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Effects of dietary supplementation with alfalfa (*Medicago sativa* L.) saponins on lamb growth performance, nutrient digestibility, and plasma parameters

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ABSTRACT

Fifty Hu male lambs (aged three to four months with a body weight of 22.3 ± 0.15 kg) were chosen to study the effects of supplementation with dietary alfalfa saponins (AS) on growth performance, nutrient digestibility, and plasma parameters. Lambs were assigned to five groups with 10 lambs per group in a completely randomized design. Different diets were offered at a 50:50 forage to concentrate ratio in which different amounts of AS were provided (0, 500, 1000, 2000, or 4000 mg/kg of dry matter intake). Diets were offered twice daily (at 08:30 and 16:30) for a period of 90 days (three months). Growth performance, apparent nutrient digestibility, body measurement indexes, and plasma parameters were determined each month. The final body weight differed among treatment groups during the initial ($P = 0.011$) and final months ($P = 0.039$) of the trial, respectively. However, no detectable effects of AS treatment on average daily gain ($P = 0.072$) or feed conversion ratio ($P = 0.113$) were found. Nutrient digestibility increased with AS dose, especially the digestibility of dry matter ($P = 0.005$), crude protein ($P = 0.005$), and acid detergent fiber ($P = 0.013$) on average. No significant differences were found among treatments for all body measurement indexes. Plasma glucose ($P = 0.016$), triglyceride ($P = 0.018$), and alanine transaminase ($P = 0.002$) levels decreased with increasing AS dose on average. These results indicated that AS plays an important role for increasing both nutrient digestibility and levels of plasma metabolites.

1. Introduction

Alfalfa (*Medicago sativa* L.) hay and silage are regarded as substantial feedstuffs for herbivores and provide abundant feed protein and physically effective neutral detergent fiber (NDF) (Beauchemin, 1991). In China, more than three million tons of alfalfa hay are produced per year. Furthermore, more than one million tons per year of alfalfa hay are currently imported to meet the growing need of domestic animal production (Palmonari et al., 2014). One of the most valuable plant secondary metabolites of alfalfa hay are saponins, which can be found in many leguminous plants. The saponins consist of a fat-soluble nucleus with either a steroid or triterpenoid structure (Cheok et al., 2014), which possess amphiphilic properties. This structure equips saponins with a

Abbreviations: ADG, average daily gain; ADF, acid detergent fiber without a heat stable amylase and expressed inclusive the residual ash; AS, alfalfa saponins; ALT, alanine transaminase; AST, aspartate transaminase; BLI, body length index; BUN, blood urea nitrogen; DMI, dry matter intake; FCR, feed conversion ratio; FBW, final body weight; GH, growth hormones; GLU, glucose; HGI, heart girth index; IGF-1, insulin-like growth factor-1; SI, somatic index; NDF, neutral detergent fiber without a heat stable amylase and expressed inclusive the residual ash; T-CHO, total cholesterol; TG, triglyceride; T3, tri-iodothyronine; T4, thyroxine

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membranolytic activity and explains their anti-bacterial, anti-tumor, and anti-inflammatory properties in animals (Wojciechowski et al., 2016). In addition, saponins can act on cholesterol and control the lipid metabolism through their capacity for binding cholesterol in the intestinal tract and other tissues (Malinow, 1984). In the animal production industry, alfalfa saponins, along with other bio-active components from alfalfa such as flavonoids, polysaccharides, and carotenoids (Hwang et al., 2001), are becoming increasingly popular due to their anti-oxidation properties and outstanding performance for promoting growth traits in weaned piglets (Shi et al., 2014) and fish (Couto et al., 2014). Due to their anti-bacterial activity, saponins also help to regulate ruminal fermentation, e.g. by decreasing the number of protozoa in the rumen, increasing N metabolism efficiency, and reducing methane emission in both *in vivo* (McMurphy et al., 2014) and *in vitro* (Rira et al., 2015). Especially, the saponins of the alfalfa root decreased ruminal protozoal populations, increased flow of total duodenal N, and reduced ruminal and total tract apparent digestibility (Klita et al., 1996).

A higher growth rate with lower feed consumption and improved meat quality are the main targets of the lamb producing industry. Fattening lambs, younger than one-year, have acute changes in their traits every month, including growth rate, digestive tract morphology changes, and variations in blood hormone levels. However, whether plant saponins supplementation enhances growth performance and animal meat quality of growing lambs has not reached a unanimous conclusion based on the relevant published reports. The reasons can be ascribed to differences of the experimental animal species, plant extract resources, and basal diet conditions. Among plant saponins, *Quillaja saponaria* extract, ranging from 30 to 90 g/kg DMI dosage, have not been shown to improve feed digestibility, growth performance, or meat quality of Barbarine lambs (Nasri et al., 2011). Furthermore, tea saponins, ranging from 120 to 360 mg/kg DMI dosage, did not affect nutrient digestibility, patterns of rumen fermentation, or plasma metabolite concentrations (Zhou et al., 2012). Moreover, reports on supplemented alfalfa (*Medicago sativa* L.) saponins to the diet of growing lambs are rare. Therefore, our study, investigated the effect of alfalfa saponins (AS) supplementation on both growth rate and nutrient digestibility of growing lambs. Plasma bio-chemical parameters reflecting the health and bioavailability of nutrients for lambs were also investigated.

2. Materials and methods

2.1. Alfalfa saponins

The alfalfa saponin extract used in this study was purchased from a commercial pharmacy (Keemei Biotechnology Co., Ltd, Xian, China) and sieved through a 5-mm sieve. The purchased commercial product (with a purity of 601.3 g/kg dry matter) was prepared via extraction of saponins from the leaves and roots of alfalfa (*Medicago sativa* L.) and purified via high performance liquid chromatography, which was calculated by the equivalent of oleanolic acid standard substance (Cheok et al., 2014). The other components of the commercial product were fibrous matter, protein, and ash.

2.2. Animals, diets, and experimental design

The experiment was approved by the Institutional Animal Care Committee of the China Agricultural University in accordance with the criteria defined in the Guide for the Care of Laboratory Animals (Beijing, P.R. China). The Hu sheep is a Chinese indigenous breed originating near Taihu Lake in China and was chosen for this experiment. The experiment was conducted at the Runlin Animal Husbandry Co., Ltd from December 2014 to February 2015. Fifty male Hu lambs (aged three to four months) with an initial body weight of 22.3 ± 0.15 kg (mean ± SD) were selected. They were kept in individual pens (1.2 m × 1.5 m) equipped with a bamboo-made slatted floor and water taps, providing free access to water. Lambs were randomly allocated to five groups of ten lambs each (n = 10). Lambs received diets formulated to meet their maintenance and growth requirements (NRC, 2007). The basal diet consisted of roughage and concentrate, and the proportions and chemical compositions of the basal diet are listed in Table 1. The total saponin and polyphenol content (calculated via the equivalent of oleanolic acid and gallic acid) in Table 1 reflect the concentration in the basal diet without AS supplementation.

Among five treatment groups, one group of lambs received the control diet (CON). The other groups (namely groups ASI, ASII, ASIII, and ASIV) received different doses of AS extract powder which were mixed into the diet (500, 1000, 2000, and 4000 mg/kg dry matter intake, respectively). The amount of AS powder added into the diet was adjusted weekly depending on the increased dry matter intake of lambs. Feed was given twice per day at 08:30 and 16:30. During each meal, firstly, the lambs received the concentrate mixed with AS powder to ensure that the lambs ingested the sufficient dose of AS extract. After the concentrate was ingested, the roughage, which was mixed with corn silage and peanut vine, was provided. A 50 g/kg dry matter refusal of diet was allowed per meal. All lambs were adapted to housing conditions and dietary treatments for two weeks before starting the 90-day growth trial.

2.3. Growth performance traits

The entire growth trial lasted for three months followed by a fourteen-day adaptation. Throughout the experiment, feed and orts were sampled once per month. At the end of each month, live body weights were recorded prior to the morning feeding. The amount of diet offered and refused was recorded daily to estimate dry matter intake (DMI, kg). Average daily gain (ADG, kg/d) was calculated between feeding period intervals. The feed conversion ratio (feed to gain, FCR) was expressed as feed consumption per unit of body weight gain.