



# Effects of soybean meal treated with tannins extracted from pistachio hulls on performance, ruminal fermentation, blood metabolites and nutrient digestion of Holstein bulls



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## ARTICLE INFO

### Article history:

Received 7 September 2014

Received in revised form 15 February 2015

Accepted 16 February 2015

### Keywords:

Holstein bull

Tannin

Pistachio hulls

Growth performance

Ruminal fermentation

Blood metabolites

## ABSTRACT

This experiment was conducted to study the effects of soybean meal (SBM) treated with various amounts of tannins extracted from pistachio hulls on performance, ruminal fermentation, nutrient digestibility, and blood metabolites in Holstein bulls. Twenty-eight Holstein bull calves ( $256 \pm 63$  kg body weight) were randomly assigned to one of four dietary treatments for 14 weeks. Each treatment group received one of four SBM treatments included in a total mixed ration (TMR) at a constant rate of 105 g/kg DM. Soybean meal was treated with pistachio extract concentrate (PEC), which contained 111.4 g/kg total phenol and 71.3 g/kg total tannin per dry matter of extract, at four experimental treatment rates of 0 (SBM-0), 5 (SBM-5), 10 (SBM-10), and 15 (SBM-15) kg PEC per 100 kg SBM on a dry matter basis. Blood samples were collected monthly and ruminal fluid samples were collected on d 83 and d 85 of treatment. Treatment of SBM with PEC did not affect final body weight or dry matter intake (DMI), but adding PEC linearly increased average daily gain (L,  $P=0.001$ ) and feed efficiency (L,  $P=0.001$ ). Inclusion of PEC linearly decreased ruminal ammonia nitrogen (L,  $P=0.02$ ) and total protozoal population (L,  $P=0.002$ ), but did not affect concentrations of total volatile fatty acids (VFA), individual VFA or pH in the rumen. Total tract nutrient digestibility and blood metabolites were unaffected by SBM treatment with PEC, except for concentrations of albumin and total protein in plasma, which increased linearly (L,  $P=0.004$  and  $P=0.001$ , respectively) with increasing PEC treatment. These results suggest that PEC supplementation decreases ruminal ammonia nitrogen concentration and total protozoa population and improves growth performance of Holstein bulls.

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## 1. Introduction

Treatment of a dietary protein such as soybean meal (SBM) to decrease its solubility and thus its hydrolysis to ammonia in rumen fluid should increase the amount of the dietary protein that bypasses ruminal degradation without sacrificing nitrogen required for ruminal microbial growth (Nishimuta et al., 1974). Protection of dietary protein from ruminal microbial

**Abbreviations:** ADFom, ash-free acid detergent fiber; ADG, average daily gain; BUN, blood urea nitrogen; BW, body weight; CP, crude protein; DM, dry matter; DMI, dry matter intake; EE, ether extract; EAA, essential amino acid; F:G, feed to gain; L, linear; NAN, non-ammonia nitrogen; NDF, neutral detergent fiber; OM, organic matter; PEC, pistachio extract concentrate; RDP, rumen degradable protein; RUP, rumen undegradable protein; SBM, soybean meal; TMR, total mixed ration; VFA, volatile fatty acid.

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degradation increases the supply of amino acids to the small intestine (Stern, 1981). Thus, the efficiency of protein utilization by the animal should be improved (Driedger and Hatfield, 1972).

Tannins have been shown to decrease ruminal degradation of crude protein (CP) and to increase the amount of CP that reaches the abomasum and small intestine (Alipour and Rouzbehan, 2010), but Patra and Saxena (2011) indicated that the responses to tannin supplementation are variable and depend on the type, source and concentration of tannin used, animal species subjected to supplementation, and the basal diet fed. Plant secondary metabolites in pomegranate-peel extract (Abarghuei et al., 2014), and grape pomace extracts (Alipour and Rouzbehan, 2010) were found to improve ruminal fermentation, enhance amino acid flow to the duodenum (Mueller-Harvey, 2006), and increase muscle deposition and milk production (Vasta et al., 2008). Dietary tannins have also been shown to positively affect average daily gain (ADG) in steers grazing winter wheat (Min et al., 2006). However, Dentinho et al. (2007) reported decreased effective rumen degradability of SBM protein treated with tannin extracts from *Cistus ladanifer* and decreased *in vitro* intestinal digestibility of protein at low phenolic doses (12.5, 25 and 50 g total phenol/kg). When using SBM treated with 10–250 g of quebracho tannins per kg, Frutos et al. (2000) also observed decreased *in vitro* intestinal digestibility of protein at the greatest treatment rate. Further research is needed to determine if tannin treatment of dietary protein improves its digestibility.

On the other hand, high amounts of pistachio byproducts have been produced in Iran at an average rate of about 310,000 metric tons annually (Behgar et al., 2011). Pistachio byproducts are produced during de-hulling of pistachio nuts after harvesting and contain high concentrations of phenolic compounds and tannins, which can affect utilization of their nutrients by animals (Reed, 1995). Commonly, extraction of secondary metabolites is carried out using costly solvents, such as methanol, ethanol or acetone (Makkar, 2003). However water has effectively been used as a solvent during extraction to decrease cost and make pistachio extract concentrate (PEC) and economically feasible treatment for animal diets (Abarghuei et al., 2014). Hence, this experiment was conducted to assess the effects of three rates of PEC treated SBM, extracted by water, on performance, feed efficiency, ruminal fermentation characteristics, blood metabolites and nutrient digestion in Holstein bulls. We hypothesized that PEC treatment of SBM would improve protein metabolism and overall feed efficiency.

## 2. Materials and methods

### 2.1. Animal care

All experimental procedures were conducted according to The Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010) guidelines. All procedures and guidelines involving animals were approved by the Animal Experiment Committee at Tehran University, Karaj, Iran.

### 2.2. Pistachio hull, tannin extraction and treatments

Five metric tons of pistachio hulls (Fandoghi variety) were obtained from the pistachio processing factory, Ghom, Iran, during the summer of 2012 and were sun dried. A two-step extraction process was performed according to procedure by Capparucci et al. (2011) with some modifications to obtain PEC. In the first extraction step, the sun dried pistachio hulls were ground through a 0.5 mm screen and soaked in water at a ratio of 1:10 (w/v) for 24 h. The solution resulting from the first extraction step contained 72.3 g/kg total phenol and 41.8 g/kg total tannin on a dry matter basis. In the second step, the pistachio extract was filtered and boiled to achieve PEC containing 111.4 g/kg total phenol and 71.3 g/kg total tannin on a dry matter basis. Chemical composition of PEC and SBM treated with various levels of PEC are shown in Table 1. Analysis of phenolic compounds was conducted in three replicates as described by Makkar (2000). Total phenol was determined by using Folin–Ciocalteu's reagents, and the concentration was measured as tannic acid equivalent using tannic acid (Merck, Germany) as a standard. Total tannins were measured as described by Makkar (2000).

A total amount of 6 kg dried SBM was ground daily through a 1 mm screen for each treatment. Then, PEC was added to ground SBM to achieve ratios of 0, 50, 100 or 150 g PEC per Kg SBM. Treated SBM was then air-dried for 12 h to reach DM content of about 900 g/kg in whole product.

**Table 1**

Chemical composition (means  $\pm$  SD) of pistachio extract concentrate (PEC) and SBM treated with various levels of PEC.

Chemical composition (g/kg DM)	PEC	SBM-0 <sup>a</sup>	SBM-5 <sup>a</sup>	SBM-10 <sup>a</sup>	SBM-15 <sup>a</sup>
Dry matter	850 $\pm$ 2.5	922 $\pm$ 2.1	919 $\pm$ 2.3	915 $\pm$ 2.2	911 $\pm$ 2.3
Crude protein	153 $\pm$ 0.8	445 $\pm$ 0.6	430 $\pm$ 0.9	416 $\pm$ 0.4	401 $\pm$ 0.5
Organic matter	777 $\pm$ 1.7	939 $\pm$ 1.1	931 $\pm$ 1.4	923 $\pm$ 1.5	915 $\pm$ 1.7
Ash	224 $\pm$ 1.7	61.1 $\pm$ 1.1	69.1 $\pm$ 1.4	77.2 $\pm$ 1.5	85.3 $\pm$ 1.7
Total phenolic	112 $\pm$ 0.4	0	56.0 $\pm$ 0.4	112 $\pm$ 0.4	168 $\pm$ 0.4
Total tannins	71.3 $\pm$ 0.3	0	35.6 $\pm$ 0.3	71.3 $\pm$ 0.3	108 $\pm$ 0.3

PEC, pistachio extract concentrate; SBM, soybean meal; SD, standard deviation.

<sup>a</sup> SBM, treated with 0, 5, 10 and 15 kg PEC per 100 kg DM of SBM.

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