

Effects of Diets with Different Ratios of Neutral Detergent Fiber to Non-Fibrous Carbohydrates on Methane Emissions in Meat Sheep: Postprint

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Abstract

This study aimed to investigate the effects of diets with different neutral detergent fiber (NDF) to non-fibrous carbohydrate (NFC) ratios (NDF/NFC) on methane emissions from meat sheep. A 4×4 complete Latin square design was adopted, with 16 Dorper × Small-tailed Han crossbred wethers randomly divided into 4 groups (n=4 per group) and fed total mixed pellet diets (with corn stover as the roughage source) at maintenance level with NDF/NFC ratios of 3.02 (Diet 1), 2.32 (Diet 2), 1.58 (Diet 3), and 1.04 (Diet 4). The experiment consisted of 4 periods, each lasting 18 days, including a 3-day adjustment period, a 7-day preliminary period, and an 8-day formal trial period, during which methane production, dietary gross energy, and apparent digestibility of nutrients were measured. The results showed that the daily methane emission of Diet 2 was significantly higher than that of Diets 3 and 4 (43.43 L/d vs. 38.88 and 35.98 L/d; $P<0.05$). Compared with Diet 1, the methane emission per kilogram of dry matter intake (DMI) of Diets 2 and 3 increased significantly (38.00 L/kg DMI vs. 42.24 and 41.69 L/kg DMI; $P<0.05$), but there was no significant difference among Diets 2, 3, and 4 ($P>0.05$). With decreasing NDF/NFC ratio, methane emission per kilogram of digestible organic matter (DOM) decreased gradually, and the methane emission per kilogram DOM of Diet 4 was significantly lower than that of Diets 1, 2, and 3 (58.78 L/kg DOM vs. 75.00, 73.35, and 64.11 L/kg DOM; $P<0.05$). With decreasing NDF/NFC ratio, methane emission per kilogram of neutral detergent fiber intake (NDFI) or acid detergent fiber intake (ADFI) increased gradually, with significant differences among all diets ($P<0.05$). In conclusion, considering the apparent digestibility of nutrients and methane emission efficiency, a corn stover diet with an NDF/NFC ratio of 1.04 is most suitable as a methane mitigation diet for meat sheep at maintenance level.

Full Text

Effects of Different Neutral Detergent Fiber to Non-Fibrous Carbohydrate Ratios in Diets on Methane Emissions from Meat Sheep

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Abstract

This study investigated the effects of different neutral detergent fiber (NDF) to non-fibrous carbohydrate (NFC) ratios (NDF/NFC) in diets on methane emissions from meat sheep. The experiment employed a 4\$×\$4 Latin square design, in which 16 Dorper × Small-tailed Han crossbred wethers were randomly divided into 4 groups of 4 sheep each. The sheep were fed total mixed pellet diets (with corn stalk as the roughage source) at maintenance level with NDF/NFC ratios of 3.02 (Diet 1), 2.32 (Diet 2), 1.58 (Diet 3), and 1.04 (Diet 4). The trial consisted of 4 periods, each lasting 18 days, including a 3-day adjustment period, a 7-day preliminary period, and an 8-day experimental period. During the experimental period, methane production and the apparent digestibility of gross energy and nutrients were measured. The results showed that daily methane emission for Diet 2 was significantly higher than that for Diets 3 and 4 (43.43 L/d vs. 38.88 and 35.98 L/d; $P<0.05$). Compared with Diet 1, methane emission per kilogram of dry matter intake (DMI) for Diets 2 and 3 increased significantly (38.00 L/kg DMI vs. 42.24 and 41.69 L/kg DMI; $P<0.05$), but no significant differences were observed among Diets 2, 3, and 4 ($P>0.05$). As the NDF/NFC ratio decreased, methane emission per kilogram of digestible organic matter (DOM) gradually decreased, with Diet 4 being significantly lower than Diets 1, 2, and 3 (58.78 L/kg DOM vs. 75.00, 73.35, and 64.11 L/kg DOM; $P<0.05$). Methane emissions per kilogram of neutral detergent fiber intake (NDFI) or acid detergent fiber intake (ADFI) increased gradually as NDF/NFC decreased, with significant differences among all diets ($P<0.05$). In conclusion,

considering both nutrient apparent digestibility and methane emission efficiency, a corn stalk diet with an NDF/NFC ratio of 1.04 is most suitable as a methane mitigation formula for meat sheep at maintenance level.

Keywords: meat sheep; NDF/NFC; methane emission; digestible organic matter

Introduction

Plant fiber fermentation in the rumen produces methane, which not only reduces dietary energy utilization efficiency but also contributes to greenhouse effects and global warming. Typically, dairy cows emit 6%-10% of dietary gross energy as methane into the environment [1], while meat sheep convert 8%-10% of dietary gross energy to methane [2-4]. Global methane emissions from ruminants account for 33% of anthropogenic methane emissions annually, contributing 15%-20% to the greenhouse effect [5]. Current research on methane mitigation measures primarily focuses on adding fatty acids, natural plants, plant-derived additives, or chemical agents to diets. However, these measures have not been widely applied in production due to lack of long-term efficacy or potential toxic side effects [6]. Studies on dairy cows and beef cattle have shown that appropriately increasing concentrate levels can improve feed utilization efficiency and reduce methane emissions [7-9], but few reports exist on the effects of concentrate levels on methane emissions from meat sheep. In 2014, China's sheep slaughter volume reached 300 million head [10]. Understanding methane emission patterns in meat sheep is crucial for developing environmentally friendly and efficient sheep production. Therefore, from a practical production perspective and using corn stalk as the roughage source, this study investigated the effects of different neutral detergent fiber (NDF) to non-fibrous carbohydrate (NFC) ratios (NDF/NFC) in diets on methane emissions from meat sheep at maintenance level, aiming to provide a theoretical basis for formulating rational diets and methane mitigation strategies for adult meat sheep.

Materials and Methods

1.1 Experimental Animals

The experiment was conducted from October 2015 to January 2016 at the Nan Kou Pilot Base of the Chinese Academy of Agricultural Sciences in Changping District. Sixteen Dorper \times Small-tailed Han crossbred wethers with an average body weight of (50.9 ± 2.8) kg were selected, weighed after overnight fasting, ear-tagged, dewormed, and randomly divided into 4 groups of 4 sheep each.

1.2 Experimental Design and Diets

A 4×4 Latin square design was employed across 4 experimental periods. In each period, each group was randomly assigned to one of four total mixed pellet diets with NDF/NFC ratios of 3.02 (Diet 1), 2.32 (Diet 2), 1.58 (Diet 3), and 1.04 (Diet 4). The experimental diets were formulated using corn stalk as roughage and corn and soybean meal as concentrate ingredients. Diet composition and nutrient levels are presented in Table 1 .

Each of the 4 periods lasted 18 days, comprising a 3-day adjustment period, a 7-day preliminary period, and an 8-day experimental period. The trial was conducted at maintenance feeding level. Prior to the formal experiment, a preliminary trial was conducted to determine feeding amounts based on body weight changes. Sheep were fed twice daily (07:30 and 16:30) with free access to water. During the adjustment period of each experimental period, sheep were individually housed in floor pens and began receiving their assigned experimental diets. At the start of the preliminary period, sheep were moved to metabolic cages to determine feed intake, initial body weight, and final body weight for establishing maintenance feeding levels.

1.3 Digestion and Gas Metabolism Trials

During the experimental period of each period, total feces and urine were collected using the total collection method. Daily feed and orts samples were collected from each sheep, and fecal output was weighed and recorded. Fecal samples were collected at 10% of total fecal weight and mixed and frozen for each sheep over the 8-day collection period. Urine was collected in plastic buckets containing 100 mL of 10% (v/v) H₂SO₄. Daily urine volume was measured and recorded, diluted to 5 L with tap water, and 15 mL urine samples were taken and mixed and frozen for each sheep over the 8-day collection period.

On days 1, 3, 5, and 7 of each experimental period, sheep were moved in 4 batches (4 sheep/batch, 1 from each group) into 4 respiration chambers. After 24 hours of adaptation, a gas metabolism system was used to measure methane and carbon dioxide emissions (GGA, Los Gatos Research, California, USA) and oxygen consumption (FC-10 oxygen analyzer, Sable Systems International, Henderson, NV, USA) for each sheep over the subsequent 24 hours [5,12]. Body weight was measured and recorded when each sheep entered and left the respiration chamber.

1.4 Sample Analysis

Feed and fecal samples were oven-dried at 65°C for 48 hours, equilibrated for 24 hours, and weighed to determine initial moisture content. Subsequently, dry matter (DM), crude ash, crude protein (CP), and ether extract (EE) contents were determined [13]. Gross energy (GE) was measured using a bomb calorimeter (Parr 6400, Parr Instrument Co., Moline, USA) [12]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined using

the method of Van Soest et al. [14] after enzymatic hydrolysis with trypsin and amylase. Urine samples were analyzed for nitrogen content and GE using the same methods.

1.5 Statistical Analysis

Data processing and analysis were performed using the general linear model procedure in SPSS 19.0 statistical software. Multiple comparisons of means were conducted using Duncan's method, with significance declared at $P < 0.05$.

Results

2.1 Nutrient Apparent Digestibility and Energy Metabolism

Table 2 shows the effects of feeding different NDF/NFC diets on nutrient apparent digestibility in meat sheep at maintenance level. As the NDF/NFC ratio decreased, apparent digestibility of DM, OM, and CP increased gradually, with significant differences among all diets ($P < 0.05$). NDF apparent digestibility increased initially and then decreased with decreasing NDF/NFC, with Diet 3 being significantly higher than Diets 1 and 2 ($P < 0.05$), while no significant differences were observed between Diet 4 and Diets 2 and 3 ($P > 0.05$). ADF apparent digestibility increased as NDF/NFC decreased, with significant differences among all diets ($P < 0.05$).

2.2 Energy Metabolism

Table 3 presents the effects of feeding different NDF/NFC diets on energy metabolism in meat sheep at maintenance level. As the NDF/NFC ratio decreased, gross energy intake and fecal energy decreased gradually, while urinary energy increased gradually, and apparent digestibility and metabolic rate of gross energy increased gradually, with significant differences among all diets ($P < 0.05$). Digestible energy and metabolizable energy generally increased as NDF/NFC decreased, with Diet 1 being significantly lower than the other three diets ($P < 0.05$), but no significant differences among Diets 2, 3, and 4 ($P > 0.05$). Methane energy was lowest for Diet 4, which was significantly lower than Diets 1 and 2 ($P < 0.05$), with no significant differences among the other diets ($P > 0.05$).

2.3 Methane Emissions

As shown in Table 4, daily methane emission decreased as NDF/NFC decreased, with Diet 2 being significantly higher than Diets 3 and 4 ($P < 0.05$), and Diet 1 being significantly higher than Diet 4 ($P < 0.05$) but not significantly different from Diet 2 ($P > 0.05$). Daily methane emission per kilogram of metabolic body weight decreased gradually as NDF/NFC decreased, with Diet 2 being significantly higher than Diets 3 and 4 ($P < 0.05$) but not significantly different from Diet 1 ($P > 0.05$). Methane emission per kilogram of dry matter intake

(DMI) increased initially and then decreased with decreasing NDF/NFC, with Diet 1 being significantly lower than Diets 2 and 3 ($P < 0.05$), but no significant differences among Diets 2, 3, and 4 ($P > 0.05$). When expressed per kilogram of organic matter intake (OMI), methane emission for Diet 2 was significantly higher than that for Diets 1 and 4 ($P < 0.05$) but not significantly different from Diet 3 ($P > 0.05$). When expressed per kilogram of digestible organic matter (DOM), methane emission for Diet 1 was significantly higher than that for Diets 3 and 4 ($P < 0.05$) but not significantly different from Diet 2 ($P > 0.05$). Methane emissions per kilogram of neutral detergent fiber intake (NDFI) or acid detergent fiber intake (ADFI) increased gradually as NDF/NFC decreased, with significant differences among all diets ($P < 0.05$). Methane emissions per kilogram of digestible NDF (DNDF) or digestible ADF (DADF) also increased as NDF/NFC decreased, with Diet 4 being significantly higher than the other three diets ($P < 0.05$), and Diets 2 and 3 being significantly higher than Diet 1 ($P < 0.05$), but no significant difference between Diets 2 and 3 ($P > 0.05$). The proportion of methane energy to gross energy increased initially and then decreased with decreasing NDF/NFC, with Diet 2 being significantly higher than Diets 1 and 4 ($P < 0.05$) but not significantly different from Diet 3 ($P > 0.05$). The proportion of methane energy to digestible energy decreased as NDF/NFC decreased, with significant differences among all diets ($P < 0.05$).

Discussion

3.1 Methane Emissions

Methane emissions from ruminants are influenced by many factors, including feed intake, diet type, dietary concentrate-to-roughage ratio, feed processing methods, animal breed, body weight, rumen fermentation patterns, and microbial flora [15-17], among which dietary concentrate-to-roughage ratio has a relatively large impact. Aguerre et al. [7] found that under ad libitum feeding conditions, daily methane emissions from dairy cows decreased gradually as the concentrate proportion increased. Benchaar et al. [18] reported that under restricted feeding conditions, dairy cows fed diets with concentrate-to-roughage ratios of 0:100, 20:80, 50:50, and 70:30 showed an initial increase followed by a decrease in daily methane emissions as the concentrate proportion increased. Similar conclusions were reached by Wang et al. [11] and Zhao et al. [19]. Differences in dietary concentrate-to-roughage ratio primarily reflect differences in NDF/NFC ratios. In this experiment, daily methane emissions decreased gradually as NDF/NFC decreased, consistent with the results of Moss et al. [20] and Chandramoni et al. [21]. This can be attributed to two main reasons: First, methane emissions are affected by dietary carbohydrates. As the NDF proportion decreases and the NFC proportion increases, the rumen fermentation pattern shifts from acetate-dominant to propionate-dominant [22], rumen pH gradually decreases, the acetate-to-propionate ratio decreases, and methane production gradually decreases. Second, as the NDF proportion in the diet de-

creases and the NFC proportion increases, the dominant rumen bacterial population changes, inhibiting protozoal growth. Since methanogens attach to the surface of protozoa in a symbiotic relationship, a reduction in protozoal numbers leads to a decrease in methanogens and consequently methane emissions [23-25].

3.2.1 Methane Emissions per Unit of Nutrient Intake

Research has shown that methane emissions are closely related to DMI [26]. Feed intake affects the passage rate and fermentation time of digesta in the rumen, thereby influencing methane emissions [26]. Increased feed intake reduces the retention time of digesta in the rumen, decreases nutrient digestibility, and consequently reduces methane emissions per unit of DMI. However, as the concentrate proportion increases, the NFC proportion in the diet also increases, which can alter the rumen fermentation pattern and thus reduce methane emissions per unit of DMI. The results of this experiment showed that as both feed intake and NDF/NFC decreased, methane emissions per kilogram of DMI and OMI increased initially and then decreased, showing a gradual decreasing trend among diets with NDF/NFC ratios of 2.32, 1.58, and 1.04.

The results also demonstrated that methane emissions per kilogram of NDFI or ADFI increased gradually as NDF/NFC decreased. However, Aguerre et al. [7] found that for lactating dairy cows under ad libitum feeding conditions, methane emissions per kilogram of DMI and OMI increased as the dietary NDF proportion increased from 47% to 63%, while methane emissions per kilogram of NDFI were not significantly affected by NDF proportion. This discrepancy with our results may be related to the digestibility of NDF and ADF. Xie [16] suggested that the proportion and digestibility of NDF in the diet affect methane emissions, primarily because the rate of methane production from NDF degradation is higher than that from NFC [27], with cellulose and hemicellulose fermentation producing 2-5 times more methane than NFC fermentation [28]. Increased degradable fiber in the rumen can enhance methane emissions. Additionally, the increased methane emissions per kilogram of NDFI or DNDF may be related to longer retention time of digesta in the gastrointestinal tract. In this experiment, feed intake decreased as NDF/NFC decreased, resulting in slower digesta passage rate and longer retention time in the digestive tract, allowing more complete fermentation of NDF and relatively higher methane production [26].

Blaxter et al. [29] reported that at maintenance level, methane energy accounts for 7%-9% of dietary gross energy and 11%-13% of dietary digestible energy. In this experiment, methane energy accounted for 8%-10% of gross energy at maintenance level, and the proportion of methane energy to gross energy increased initially and then decreased as NDF/NFC decreased. Hua et al. [8] investigated the effects of different concentrate-to-roughage ratios on methane emissions from Huanghuai White goats at 1.2 times maintenance energy level using *Leymus chinensis* as the roughage source, finding that the proportion of methane energy to

gross energy decreased gradually as the concentrate proportion increased. Moss et al. [20] found that at 1.1 times maintenance level, the proportion of methane energy to gross energy increased gradually as soybean meal supplementation (i.e., concentrate proportion) increased, which is inconsistent with our conclusion. This discrepancy may be related to the starch proportion in the diet. Our experiment used corn and soybean meal as concentrate sources, resulting in a relatively high dietary starch proportion that produced more propionate upon degradation, reducing the acetate-to-propionate ratio and consequently decreasing methane emissions.

3.2.2 Methane Emissions per Unit of Digestible Nutrients

This experiment found that as NDF/NFC decreased, the apparent digestibility of DM, OM, CP, and ADF increased significantly (Table 2). According to previously established regression equations, nutrient digestibility is negatively correlated with methane emissions per unit of organic matter, with DOMI being closely related to methane production [26]. In this experiment, OM apparent digestibility increased from 56% to 75% (Table 4), while methane emissions decreased from 74.97 L/kg DOM to 58.78 L/kg DOM, and the proportion of methane energy to digestible energy decreased from 16.63% to 12.42%. Zhao et al. [19] found that as the dietary concentrate-to-roughage ratio increased from 8:92 to 64:36, methane emissions per kilogram of DOM decreased from 48.80 L/DOM to 37.83 L/DOM, and the proportion of methane energy to digestible energy decreased from 12.27% to 7.71%. Similar conclusions were reached by Zhao et al. [30], all consistent with our results showing that methane emissions per kilogram of DOMI decreased gradually as dietary concentrate level increased.

In this experiment, both methane emissions per megajoule of digestible energy intake and the proportion of methane energy to digestible energy decreased as the dietary NDF proportion decreased. This indicates that under restricted feeding conditions, reducing dietary NDF proportion and increasing concentrate proportion can improve the utilization efficiency of digestible energy in meat sheep. This is consistent with the findings of Wang et al. [11] and Liu [31]. In practice, NDF proportion can be appropriately reduced and concentrate proportion increased under restricted feeding conditions to improve digestible energy utilization efficiency.

Conclusions

1. At maintenance level, as the NDF/NFC ratio in corn stalk diets decreased, daily methane emissions and methane emissions per kilogram of metabolic body weight in meat sheep decreased gradually, methane emissions per kilogram of DOM decreased gradually, while methane emissions per kilogram of NDF intake and per kilogram of digestible NDF intake increased

gradually.

2. At maintenance level, as the NDF/NFC ratio in corn stalk diets decreased, the apparent digestibility of all dietary nutrients in meat sheep increased gradually.
3. Considering both nutrient apparent digestibility and methane emission efficiency, a diet with an NDF/NFC ratio of 1.04 is most suitable as a methane mitigation formula for meat sheep at maintenance level.

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