition, such fermented products contain numerous bioactive ingredients with diverse physiological functions, including growth promotion, antioxidation, immunity improvement and antibacterial action (McDonald *et al.* 1991). Thus, the fermented products benefit poultry by improving its digestive enzyme activities and anti-oxidative functions in serum, which enhances poultry growth and promotes physiological functions (Zhuang *et al.* 2009).

The Gushi goose is an important and emerging poultry species, and its production is becoming more widespread in Henan Province (China). However, only a few studies have been reported on the effects of AM or FAM on the species owing to the absence of feeding standards for geese. A number of studies suggest that maize and AM are used in the production of other geese (Wang *et al.* 2010). In addition, the increasing demand and high price of soybean meal (SBM) with the expansion of intensive livestock production have increased dramatically in recent years, necessitating a search for alternative protein sources. One recent research shows that FAM is likely to be one of the alternatives to SBM (Laudadio and Tufarelli 2010). Although studies have shown that alfalfa silage, as a feed ingredient, effectively promotes growth and enhances the blood physio-biochemical indexes of lactating dairy cow (Plaizier 2004), Boer goat (Yan *et al.* 2010), and dairy cow (Wang *et al.* 2015), information on the application of FAM in artificial feeds is limited. Thus, the present study evaluated the effect of different replacement ratios of SBM by FAM on the bird's growth performance, serum antioxidant level, digestive enzyme activities, and cecal microflora content.

## 2. Materials and methods

### 2.1. FAM preparation

The alfalfa used for the study was harvested at the flowering stage on June 20, 2014 (Henan University of Technology, Henan Province, China). The plants were rinsed twice under running water to remove dirt, drained through a strainer at 30°C for 24 h, and then ground to a particle size of <2.0 mm using a 2.0-mm sieve. The alfalfa underwent SSF with *B. subtilis* ACCC 01746.

*B. subtilis* was generously provided by Dr. Hu Yuanshen (College of Bioengineering, Henan University of Technology). The strain was precultured on potato dextrose agar and later cultured in culture broth medium (containing 0.25% yeast extract, 0.25% $K_2HPO_4$, and 0.4% $KH_2PO_4$ at pH 7.0) at 35°C for 24 h. These culture conditions yielded a *B. subtilis* suspension with a density of $1\times10^6$ cells $mL^{-1}$ culture. For SSF, 50 kg of sterilized AM, 100 mL of culture, and 20 L of distilled water were mixed until uniformity (50% moisture) and then cultivated at 32–35°C, which is the optimal growth temperature range for *B. subtilis*. The mixture underwent anaerobic fermentation for 7 days (pH was maintained at a natural level). The FAM was then dried in a hot air oven at 30°C for 20 h and refrigerated until mixing with the diets. Dried FAM sample (1 g) was diluted with 90 mL of saline. Further dilutions were made as required. The standard pour plate method was employed to determine viable cell counts, and the microbial species were determined by the colony morphology, and provided a strain density of $1.52\times10^8$ cells $g^{-1}$ FAM. AM (10 g) and FAM (10 g) were sampled to analyze the alfalfa saponin and chemical composition. Dry matter, crude protein, crude fiber, crude ash, calcium, and phosphorus were determined following the methods of the AOAC (2000). Amino acid contents of the samples (AM, FAM, SBM, and experimental diets) were analyzed using a high-performance liquid chromatography autoanalyzer (Waters PICO TAG amino acid autoanalyzer; Millipore, MA, USA) following standard procedures. A similar procedure as described for soybean meal was followed for FAM treatment. The differences in the ingredients of AM, FAM, and SBM are shown in Table 1.