



# Influence of N shortage and conjugated linoleic acid supplementation on some productive, digestive, and metabolic parameters of lactating cows

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## ABSTRACT

The effects of a shortage of dietary N and of a rumen protected CLA (rpCLA) supplement on DMI, rumination, rumen fluid characteristics, milk yield (MY), and milk N/N intake ratio (ENU) were studied in mid-late lactating cows. Twenty cows housed in 4 pens in groups of 5, homogeneous for parity, days in milk (DIM) and MY, were fed on 4 different diets: with 150 g (CP15) or 123 g (CP12) of CP/kg DM, with or without an rpCLA supplement (containing 6.34 g/d of C18:2c9,t11 and 6.14 g/d of C18:2t10,c12). A 4 × 4 Latin Square experimental design was used with periods of 3 wks, although the sequence of the 4 treatments (CP15 or CP12, with or without rpCLA) was such that each group received CP15 or CP12 for 6 consecutive wks. The CP12 diet was formulated from CP15 by replacing soybean meal with barley grain to maintain similar energy, fiber content and feed particle size. Rumination activity, DMI, and MY were recorded daily. Rumen fluid was analyzed for VFA and ammonia N content, and milk for quality traits. Nutrient digestibility was estimated using Lignin(sa) as a marker. Period, treatment and group (random) were included as sources of variation in the statistical analysis. Dietary CP restriction tended to reduce DMI (−7.7%;  $P=0.09$ ) and digestibility, but increased time spent in rumination (+10%;  $P=0.009$ ), decreased rumen fluid ammonia N (−36%;  $P<0.001$ ), and reduced MY (−4.8%;  $P=0.047$ ) and milk protein content (−4.7%,  $P=0.026$ ); it had no influence on the ratio between energy-corrected milk yield and DMI. CP restriction reduced N intake by 122 g/d and N in milk by 14 g/d, did not influence N in feces, but increased ENU from 0.31 to 0.36 ( $P<0.01$ ). A low marginal response of 115 g milk N/kg to the increased N intake from soybean meal was found. The addition of rpCLA tended to reduce DMI (−8.1%;  $P=0.07$ ) and decreased milk fat content (−15%;  $P=0.002$ ), but decreased N in milk only when added to CP12 (CP × rpCLA interaction,  $P=0.016$ ). A shortage of N supply increases ENU without apparent alteration of BW, BCS and blood metabolites. Long-term investigations to clarify the role of body N reserves and of a shortage of CP supplies on N partitioning are needed.

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**Abbreviations:** ADF, acid detergent fiber; AST, aspartate aminotransferase; BCS, body condition score; BW, body weight; CK, creatinine kinase; ECM, energy corrected milk yield; EE, ether extract; CLA, conjugated linoleic acid; CP, crude protein; DM, dry matter; DMI, dry matter intake; GGT, gamma-glutamyltransferase; Lignin(sa), sulphuric acid lignin; ME, metabolizable energy; MY, milk yield; NE<sub>L</sub>, net energy lactation; NEFA, non-esterified fatty acids; OM, organic matter; RDP, rumen degradable protein; RUP, rumen undegradable protein; MP, metabolizable protein; MY, milk yield; rpCLA, rumen protected CLA; VFA, volatile fatty acids.

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## 1. Introduction

Research to evaluate the effects of a shortage of protein supply, with respect to nutrient recommendations, on the production and quality of products is gaining interest in all domestic species due to concerns about the environmental impact of farming and the increasing cost of protein sources (Steinfeld et al., 2006; Schiavon et al., 2012). A shortage of rumen degradable energy, protein, or both would reduce feed digestibility, DMI and production outcome (Tedeschi et al., 2000; Valkeners et al., 2008). In their meta-analysis data from northern Europe and the USA, Huhtanen and Hristov (2009) found mean efficiencies of N utilization (N in product/N intake; ENU) of 0.28 and 0.25, respectively, with large variations in the two datasets. However, they observed that increasing milk yield would increase ENU, although this increase was considerably smaller than that obtainable by reducing CP intake, and they also observed a positive effect of starch on ENU, whereas the effect of aNDF was negative at any given level of energy and protein intake, in agreement with Colombini et al. (2010). Supplementing low protein diets with rumen protected CLA (rpCLA) was found to improve ENU in beef cattle (Schiavon and Bittante, 2012). von Soosten et al. (2012) found that rpCLA exerted a protective effect against excessive use of body reserves in early lactating primiparous Holstein cows, and increased protein accretion, and they suggested a role of CLA in metabolic mechanisms of N partition in different body functions.

This experiment aimed to analyze and quantify the effects of corn silage based-diets with low N content, with or without the addition of rpCLA, on some productive, digestive and metabolic parameters, and ENU.

## 2. Materials and methods

All the experimental procedures involving animals were approved by the “Ethical Committee for the Care and Use of Experimental Animals” of the University of Padua (#29562, CEASA, Legnaro, Italy).

### 2.1. Animals, diets, and experimental design

Two wks before the start of the experiment 20 lactating Holstein cows were divided into four experimental groups and housed in four pens with straw-bedded cubicles, 5 cows per pen. The groups were balanced for milk yield ( $30.0 \pm 1.4$  kg/d), DIM ( $174 \pm 6$  d), parity ( $2.0 \pm 0.36$ ), BW ( $641 \pm 26$  kg) and BCS ( $2.9 \pm 0.07$ ). The cows had 2 wks of adaptation to the experimental conditions before the start of the experiment.

Four total mixed rations (TMR) were compared. The control diet (CP15), which was representative of the diets used in north-eastern Italy (Dal Maso et al., 2009), was based on corn silage and cereal grains (Table 1). It was formulated according to NRC (2001) recommendations to provide, for an expected 21 kg/d of DMI, the amount of MP required for 30.0 kg/d milk yield with 35, 34, and 47 g/kg of protein, fat and lactose, respectively, and a slight excess of NE<sub>L</sub>, so that MP was intended to be the first limiting factor. The low-protein diet (CP12) was formulated from the CP15 diet by simply replacing 65 g/kg DM of soybean meal with 75 g/kg DM of barley grain and reducing the other ingredients proportionately so that dietary CP was reduced from 150 to 123 g/kg DM and dietary starch increased from 227 to 263 g/kg DM. On the basis of ingredient composition, the NRC (2001) model predicted an average of 30.0 kg/d of metabolizable energy (ME)-allowable milk and 20.3 kg/d of metabolizable protein (MP)-allowable milk for a DMI of 21.2 kg/d of CP12.

To minimize differences in composition among treatments, a common basal diet, corresponding to the composition of the CP12 diet without barley grain, was prepared daily using a mixer-wagon equipped with a computer-assisted weighing scale, which was calibrated monthly. The amounts of barley grain or soybean meal required to complete the CP12 and CP15 rations were supplied by top dressing and mixing after distribution of the common basal diet in the manger of each pen. The other two experimental diets, CP15<sub>rpCLA</sub> and CP12<sub>rpCLA</sub>, differed from CP15 and CP12 only in the addition by top dressing and mixing, together with barley or soybean, of 80 g/d per cow of an rpCLA commercial mixture (SILA, Noale, Italy) consisting of methyl esters of CLA bound to a silica matrix and coated with hydrogenated soybean oil. Mixing procedures were completed in few minutes by an operator, when the cows were kept away from the mangers of each pen. The composition of the lipid-coated rpCLA was, per kg of CLA supplement, 800, 178, and 22 g of lipids, ash and moisture, respectively, 655 g of palmitic/stearic acids and linoleic acid, 99 g of C18:2c9,t11, 96 g of C18:2t10,c12, and 150 g of other FAs and glycerol. A detailed description of the chemical composition of the rpCLA is given in Schiavon et al. (2011). Cows were milked at 5:30 a.m. and 5:00 p.m. and received their ration in a single daily dosage after the morning milking, at approximately 6:30 a.m., although they had free 24-h access to the manger.

A 4 × 4 Latin square experimental design was used with periods of 3 wks, the first 2 wks for adaptation and the third for data collection. The sequence of treatments was designed to ensure that each group received the CP15 or the CP12 diet for 6 consecutive wks, the rpCLA added or withdrawn without a simultaneous change in the CP content of the diet, and, conversely, the change in CP made without a simultaneous change in rpCLA status, as illustrated in Table 2. This schema was also intended to minimize sequence effects and avoid confounding carry-over effects, although such effects on DMI and MY at different CP levels have been commonly found to be minimal (Huhtanen and Hetta, 2012). Each group of cows was fed on experimental diets for 2 wks prior start of the experiment as described in Table 2.

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