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Effects of dietary protein levels and calcium salts of long-chain fatty acids on nitrogen mobilization, rumen microbiota and plasma fatty acid composition in Holstein bulls

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ABSTRACT

The aim of this study was to investigate the impact of dietary crude protein (CP) levels and calcium salts of long-chain fatty acids (CSFA) on the nutrient intake and digestibility, nitrogen deposition, rumen fermentation characteristic and microbiota, plasma biochemical indexes and fatty acid composition in the Holstein bulls. Eight Holstein bulls was used in a 4 × 4 Latin Square design with 2 × 2 factorial diets, including two levels of CP (133 or 112 g/kg dry matter), and with or without 2.32 g/kg CSFA. The high CP level diets increased the CP intake and the CP apparent digestibility and the urinary nitrogen excretion ($P = 0.001$). Dietary supplementation with CSFA promoted the apparent digestibility of organic matter ($P = 0.012$). The diets of CSFA and high CP level raised the rumen ammonia nitrogen concentration ($P = 0.009$), and enhanced the isovalerate and valerate concentrations, respectively. The high CP diets improved the abundance of *Butyrivibrio fibrisolvens* ($P = 0.037$) and *Megasphaera elsdenii* ($P = 0.023$), while the CSFA reduced the abundance of methanogens ($P = 0.047$). High CP increased urea concentration ($P = 0.008$). Dietary supplementation with CSFA increased the cholesterol and the low-density lipoprotein cholesterol concentration in the plasma, and the proportion of C16:0, C18:1n9c and Δ^9 desaturase C18, while it reduced the ratio of C21:1 and C22:0. The low CP diets reduced the protein waste and environmental pollution in the final stage of fattening Holstein bulls. Further study needs to be done to investigate the effect of CSFA on the CH_4 emission in terms of microbial mechanism in the rumen.

1. Introduction

In the process of fattening beef cattle, the gradual reduction of muscle protein deposition is helpful to increase the fat deposition and improve the beef quality. Recent studies have shown that administering diets with 140 g/kg crude protein (CP) levels in dry matter (DM) basis has no effects on the nitrogen retention and growth performance compared with 10 g/kg CP levels in finishing Nellore bulls at age of 20 months (Menezes et al., 2016), which means that high protein diets may lead to the protein waste and the

Abbreviations: CSFA, calcium salt of long-chain fatty acid; CP, crude protein; VFA, volatile fatty acid; DM, dry matter; OM, organic matter; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; NH_3-N, ammonia nitrogen; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol

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increase of feed cost. It is necessary to adjust beef cattle dietary protein levels to avoid protein waste and nitrogen emission in the final fattening stage. At the same time, appropriate feeding strategies should be used to increase the fat deposition by altering lipid metabolism.

Raising energy concentration and adding plant oil in the diets are always used to increase beef cattle fat deposition. But the excessive concentrate has the risk of rumen acidosis (Wetzels et al., 2017), and adding oil such as soybean oil is harmful to the rumen microorganisms (Gomez-Cortes et al., 2008) and the fiber digestibility (Manso et al., 2006). The calcium salts of long-chain fatty acids (CSFA) as one kind of rumen protecting fat, which has no negative effects on the fiber digestibility (Manso et al., 2006). However, the CSFA characteristics of rumen protection make scholars neglect the effect of it on the rumen microorganism, because fatty acid of calcium salts are not completely inert in the rumen (Vannevel and Demeyer, 1996). Besides, lipid metabolism firstly occurs in the liver and then through plasma transport to adipose tissue (Bell, 1979). Therefore, the plasma lipid index is an important indicator to reflect the fat metabolism.

The required protein level at fattening stage is dependent on cattle breeds and ages. Specialized beef breeds have persistent lean meat growth. while dairy breeds deposit fat in the early stage (Gallo et al., 2014; Spanghero et al., 2017). Consequently, the required protein level should be studied in the beef production of Holstein bulls. Previous studies have shown that the protein intake and digestibility have a linear dependence on the protein levels in dietary (Obeid et al., 2007; Menezes et al., 2016). However, in *vitro* study demonstrated a contrary result that diet protein levels have no effect on the protein disappearance (Amaral et al., 2016). The high protein diets can increase the concentration of rumen ammonia nitrogen and urea nitrogen (Da Silva et al., 2016), which are beneficial to microbial protein synthesis (Amaral et al., 2016). Dietary with high protein level also reduces the efficiency of protein utilization (Pina et al., 2009). However, there are no reports to reveal the effects of protein levels on the rumen microorganisms and plasma fatty acid profile.

Therefore, we hypothesized that the low protein diets have no effects on the animal feed nutrients intake and digestibility, and the CSFA is beneficial for the fat metabolism and rumen metabolism in the finishing Holstein bulls. The aim of the present experiment is to define the impacts of the CSFA and CP on the intake, apparent digestibility of nutrients, rumen fermentation characteristics, rumen microbiota populations, hematologic indexes and fatty acid composition in Holstein bulls.

2. Materials and methods

The experimental procedure was conducted by the Animal Welfare and Ethics Committee of China Agricultural University. Animal care and handling were followed the guidelines by the regulations for the Administration of Affairs Concerning Experimental Animals (The State Science and Technology Commission of P. R. China, 1988).

2.1. Experimental design and animal diets

In this study, 8 Holstein bulls (20 months old, and 485 ± 20 kg of body weight) were selected and randomly divided into 2 groups. The 4 bulls in each group received one of 4 experimental diets under the 4 × 4 Latin Square experimental design. Four diets are shown in Table 1, including LN: low CP diets with no CSFA; HN: high CP diets with no CSFA; LC: low CP supplemented with CSFA diets and HC: high CP level diet with CSFA supplement. These four diets had the same level of total digestible nutrients (750 g/kg) except for the added of CSFA, Dietary formulations were expected to average daily gain was 1.7 and 2.1 kg in low and high-protein diets according to the Nutrient Requirements of Beef Cattle (NASEM, 2016). The fatty acid composition of CSFA is shown in Supplementary Table 1. Each period contained 25 days, and the first 20 days were the adaptation, the other 5 days were the sampling time. The bulls were individually housed and fed twice per day at 7:30 and 17:00, offered fresh drinking water *ad libtum*. The feed intake was recorded daily.

2.2. Sampling and chemical analyses

The Orts and feed samples were collected on the 21st to 23rd days of each cycle, the rectal faeces were gathered at 6:00, 12:00, 18:00 and 24:00, respectively. About 10 mL blood was collected through the jugular vein using a vacuum blood tube containing sodium heparin at 7:00 on 24th day, and the plasma was separated by centrifugation 3000 × g for 10 min and stored at −20 °C. Representative samples of rumen contents were collected at 10:00 on the 25th day *via* esophageal tubing and by retaining particles attached to the metal strainer. The rumen liquid used for determination of volatile fatty acids (VFA) was separated by 3000 × g centrifugation 15 min. The 250 g/L partial phosphoric acid solution, which contained 2-ethylbutyric acid as the internal standard, was added into the rumen liquid in a concentration of 0.25 ml/mL rumen fluid before stored at −20 °C. While rumen contents for the rumen microorganism analysis were stored in liquid nitrogen. About 60 ml urine was gathered at 5:00 on 24th or 25th day, and 10 mL of 0.5 mol/L dilute sulfuric acid was added into the urine.

The neutral detergent fiber (aNDF; Van Soest et al., 1991), ether extract (EE; method 920.39), DM (method 934.01), organic matter (OM; method 942.05), acid detergent fiber (ADF, ; Van Soest et al., 1991) and CP (method 990.03) of feed, orts and feces were analyzed by the methods of AOAC (AOAC International, 2002). For the determination of EE in CSFA, it needed to treat with 0.5 v/v sulfuric acid for acid hydrolysis before extraction. The acid insoluble ash of the samples as the endogenous marker was determined by the method as before (VAN Keulen and Young, 1977). The apparent digestibility of the nutrients was calculated using the endogenous tracer method (Huhtanen et al., 1994). The urea nitrogen was measured by previous studies (Da Silva et al., 2016).