Meat Science 105 (2015) 1–7

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Effects of dietary supplementation with green tea polyphenols on digestion and meat quality in lambs infected with *Haemonchus contortus*

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ARTICLE INFO

Article history:
Received 1 July 2014
Received in revised form 20 January 2015
Accepted 9 February 2015
Available online 14 February 2015

Keywords:
Tea polyphenols
Haemonchus contortus
Meat quality
Lamb

ABSTRACT

Ujumqin sheep are susceptible to infection by the gastrointestinal nematode *Haemonchus contortus*, which reduces productivity and total meat yield in sheep. Thus, the effects of green tea polyphenol (GTP) supplements (0, 2, 4, or 6 g of GTP/kg feed) on dietary nutrient digestibility and meat quality in lambs infected with *H. contortus* were examined; control lambs were not infected. *H. contortus* infections did not affect digestion but the apparent digestibilities of nutrients were decreased by dietary 2 g of GTP/kg feed supplementation. There was an interaction between treatment and sampling time on plasma total protein, urea nitrogen, and amino acid concentrations. The antioxidant activity and meat color of INFGTP0 lambs decreased. In conclusion, *H. contortus* infections in lambs decreased meat quality, but appropriate levels of dietary GTP supplementation diminished these negative effects though lower dose of GTP supplement showed negative effects on digestion.

1. Introduction

Gastrointestinal nematode (GIN) infection in sheep and goats has resulted in a major health, welfare, and economic problem worldwide (Marume, Chimonyo, & Dzama, 2011; Miller & Horohov, 2006). The blood-feeding nematode *Haemonchus contortus* is especially harmful because it decreases feed efficiency and causes anemia and even death in host animals (Getachew, Dorchies, & Jacquiet, 2007). In Northern China, *H. contortus* infection has been identified as an important problem for sheep production because of its adaptability to the climate conditions of this region (Yin et al., 2013).

Over the past five decades, repeated use of chemical anthelmintic drugs to control GINs has caused prevalent drug resistance GIN populations, drug residues in animal products and loss of productivity in host animals (Jackson & Coop, 2000; Tsiboukis, Sazakli, Jelastopulu, & Leotsinidis, 2013), which have stimulated interest in alternative approaches to parasite control that are less reliant on chemotherapeutics. Thus, many studies have explored the use of plant-derived phenolic compounds, especially tannins, which show direct and indirect anthelmintic activity for GIN control in ruminants (Hoste, Jackson, Athanasiadou, Thamsborg, & Hoskin, 2006; Marume, Chimonyo, & Dzama, 2012; Vargas-Magaña et al., 2014).

In a recent study, Brogna et al. (2014) reported that feeding tannins and saponins extracted from quebracho did not detrimentally affect meat quality in sheep infected with *H. contortus*, but the ratio of C14:1 *cis*-9 increased and the cholesterol concentration decreased. However, although GIN infections affect some parameters of meat characteristics, including the meat color stability, fatty acid composition and conformation of small ruminants (Arsenos et al., 2007, 2009), an important question that has not yet been addressed is to what extent *H. contortus* infection affects meat quality in sheep.

Feeding of ruminants with high-polyphenols plants could improve meat quality by increasing antioxidants into the circulatory system, distributed and retained in the meat and therefore increasing sensory, flavor and juiciness scores of meat (Moyo, Masika, & Muchenje, 2014; Qwele et al., 2013). Green tea extracts contain polyphenolic compounds, including flavanols, flavandiols, flavonoids, and phenolic acids, which account for 30% of the dry weight of green tea leaves (Mukhtar & Ahmad, 2000). Green tea polyphenols (GTPs) improve growth performance and meat quality parameters, including meat color, tenderness and shelf life due to their antioxidant properties in cattle, sheep and goats in vivo and in vitro (Mitsumoto, O'Grady, Kerry, & Buckley, 2005; Wang & Xu, 2013; Zhong et al., 2009). However, further studies are required to determine whether GTPs exert positive effects on growth and meat quality in sheep infected with GINs.

Thus, the objectives of the present study were to determine the effects of dietary supplementation with GTPs on digestibility and meat quality parameters in lambs artificially infected with infective larvae (L3) of *H. contortus*.

2. Materials and methods

2.1. Chemicals, reagents, animals, diets and management

The use of the animals and the experimental procedures were approved by the Animal Care Committee at the Institute of

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http://dx.doi.org/10.1016/j.meatsci.2015.02.003
0309-1740/

Geography and Agroecology in the Chinese Academy of Sciences in Jilin, China.

All lambs in the same flock with similar age of 3 months old were anthelmintic treated using a combination of moxidectin injection (0.8 mg/kg BW), levamisole injection 10% (11.2 mg/kg BW), and albendazole tablet (21.6 mg/kg BW) to remove any background GIN infection at 30 days prior to the following 56 day feeding period. Then, 30 male Ujumqin lambs with a similar body weight and an average age of 120 ± 10.3 days were selected from the dewormed flock and randomly assigned to one of five groups for a feeding period of 56 days. All chemicals, reagents, treatments, diet, and management of animals were the same as described by Zhong, Li, Sun, and Zhou (2014). The 5 treatment groups were as follows: (1) lambs uninfected with *H. contortus* served as the control group (UNINF); (2) lambs infected with *H. contortus* and supplemented no GTP (INFGTP0); (3) lambs infected with *H. contortus* and supplemented 2 g of GTP/kg feed (on a dry-matter [DM] basis) (INFGTP2); (4) lambs infected with *H. contortus* and supplemented 4 g of GTP/kg feed (INFGTP4); and (5) lambs infected with *H. contortus* and supplemented 6 g of GTP/kg feed (INFGTP6).

2.2. Sampling procedures

Total feces of each lamb were collected for 5 consecutive days on days 51 to 55 of the experimental period. Feces from each lamb on each day were composited, weighed, and acidified with 0.1 g/kg sulfuric acid at a ratio of 3 mL sulfuric acid/kg feces. Then, a 100-g subsample of daily acidified feces was stored at −20 °C until analyzed for nutrients. At the end of the experiment, subsamples of the diets and feces were dried at 105 °C for 3 h to analyze the DM content. Sub-samples of the feed and feces were dried at 65 °C for 48 h, ground through a 1-mm screen (Wiley mill, Arthur H. Thomas Co., Philadelphia, PA, USA), and stored at 4 °C for analysis of chemical composition.

Before the morning feedings on days 0, 14, 28, 42, and 56 of the experimental period, 5-mL blood samples were collected according to the same procedure of Zhong et al. (2014) for analysis of biochemical parameters and amino acid (AA) profiles.

At the end of the feeding period, all lambs were electrically stunned and slaughtered by exsanguination using commercial procedures conducted according to the recommendations of the Animal Ethics Committee at the Institute of Geography and Agroecology. The electric stunning was applied using a head-only stunner with the stunning electrodes between the eyes and ears on either side of the head. The current was delivered at a constant voltage of 220 V, 1.0 A for 3 s with scissor tongs (NBT m.p.s. B.V., Stork). The lambs were bled within 20 s after stunning and then were hung to remove their skin, head (at the occipito-atlantal joint), forefeet (at the carpal–metacarpal joint), and hind feet (at the tarsal–metatarsal joint). The viscera, including the heart, lungs, liver, spleen, kidneys, rumen, and pancreas, were removed. Afterwards, carcasses were weighed and chilled under commercial conditions at 4 °C for 12 h in total darkness. Thereafter, the left side of carcass was used for measurements of meat quality parameters. After carcass refrigeration at 4 °C for 24 h, approximately 300 g of the left longissimus thoracis et lumborum (LTL) was sampled. Some LTL subsamples (100 g each) were immediately stored at 4 °C for the determination of shear force, pH, and meat color at 24 h postmortem. The remaining LTL subsamples (200 g each) were sliced, individually vacuum-packed, and stored at −20 °C for the measurement of total heme pigment (THP) concentrations, AA profiles, and lipid oxidation levels.

2.3. Analytical procedures

Diet and fecal subsamples were initially dried at 105 °C for 3 h to determine the DM content. Before the laboratory analyses, all feed and fecal sub-samples were additionally dried at 65 °C for 48 h and ground to pass through a 1-mm screen for analysis of chemical composition. The total ether extract (method no. 920.39; Association of Official Analytical Chemists (AOAC, 1995)) and nitrogen (N) content (method no. 988.05, AOAC, 1995) in the diets and feces were analyzed. The crude protein (CP) content was calculated as 6.25 times the N content. The levels of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the method of Van Soest, Robertson, and Lewis (1991) using heat-stable amylase and sodium sulfite for NDF determination, and expressed exclusive of residual ash for all samples of the diets and feces.

The plasma was used to determine the total protein, albumin, globulin, total cholesterol, triglyceride and plasma urea nitrogen (PUN) concentrations using an automated biochemical analyzer (Hitachi, 7020, Beijing, China) and commercial kits (Jiancheng Biology Co., Nanjing, China). Plasma AA concentrations were determined according to the method of Iwaki et al. (1987) using cation exchange chromatography and lithium citrate buffer solutions (LKB Biochrom amino acid analyzer, model 4151 Alpha Plus, Pharmacia, Uppsala, Sweden).

The pH values of the LTL samples were measured using a portable pH-meter (Testo 205, Testo AG, Lenzkirch, Germany) with a plastic body, spear-tipped probe coupled with a temperature probe. Shear force analysis was conducted according to the guidelines of the American Meat Science Association (AMSA, 1995) using a Warner-Bratzler Shear Device (C-LM3B, Beijing, China). The lipid oxidation levels of the LTL samples were measured using the 2-thiobarbituric acid reactive substance (TBARS) method modified as described by Schmedes and Hölmer (1989), and results were expressed as micrograms of malonyl dialdehyde (MDA) per gram of meat. A Minolta color-meter (model CR410, Minolta Camera Co. Ltd., Osaka, Japan) was used to measure the meat color parameters of lightness (L^*), redness (a^*), and yellowness (b^*). The THP content in the meat was measured at 24 h postmortem according to the method of Ockerman (1985). The subsamples of the LTL were freeze-dried and the dry matter was used for the preparation of acid hydrolysates to estimate AA concentrations according to the method of Gilka et al. (1989).

2.4. Statistical analyses

The data gathered on digestibility of diet nutrients and meat quality parameters were analyzed using a General Linear Model followed by Duncan's multiple range tests (SAS, 2002). The data gathered on blood metabolites were analyzed with SAS (2002) using the MIXED model procedure described by Littell, Milliken, Stroup, and Wolfinger (1996), with a model consisting of dietary treatment, sampling time, and treatment × time interaction as fixed effects and with an animal as the random effect. The measurements obtained from each lamb at different sampling times were treated as repeated measures. The means were separated using the least square mean and presented with the standard error of the mean (SEM). The results were considered statistically significant at $P \leq 0.05$.

3. Results

3.1. Diet digestibility

The apparent digestibility of dietary nutrients is shown in Table 1. The *H. contortus* infection in lambs without GTP supplementation did not affect the apparent digestibility of DM, CP, NDF, ADF, and fat. However, diet digestion in infected lambs was influenced by the dietary GTP supplements. The apparent digestibility of DM ($P = 0.026$), CP ($P = 0.037$), and fat ($P = 0.011$) in the INFGTP2 group was reduced significantly compared with that in the UNINF group.

3.2. Blood metabolites

Blood biochemical parameters and plasma AA profiles are shown in Table 2. There was an interaction between treatment and sampling time