Livestock Science 172 (2015) 5–15

Contents lists available at ScienceDirect

Livestock Science

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

Effects of nitrate and fumarate in tree leaves-based diets on nutrient utilization, rumen fermentation, microbial protein supply and blood profiles in sheep

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ARTICLE INFO

Article history:
Received 30 May 2014
Received in revised form
11 December 2014
Accepted 12 December 2014

Keywords:
Nitrate
Fumarate
Tree leaves
Sheep
Nutrient utilization
Rumen fermentation

ABSTRACT

This experiment was conducted to study the effects of nitrate and fumarate on nutrient utilization, rumen fermentation and blood biochemical profile in sheep fed on tree leaves-based diet. Thirty two matured male Chokla breed of sheep (2–3 years old and 46.9 ± 0.95 kg average body weight) were equally distributed in a randomized block design in four groups with similar average age and mean body weight. All groups were fed diets containing roughage and concentrate in a 70:30 ratio. Control group (C) was fed with cenchrus (*Cenchrus ciliaris*) straw as an only roughage source, whereas other three groups (T1, T2 and T3) were fed with cenchrus straw, ardu (*Ailanthus excelsa*) leaves and khejri (*Prosopis cineraria*) leaves (50:25:25) as roughage sources. Animals in the T1 group were not supplemented with any feed additive; whereas, animals in the T2 and T3 groups were added with 2% potassium nitrate of the concentrate mixture and 2% fumarate of the dry matter (DM) intake, respectively. There were no effects ($P > 0.10$) of any treatments on DM intake, BW change or nutrient utilization. N retention increased in treatment groups compared with control due to greater amount of N intake. Concentrations of glucose, haemoglobin, albumin, blood urea nitrogen and creatinine, and different blood enzyme concentrations were not affected ($P > 0.10$) by any treatments, but concentrations of total protein, globulin and cholesterol in blood were higher for T1 and T3 than for C and T2 treatments. Microbial N supply in terms of digestible OM intake or digestible OM retained in the rumen did not differ among treatments. Total amylase and carboxymethyl cellulase concentrations were similar among the groups. However, total xylanase activity increased in T3 group than other groups. A reduction in average propionate concentration ($P=0.020$) was observed for the T2 treatment in comparison with the C and T1 groups. Concentrations of ammonia N, total N and trichloroacetate-perceptible N were lower in control than other treatments, but concentrations of soluble N were not affected ($P > 0.05$) by any treatments. In conclusion, fumarate (2% of DM) may be included in the diet of sheep fed with ardu–khejri leaves without affecting nutrient utilization and rumen fermentation, whereas ardu–khejri leaves diet containing nitrate (0.6% of DM) may affect rumen fermentation.

1. Introduction

Greenhouse gas (GHG) emissions have become an increasingly important focus worldwide due to their effects on global warming and climate change (IPCC, 2007). Methane

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http://dx.doi.org/10.1016/j.livsci.2014.12.008
1871-1413/

is the second largest anthropogenic GHG, which contributes 14.3% of total anthropogenic GHG emissions (IPCC, 2007). Indian livestock accounts for about 13.9% of the global enteric methane emission (global enteric methane emission of 85.63 Tg/year) and the enteric methane emission has been projected to be 12,848 Gg in 2012, and this could be increased to 14,553 Gg in 2020 without proper mitigation measures (Patra, 2012). Most of the CH_4 from ruminant livestock originates from microbial fermentation of carbohydrates in the rumen, which also accounts for a substantial gross energy loss of feeds depending upon the types of diets. Therefore, inhibition of CH_4 production in the rumen had been attempted to increase the utilization of feed energy for production purpose and mitigate GHG emissions. A number of methane mitigation strategies have been evaluated in ruminants.

Foliages from trees and browses comprise important feed resources for small ruminant production in tropical countries especially for landless and marginal farmers. In these regions of developing world, feeds from conventional resources are limited and often too expensive for the low input–output livestock production system. These multipurpose tree foliages contain low to moderate levels of N, minerals and vitamins (Patra, 2009) that are deficient in many low-quality roughages. Thus, the multipurpose tree foliages have been suggested as a solution to feeding of ruminants in the tropical areas, especially as supplementary feeds to low-quality forages (Patra, 2009, 2010). *Prosopis cineraria* (khejri) and *Ailanthus excelsa* (ardu) foliages are widely used for small ruminant production for these purposes (Bhatta et al., 2007). In a previous study, *P. cineraria* and *A. excelsa* foliages containing plant secondary compounds namely tannins have shown to lower enteric methane production in vitro (Pal et al., 2014). Thus, tree leaves may be used as supplementary feeds as well as methane mitigation.

Nitrate suppresses methane production by acting as a hydrogen sink as well as directly inhibiting the methanogens (Patra and Yu, 2013). Addition of fumarate that is an intermediate of propionate production enhances propionate concentration with a stoichiometric decrease in hydrogen availability for methane synthesis (Kolver et al., 2004). Fumarate and nitrate have been evaluated widely for nutrient utilization and rumen fermentation in ruminants (Kolver et al., 2004; Patra and Yu, 2013), but not in tree leaves-based diets. *P. cineraria* and *A. excelsa* foliages have shown to lower enteric methane production additively along with nitrate and fumarate in vitro (Pal et al., 2014). Thus, the objective of this study was to investigate the effects of combinations of *A. excelsa* and *P. cineraria* leaves plus nitrate or fumarate on nutrient utilization, rumen fermentation, microbial protein supply and blood biochemical profile in sheep.

2. Materials and methods

2.1. Animals, experimental design, management and diets

Thirty two matured 2–3 years old male Chokla breed of sheep (*Ovis aries)* were used as experimental animals. Prior to the beginning of the study, the sheep were fed with cenchrus (*Cenchrus ciliaris*) straw and a concentrate mixture (70:30 ratio) for 21 days adaptation period. After adaptation, the animals were equally distributed in a randomized block design in four dietary groups with similar average age and mean body weight. All groups were fed diets containing roughage and concentrate in 70:30 ratio. Control group (C) was fed with cenchrus straw as an only roughage source, however, other groups were fed with cenchrus straw, ardu and khejri leaves (50:25:25) as a roughage source. Among the leaves feeding groups, sheep was fed either without any additive (T1), or with 2% potassium nitrate of the concentrate (T2) or 2% fumarate of the DM intake (T3). Sheep in C, T1 and T3 groups were fed with the concentrate-1 and T2 with concentrate-2 (Table 1). Potassium nitrate at the dose of 2% of concentrate-2 and fumarate as di-sodium fumaric acid at 2% of DM intake were fed to each animal of the respective group mixing with the concentrate mixture prior to feeding. Concentrate-1 and -2 had similar protein, but concentrate-2 contained potassium nitrate at 2% of DM replacing 50% protein ingredients. The experimental feeding lasted for 45 days. All the sheep were kept in a well ventilated shed with facilities for individual feeding and watering in separate feeding troughs. Sheep were maintained under proper vaccination and deworming schedules to ensure that all the animals were in apparently healthy condition and free from any disease at the onset of experiment.

Measured amounts of the concentrate mixture followed by roughages ad libitum (extra 10% of the previous day's intake) were offered in the morning. The amount of feed offered was based on previous day voluntary feed intake in order to maintain the roughage to concentrate ratio of 70:30 throughout the experiment. Body weight of the individual sheep was taken weekly in the morning before feeding in order to assess the changes in body weight. Animals had access to ad libitum fresh and clean drinking water during the day. Other care and management were also identical for all groups. Body weights of the animals were taken at the beginning (day 1) and end (day 45) of the experimental feeding.

2.2. Collection of blood and rumen fluid samples

Blood was collected from 5 animals in each group both at the beginning and on day 43 of experimental period into sterile centrifuge tubes. Immediately after collection, the tubes were placed in an ice bath and serum was harvested subsequently by centrifuging the blood at 3500 rpm for 10 min in a centrifuge machine. The serum samples were stored at −20 °C in multiple small sterile containers for subsequent analyses. Haemoglobin content in the blood and urea and glucose in serum were determined on the day of collection.

The rumen fluid samples were collected for 2 consecutive days (on day 44 and 45). The samples were collected from 5 animals in each group using flexible stomach tube before morning feeding and 4 h post feeding. The pH of rumen fluid was determined immediately using digital pH meter (Electronics Corporation of India Limited, Hyderabad). About 15 ml of rumen fluid was preserved for enzyme estimation in sterile bottles at −20 °C. The remaining rumen fluid was strained through four layers of muslin cloth. Strained rumen