Milk performance and oxidative status responses to rumen protected methionine supplementation in genotyped α-S1 casein lactating dairy goats fed two levels of metabolizable protein diets

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Title: Milk performance and oxidative status responses to rumen protected methionine supplementation in genotyped α-S1 casein lactating dairy goats fed two levels of metabolizable protein diets.

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Highlights

- Rumen-protected methionine (RPM) increased raw milk production in goats fed low protein diets (14% CP), and milk protein content in goats fed high protein diets (16% CP).
- These differences in productive responses were affected by the genetic variant for alpha-S1 casein content in goat milk.
- Goats fed RPM tended to have a higher plasma oxidative status than those fed no RPM.
- Specific requirement for dietary methionine have to be studied for dairy goats relatively to their genetic variant for alpha S1 casein in milk.

Abstract

In dairy goats, responses to rumen-protected Methionine (RPM) supplementation on milk performance and milk protein synthesis were inconsistent. The objective of the study was to evaluate the effects of supplemental RPM in diets differing in CP content on milk performance, milk composition, plasma concentration of amino-acids (AA) and parameters of oxidative status in goats genotyped for their genetic variant for alpha-S1 casein (CNS1). Sixteen goats (Body weight = 66.6 ± 8.5 kg, days in milk = 89 ± 5) were grouped into 2 blocks (High vs Low) according to their secretion of CNS1 in milk, and within each CNS1 block, goats were selected to make blocks comparable for milk yield, milk protein content, body weight and days in milk. Goats within CNS1 blocks were allotted to 2 periods cross-over (4 wks for each period with one washout week) nested in 2 x 2 factorial design with dietary CP (LP vs HP, 14 vs 16% CP in TMR fed ad libitum) and RPM supplementation (CTL vs RPM, 0 vs 4 g/d). Dry matter intake and milk yield were individually recorded daily, whereas milk samples for milk composition determination, and blood samples were obtained individually twice weekly, and once weekly, respectively. Blood was sampled for glucose, NEFA, BHB, urea, insulin, total or oxidized glutathion, superoxide dismutase, catalase, malondialdehyde, and protein carbonyl. At the end of each period, blood was sampled during two consecutive wks to determine plasma AA profile. Goats fed HP diet had higher CP, PDIE, and LysDi (lysine digestible in intestine) intake than those fed LP diet, whereas goats supplemented with RPM had higher MetDi (methionine digestible in intestine) intake ($P < 0.001$) than those fed the CTL diet. Goats fed HP diet had similar milk performance, milk composition and yields, and similar feed, energy and PDI efficiencies, but higher milk urea concentration than those fed LP diet. Goats supplemented with RPM had higher milk yield only when fed the LP diet (RPM x dietary CP: $P < 0.02$). Compared to goats fed CTL diet, those supplemented with RPM had higher milk protein content, but this was observed only in

those fed HP diet (RPM x dietary CP: $P < 0.008$), especially for Low CNS1 goats (RPM x CNS1: $P < 0.05$; RPM x dietary CP x CNS1: $P < 0.11$). Efficiencies of PDI, feed and energy were not altered by RPM supplementation, but CNS1 x dietary CP x RPM interaction was significant for these efficiencies. Goats supplemented with RPM tended to have higher plasma MDA ($P < 0.07$) and total glutathione concentrations ($P < 0.09$). In conclusion, increasing dietary CP failed to improve DMI, milk performance, milk protein content and protein yield, which suggest that Net Energy for lactation was a limiting factor of our experimental diets. With RPM supplementation, increase in milk production in the low CP group, and increase in milk protein content in the high CP group suggests that changes in metabolic partition of nutrients between milk secretion vs milk protein synthesis occurs according to the level of limitation of methionine in diet, which may affect the methionine requirement for goat milk production.

Key words:

Dairy goat, Rumen protected methionine, Metabolizable protein.

1. Introduction

Dietary nitrogen (N) is used inefficiently in lactating ruminant for milk protein secretion, with substantial N excretion in manure which leads to negative environmental effects, such as contribution to greenhouse gas emissions through NH_3 release, and water pollution leading to eutrophication (Reed et al. 2015). Balancing essential amino-acids (AA) in diets and decreasing crude protein (CP) concentration may constitute appropriate ways to increase milk performance, protein yield, and protein efficiency while lowering N excretion (Spek et al., 2013). Methionine (Met) is usually considered as a limiting AA for milk protein synthesis (Rulquin et al., 2007; Schwab and Broderick, 2018) and recommendation for that AA has been recently reviewed (INRA, 2018). In dairy cows, balancing diets through rumen-protected Met (RPM) improved the efficiency of dietary CP for milk protein secretion (Zanton et al., 2014), with marginal or no changes in the N excretion to the environment (Broderick et al., 2008). Beneficial effects of Met supply is not restricted to milk performance but also to animal health (Sun et al 2016). Indeed, Met is a regulator of protein synthesis and is a precursor in trans-sulfuration and methylation reactions, to provide cysteine and choline (Zhou et al, 2016b). Met has also been implicated in liver lipoprotein secretion (Bauchart et al. 1998), glutathione synthesis, and synthesis of compounds implicated in the management of oxidative stress and inflammation in cows (Osorio et al., 2014; Zhou et al., 2016a). In dairy ewes, supplementation with Met improved oxidative status, with a reduction of the plasma malondialdehyde concentration and an increase in plasma glutathione transferase activity (Mavromatis et al., 2021), but no data on oxidative status are available for dairy goats supplemented with Met.

In high producing dairy goats, a limited set of publications validates the use of RPM or RP-Lys on milk performance. Some studies reported the combination of both AA making impossible to attribute the observed effects to a specific AA (Foda et al., 2009; Madsen et al.,

2005). When only RPM was supplied, inconsistent responses were observed with positive effects on milk protein yield (MPY) or milk protein contents (MPC) (Flores et al., 2009; Piccioloi-Capelli et al., 2016; Boutinaud et al., 2020; Lemosquet et al. 2020), but also with no effects on MPY or MPC (Alonso-Melendez et al. 2016; Al-Qaisi and Titi, 2014). These discrepancies may be related to physiological stage of animals, duration of supplementation, form of protection of Met, energy or CP content of diet, or co-limiting dietary AA. In cows, the interaction between Met supplementation and dietary CP appears to be inconsistent, with no (Leonardi et al., 2003; Haque et al., 2012) or significant interaction on milk protein yield (Cabrita et al., 2011; Lee et al., 2012). Recently, Lemosquet et al. (2020) reported that Met supplementation in dairy goats increased milk yield only when fed adequate amount of energy, and increased MPC, MPY and casein content at low and adequate net energy supply, but to our knowledge, there is no study on the interaction between diet CP content and Met supplementation on milk performance in dairy goats. Moreover, goats have been characterized by a high variability in milk protein content according to their genotype for casein-alpha S1 (CNS1, Grosclaude et al., 1987), and this have been demonstrated to affect their response to dietary CP levels (De la Torre et al., 2008; Schmidely et al., 2002). The MPY response to RPM supplementation of dairy goats exhibiting low secretion of CNS1 have been shown to be low in relation with low Met requirement (Jacobsen, 2015), but no study is available for goats exhibiting alleles associated with a high secretion of CNS1.

Therefore, the first objective of the present study was to determine the effects of supplementing diets differing in CP content (LP=14 vs HP =16% CP) with RPM on milk performance, milk composition, and N and net energy feed efficiencies in Alpine goats that have been genotyped for their genetic variant for CNS1. We hypothesized firstly that milk performance or MPY responses to RPM supplementation could be more efficient in low CP diets, and secondly more efficient in goats with high milk protein yield. A secondary

objective was to characterize plasma biomarkers to assess oxidative status response to RPM supplementation.

2. Material and methods

The entire experiment was conducted in accordance with the National Legislation on Animal Care (certified by the French Ministry of Higher Education, Research and Innovation, Ethic Committee COMETHEA, authorization APAFIS #9833).

2.1. Animals, diets and experimental design

Forty-four multiparous alpine goats were characterized for their genotype of milk CNS1 from blood DNA extraction (Labogena, 78352 Jouy-en-Josas, France) before the start of the trial. Goats were grouped into 2 blocks with block **High** for the goats expressing Ab/Ab, Ab/Ac, Ac/Ad, Ab/B, Ac/B allelic genotypes, and block **Low** for those expressing Ab/E, Ac/E, Ad/E and E/E allelic genotypes (Grosclaude et al., 1987). Within each block, eight goats were selected and balanced for ~~raw~~ milk yield (~~RMY~~ MY), MPC, BW and DIM. At the beginning of the trial, ~~RMY~~ MY was 4.33 ± 0.43 vs 4.36 ± 0.77 kg/d, MP) was 31.3 ± 1.9 vs 35.9 ± 1.5 g/kg, BW was 67.5 ± 7.5 and 66.7 ± 9.2 kg, DIM was 90 ± 5 and 87 ± 6 days in High CNS1 vs Low CNS1 blocks, respectively. During the whole trial (11 wks including a 2-wk adaptation period and 9-wk cross-over experimental period), goats were housed in individual pens and had free access to water and trace-mineralized salt blocks. Goats were fed twice a day with one-third in of their respective diet distributed in the morning (0730 h) and two-thirds in the afternoon (1630 h).

The experimental design is presented at the figure 1. During the 1st wk of the adaptation period, goats were progressively switched from their previous grass hay-based diet (Forage: concentrate ratio 70:30, 13% CP) to the experimental low-protein diet (LP, table1). At the end of the 1st week, goats within CNS1 blocks were assigned to their respective experimental diets

LP vs HP (14 vs 16% CP, table 1) according to DMI, milk yield, and MPC and progressively switched during the 2nd wk from the LP diet to the LP or HP diets. At the end of the 2nd wk, half of goats in each experimental diet received 2.4 g metabolizable methionine /d for 4 wks, whereas the others were considered as control. Assigned Methionine (**RPM**) or control treatments (**CTL**) will be pursued for 4 wks (Period 1), and crossed-over for 4 additional wks (Period 2) after one wk washout period. Methionine supplementation was realized by hand mixing daily 4 g/d of Smartamine (Adisseo, Commentry, France) in the morning feeding, representing an amount of 2.4 g/d of metabolizable methionine (~~**RPM**~~), assuming that DL-Methionine content in Smartamine is 75%, with a pH sensitive coating considered to have 80% bioavailability (Graulet et al., 2005). This amount was supplied to meet calculated concentration of Methionine digestible in intestine (MetDi) relatively to PDIE for dairy cows of 2.5 g metabolizable Methionine/100 g PDIE (Protein Digested in the small Intestine supplied by RUP and by microbial protein from rumen-fermented OM) in the RPM group (INRA, 2007) whereas CTL group were below that recommendation (90%) assuming an average DMI of 3.5 kg DM/d. Both adaptation and experimental diets were fed as TMR and distributed to have at least 10% orts.

2.2. Experimental measurements and sampling

From wk 1 onwards, feed distributed and orts were recorded daily. One diet sample was collected at wk 2, 6, and 11 for the determination of feed and orts composition. Goats were weighed twice a day at milking parlor and data were averaged individually by wk. During the whole trial, 2 milk samples of 30 mL (one at the evening milking and one at the following morning milking) were obtained individually twice a week. Samples were preserved with the addition of bronopol (Grosseron SA, Saint-Herblain, France) and stored at 4°C until analysis. ~~Total protein, fat, lactose, SCC and urea were analyzed by infrared spectrophotometry in a Milk Recording Organisation laboratory (Syndicat Interdépartemental de l'Élevage, Le Mée,~~

France). At wk6 and 11, 2 milk samples were individually collected and frozen at -18°C for analysis of milk total casein.

From wk 3 to 11 (except during the washout wk), blood was sampled once weekly by jugular venipuncture into heparin vials in the morning (0700 h), before the milking and before the distribution of the diet. Blood samples (2 x 10 mL) were centrifuged at 1,780 × g for 10 min at 4°C immediately after collection. Plasma was removed and stored at −18°C until glucose, NEFA, BHB, urea, insulin, total glutathione (GSH), oxidized GSH, superoxide dismutase (SOD), catalase, malondialdehyde (MDA), and protein carbonyl was analyzed. Two additional blood samples were collected during the last two wks of each cross-over period (wk5 and 6, and wk 10 and 11) to analyze plasma free AA concentrations, plasma non-proteinogenic AA and AA derivatives.

2.3. Laboratory analysis

Dietary feed values (Table 1) were calculated according to INRA feeding system (INRA, 2007) from analyzed chemical composition and in vitro digestibility measurements (Upscience-Labs, Saint-Nolf, France). Feed and orts samples were dried in a forced-air oven at 90°C for 24 h to determine DM content and then ground and stored at room temperature until analysis of OM, NDF, ADF (Van Soest et al., 1991), and starch (Gluco-sequant glucose/HK; Roche/Hitachi Diagnostics, Mannheim, Germany) content. Feed DM, ash, and enzymatic starch were analyzed following International Organization for Standardization (ISO) standards: ISO (1999; 6496), ISO (2002; 5984), and ISO (2004; 15914), respectively. Feed and orts were ground at 0.5-mm screen and analyzed for AA content by HPLC (Adisseo, Commentry, France) after protein hydrolysis with 6 *N* HCl at 110°C for 24 h under reflux (Commission Regulation (EC) No 152/2009). Sulfur AA (Met and Cys) were analyzed separately by oxidation using performic acid before the protein hydrolysis. For DL-Trp analysis, samples were hydrolyzed in an autoclave using Barium Hydroxide in the absence of

oxygen. After the acidification of an aliquot fraction, the tryptophan was separated by HPLC using a flurometric detector (Commission Regulation (EC) No 152/2009).

Plasma glucose, NEFA, and BHBA were analyzed by an autoanalyzer (Cobas-Mira Roche/Hitachi, Mannheim, Germany) with enzymatic assay-kits (Gluco-sequant glucose/HK, Roche/Hitachi Diagnostics; NEFA-HR(2), Wako Chemicals GmbH, Neuss, Germany). Plasma β-Hydroxybutyrate was analyzed by the method of Barnouin et al. (1986). Plasma insulin was analyzed using an ELISA kit (10-1202-01; Mercodia AB, Uppsala, Sweden). Plasma AA, non-proteinogenic AA and AA derivatives concentrations were analyzed by UPLC/MS (Waters Acquity Ultra Performance LC system, Waters Corp.) as described by Haque et al. (2012). Enzymatic assay kits were used for colorimetric determination of plasma SOD (Ransod, SD 125, RANDOX, Cremmlin, GB), total GSH and oxidized GSH (glutathione (GSSG/GHS), ref ADI-900-160, ENZO, Farmingdale, USA), catalase (EnzyChrom^{TM} Catalase Assay kit, ref ECAT-100MDA, GenPrice Inc, San José, USA), MDA (OxiSelect^{TM} TBARS, STA-330, Cell Biolabs Inc, San Diego, USA), and PC (OxiSelect^{TM} Protein Carbonyl, STA-307, Cell Biolabs Inc, San Diego, USA).

Total protein, fat, lactose, SCC and urea in milk were analyzed by infrared spectrophotometry in a Milk Recording Organisation laboratory (Syndicat Interdépartemental de l'Élevage, Le Mée, France). Milk casein N content was determined by difference between total N and non-casein N according to method ISO 17997-2 (IDF 29-2/2004).

2.4. Calculations and statistical analysis

The PDI gross efficiency for milk protein synthesis was evaluated by the ratio between MPY (g/d) and dietary intake of PDIE (g/d). The PDI metabolic efficiency was evaluated by the ratio between MPY and the difference between PDIE intake minus the PDIE requirement (g/d, INRA (2007)). Data were analyzed using PROC MIXED of SAS (SAS Institute, 2004) according to a cross-over design (including RPM (CTL vs RPM), Period (1 vs 2), and

sequence (SEQ) of RPM supplementation as factors) nested within a factorial design with CNS1 Block (Low vs High) and dietary CP (LP vs HP) as factors. Period (1 vs 2), Seq (RPM/CTL vs CTL/RPM), RPM supplementation (CTL vs RPM), Genotype (Low vs High), dietary CP (LP vs HP), dietary CP x RPM interaction, Genotype x RPM interaction, dietary CP x Genotype and dietary CP x Genotype x RPM interaction were considered as fixed factor. When data were repeated within each period, time, time x RPM, and time x dietary CP were also included as fixed factors in the model, with goat(Seq) as a random effect and a compound-symmetric covariance structure as proposed by Choi et al. (2014). Least squares means are reported and treatment effects were declared significant at $P < 0.05$ and tendencies at $P \leq 0.10$.

3. Results

Most of the interactions between time and RPM supplementation, or time and dietary CP were not significant. They were removed in the presentation of the results to focus on the main factors (RPM supplementation, dietary CP, and genotype) and on their interactions.

3.1. Milk performance

DM and nutrient intake are presented in table 2. Goats fed HP diet had higher CP ($P < 0.02$), PDIE ($P < 0.005$), LysDi ($P < 0.001$), Histdi ($P < 0.002$) and LeuDi ($P < 0.003$) intake than those fed the LP diet. As expected, goats supplemented with RPM had higher MetDi intake (+2.2 g/d in average, $P < 0.001$) than those fed the CTL diet, with no other difference in DMI or nutrients intake.

Milk performance and milk composition are presented in table 3. Goats fed HP diet had similar milk performance, milk composition and yields, similar PDI efficiencies, similar feed and energetic efficiencies, but higher milk urea concentration than those fed LP diet ($P < 0.01$). Goats supplemented with RPM had higher ~~raw~~ milk yield (+0.1 kg/d) only when fed

LP diet (RPM x dietary CP interaction: $P < 0.02$). Compared to goats fed CTL diet, those supplemented with RPM had higher MPC, but this was observed only in goats fed HP diet (interaction RPM x dietary CP: $P < 0.008$), and this effect was more pronounced in Low CNS1 block (interaction RPM x CNS1: $P < 0.05$; interaction RPM x dietary CP x CNS1: $P < 0.11$): in the Low CNS1 block, difference in MPC and MPY between goats fed LP x CTL diet and those fed HP x RPM diet was 2.7 g/l and 17.7 g/d, whereas it was only 0.8 g/l and 11 g/d in the High CNS1 block, respectively. Similarly, in Low CNS1 block, difference in milk casein concentration in goats fed LP x CTL diet and those fed HP x RPM diet was 3 g/L, whereas it was only 0.2 g/l in the High CNS1 block (interaction RPM x dietary CP x CNS1: $P < 0.04$). In High CNS1 block, goats supplemented with RPM had higher feed efficiency and energy efficiency than those fed CTL diet whatever the CP content, whereas in the CNS1 Block, those supplemented with RPM had lower feed efficiency and energy efficiency that those fed CTL diet, but this was observed only for goats fed HP x CTL diet (interaction RPM x Dietary CP: $P < 0.04$ and $P < 0.003$ for feed or energy efficiency, respectively; interaction RPM x CNS1: $P < 0.01$ and $P < 0.01$, respectively; interaction RPM x dietary CP x CNS1: $P < 0.001$ and $P < 0.02$, respectively). Efficiencies of PDI were not affected by RPM supplementation, but interaction between CNS1 x dietary CP x RPM was significant ($P < 0.001$ for PDI gross efficiency and $P < 0.002$ for PDI metabolic efficiency). Indeed, in goats fed HP diet (but not LP diet), those supplemented with RPM in the Low CNS1 block had lower PDI gross and metabolic efficiencies (-0.03 g MPY/g PDIE intake, and -0.04 g MPY/g (PDIE intake-PDIE requirement) than the CTL ones; conversely, when fed HP (but not LP diet) goats supplemented with RPM had higher PDI gross and metabolic efficiencies (+0.02, and + 0.02 unit). Compared to goats fed CTL diet, those supplemented with RPM had higher milk urea concentration, without any interaction with other factors.

263 Goats of High CNS1 block had higher ~~raw~~ milk yield, higher MPY (+18 g/d, $P < 0.05$)
264 and MPC (+2.8 g/kg, $P < 0.02$), and higher milk casein content (+ 3.3 g/kg, $P < 0.02$) than
265 those of Low CNS1 block. They also had higher PDI gross efficiency (+0.06 unit, $P < 0.03$)
266 or PDI metabolic efficiency (+0.05 unit, $P < 0.05$) than those of Low CNS1 block.

267 *3.2. Plasma characteristics and oxidative status*

268 Plasma characteristics and parameters of oxidative status are presented in table 4.
269 Goats fed HP diet had higher plasma urea than those fed LP diet ($P < 0.006$), with no effect
270 on other plasma characteristics. Goats supplemented with RPM had higher plasma BHB
271 concentration ($P < 0.05$), and a trend to have higher plasma urea concentration ($P < 0.07$) than
272 CTL. Interaction between RPM and dietary CP was significant with an increase in plasma
273 insulin in goats fed HP x RPM diet but a decrease in those fed LP x RPM diet. Parameters
274 characterizing oxidative status were weakly altered by CP content except for an increase in
275 total GSH in the HP diet. Goats in the High CNS1 block had higher MDA plasma
276 concentration ($P < 0.02$). Goats supplemented with RPM tended to have higher plasmatic
277 MDA ($P <0.07$) and total GSH concentrations ($P < 0.09$).

278 *3.3. Plasma Amino-acids and derivatives*

279 Plasma AA data are presented in table 5. Dietary CP did not affect plasma
280 concentrations of non-essential (NEAA) or essential AA (EAA), except for Arg ($P < 0.05$).
281 Compared to CTL goats, those supplemented with RPM tends to have higher plasma
282 methionine ($P < 0.10$) and they had lower Ser ($P < 0.03$) and Tyr ($P < 0.04$) concentrations.
283 Compared to Low CNS1 goats, High CNS1 goats had higher plasma concentration for all
284 EAA (except His and Lys) and for numerous NEAA. Interactions between dietary CP, RPM
285 and genotype for CNS1 were not significant on plasma EAA concentrations, except for Lys

with a trend to a decrease in plasma Lys after RPM supplementation in LP diet, but an increase in goats fed HP diet ($P < 0.06$).

Plasma non-proteinogenic AA and AA derivatives data are presented in table 6. Compared to goats fed LP diet, those fed HP diet had lower plasma concentrations of α-aminoadipic ($P < 0.003$) and α-aminobutyric acid ($P < 0.05$). Compared to CTL goats, those supplemented with RPM had lower plasma concentration of α-aminoadipic acid ($P < 0.02$) and higher α-aminobutyric acid ($P < 0.03$) and cystine ($P < 0.001$).

4. Discussion

4.1. Diet formulation

We have planned 2 different CP contents in the experimental diets to test the hypothesis that supplementation with RPM improved performance through higher efficiency in dairy goats fed low CP than high CP diets, as observed in dairy cows. As expected, LP and HP diets differed for their CP content (13.9 vs 15.8 % CP), PDIE content (100 vs 115 g/kg DM, where PDIE is the protein digested in the small intestine supplied by dietary RUP and by microbial protein synthesized from rumen-fermented OM) and PDIN content (92 vs 109 g/kg DM, where PDIN is the protein digested in the small intestine supplied by dietary RUP and by microbial protein synthesized from rumen NH_3). In these conditions, PDIN appears to be a limiting factor for milk performance in LP diets as dietary recommendations (INRA, 2007) suggest a minimal content of 100 g PDI /kg DM to meet animal requirement. However, a recommended deficit in dietary degradable protein ((PDIN –PDIE) / UFL between -7 and 0 g/UFL with 1 UFL = 1 Feed Unit for Lactation corresponding to 7.11 MJ of Net energy for lactation) is acceptable to account for the use of ruminal NH_3 recycled via the salivary urea (INRA, 2007). In LP diet, this deficit was between -8 and -9 g/UFL, which is slightly below the recommended deficit for goats producing 3.9 to 4.4 kg milk as in our trial. Consequently,

it cannot be excluded that the lack of MPC and MPY responses to RPM supplementation, especially in LP diet (see below), could be related to a lack of dietary fermentable N.

4.2. Effect of CP and PDI on milk performance and composition, and plasma parameters

Increasing CP content from 14% to 16% and consequently PDIE/UFL ratio from 65 to 76 g /UFL increased numerically DMI (+0.1 kg DMI/d, i.e. +2.9%). This numerical increase is however in line with that predicted by INRA (2018): increasing dietary CP content by 18 g/kg DM would result in an 3% increase in intake capacity. In cows, several studies have shown a similar positive effect of PDIE/UFL on the intake capacity (review of Daniel et al., 2016), independently of any impact on ruminal digestion, indicating that a shortage in PDIE supply compared to energy availability limits intake capacity. However, response of DMI to an increase in PDIE/UFL ratio may be partially limited by the availability of fermentable N, especially when (PDIN-PDIE)/UFL ratio is decreased (Rico-Gomez and Faverdin, 2001; Huthanen and Hetta 2012). Therefore, despite the increase in PDIE/UFL in HP diet compared to LP diet, the relative decrease in PDIN-PDIE / UFL (2 g (PDIN-PDIE) / UFL between HP and LP diet) may have limited the potential increase in DMI. Consequently, goats fed LP or HP diets had similar intakes of NEL, starch and fat, but different CP and PDIE intake by design. Moreover, as HP diet had slightly higher LysDi and HistDi concentration than LP diet, goats fed HP diet had significantly higher LysDi and HistDi intake than those fed LP diets.

Goats fed HP diet had similar milk performance, PDI efficiencies, MPC and MPY than those fed LP diet, in contrast to studies in goats (Schmidely et al. 2002; De la Torre et al., 2009) or in cows (Hacque et al. 2012) fed similar changes in dietary CP contents. In these trials, increase in milk yield or MPY with higher dietary CP content was associated with an increase in DMI, that provided more energy and amino-acid available for mammary milk

335 production or protein synthesis. In our experiment, despite the increase in CP, PDI and AA
336 intake, no increase in milk yield, MPC or MPY was observed suggesting that NEl intake was
337 the first limiting factor of HP and LP diet especially for lactose synthesis (Lemosquet et al.,
338 2010), and lead to inefficient use of dietary protein for milk synthesis. As previously reported
339 in goat, PDI gross and metabolic efficiencies were reduced as dietary CP content increased,
340 but this was observed only in goats expressing the high genetic variant for CNS1 (Schmidely
341 et al., 2002; De La Torre et al., 2009) that also add the highest plasma urea. As the plasma
342 concentration of total AA or individual AA was not affected by dietary CP content despite a
343 higher AA intake with no change in protein yield, the higher plasma urea concentration
344 reflects partly an increase of AA catabolism in the liver and/or a greater use of some or all AA
345 for protein synthesis in the peripheral tissues. It also reflects possibly a less efficient use of
346 ruminal NH_3 by rumen microbes at the higher CP intake in the goats fed HP diet (Schwab and
347 Broderick, 2018).

348 Plasma parameters related to oxidative status were not affected by dietary CP content,
349 except for an increase in plasma oxidized glutathione in goats fed HP diet. To our knowledge,
350 no data are available in ruminant on the effect of dietary CP content on oxidative status and
351 especially on glutathione and its oxidized derivatives. In our study, feeding more CP was
352 associated with a trend in a higher intake of methionine and cysteine, 2 amino acids with
353 sulfur atoms that are particularly prone to oxidative damage (Celi and Gabai, 2015). However,
354 this increase in oxidized glutathione in goats fed HP diets was associated in our trial with no
355 change in Protein-Carbonyl plasma concentration which is considered as a marker of protein
356 oxidation, and even a decrease in alpha-aminoadipic acid which is a marker of lysine
357 oxidation (Estevez et Jiong, 2019). We also found a significant decrease in α-amino-butyric
358 acid (a non-proteinogenic AA) plasma concentration, that is a product of methionine,
359 threonine, serine, and glycine metabolism, finally deriving from alpha-ketobutyrate (Chiarla

et al., 2011). In human, higher plasma α-amino-butyric acid concentration reflects liver dysfunction, and increased protein catabolism and oxidation (Chiarla et al. 2011). In dairy ruminants, data on α-ketobutyrate are scarce and inversely related to DMI and energy/protein catabolism (Klein et al 2010). Taken all together, these data do not provide a clear figure on oxidative status of the effect of feeding high CP level in the ruminant. Moreover, our trial was conducted in healthy goats at established lactation period, which probably do not constitute factors to alters oxidative status (Abuelo et al, 2013).

4.3. Effect of Methionine supplementation on milk Performance and composition, and plasma parameters

Balancing diets for metabolizable methionine through supplementation with RPM did not result in an increase in DM intake as previously observed in goats fed different forms of RPM (Alonso-Melendez et al 2016: Al Qaisi and Titi, 2014; Flores et al., 2009) or in cows (Haque et al., 2012). This lack in DMI response to supplementation with RPM was observed independently of the dietary CP content, whereas Madsen et al. (2005) reported an increase in DMI after supplementation with RPM in goats fed high CP (18% CP/DM) vs low CP (13%CP/DM) diets. In cows, inconsistent responses in DMI to supplementation with RPM have been observed that could be due to the form of methionine protection, deficiency of Met in the diet, and the availability of other co-limiting AA (Zanton et al., 2014). As expected, LP and HP diets in our trial were methionine deficient as their MetDi/PDIE ratio was 2.05 and 1.90 % PDIE respectively, which is below the value of 2.50 % to cover methionine requirement for mammary protein synthesis in cow (Rulquin et al., 2007). In these conditions, supplementation with RPM alleviates the deficiency of LP and HP diet for their methionine / PDIE ratio, but both diets remained deficient for lysine (see below). Consequently, goats supplemented with RPM had higher intake of Methionine, but similar intake of NEL, and

other EAA than the CTL goats, and this was independent of dietary CP content. This probably was reflected from the lack of response of ~~raw~~ milk yield and MPY to RPM, even though an interaction was observed between supplementation with RPM and dietary CP content, indicating that supplemental RPM was partitioned toward ~~raw~~ milk synthesis in low CP diets, and to a lower extent, toward MPY in the high CP diet resulting in an increase in MPC (see below). ~~Raw~~ Milk yield was not increased with different forms and doses of RPM supplementation when DMI and consequently NEl intake were not altered by supplementation with RPM in goats (Alonso-Melendez et al 2016: Al Qaisi and Titi, 2014; Flores et al., 2009), whereas when DMI was increased, a positive response of milk yield, and MPY was observed (Madsen et al., 2005), with no changes in MPC. However, in our trial, MPC was increased with RPM supplementation in goats fed high CP, but not in those fed low CP (significant RPM x CP interaction) which was at the opposite of our initial hypothesis. This is probably related to the fact that Met in high CP diet was more limiting that in low CP diet, which resulted in a more efficient use of RPM in high CP than in low CP diet. This is in line with the lower increase in milk and plasma urea associated with RP supplementation in high CP than in low CP diet. The lack in MPY and MPC responses could also be due to the possible limitation of Lys as first limiting AA in our experimental diets. Indeed, contrarily to what was expected, LP and HP diets were also slightly deficient for lysine as their digestible LysDi/PDIE ratio was 6.4% for both diets which is below the recommendation of 7.3% PDIE (Rulquin et al., 2007). That could have also contributed to prevent any increase in DM intake and any increase in MPC, even though in both diets, the ratio of lysine to methionine was near 2.9 which have shown to be the optimal ratio for mammary milk protein synthesis (IINRA, 2007; 2018). Madsen et al., (2005) also reported an increase in DMI, milk yield and MPC in goats fed rumen protected lysine and methionine in combination with a Met/Lys ratio equal to 2.5. These data suggest that in our trial, the low responses of MPY and MPC were due to

410 limiting supply of these two AA (possibly Lysine as the first limiting amino acids) for
411 mammary milk production when the goats were fed high or low CP diets. The lack of
412 response in MPY and MPC may be also due to limiting NEL supply by both diets to optimize
413 mammary protein synthesis. Indeed, Boutinaud et al (2020) reported an increase in MPY and
414 mRNA S1 casein to supplementation with RPM only in dairy goats fed adequate NEl supply
415 (1.84 Mcal/kg DM) but not in those fed low NEl supply (1.48 Mcal/kg DM). The energetic
416 contents of the experimental diets were only 1.52 to 1.55 Mcal/kg which may have limited the
417 increase in protein synthesis after RPM supplementation. Finally, it cannot be excluded that
418 the low response in milk yield and/or MPY was due to the short time of each experimental
419 period of the cross-over design.

420 Supplementation with RPM did not affect the milk fat yield, even though an
421 interaction was observed between RPM and dietary CP: goats supplemented with RPM in low
422 CP diet had higher milk fat yield than those fed high CP diet. This is essentially due to the
423 changes in ~~raw~~ milk yield (cf. above) as no change in milk fat content was observed with
424 RPM supplementation whatever the dietary CP content. Inconsistent responses in goats were
425 observed for milk fat content and milk fat yields (Alonzo-Melendez et al. 2016; Al Quaizi et
426 al., 2014; Flores et al., 2009; Madsen et al., 2005). These variations may be associated with
427 the partition of methionine between different functions and biochemical pathways. Indeed, in
428 the liver, methionine is implied as an intermediate in methyl group transfer for choline and
429 phosphatidylcholine synthesis, that are constituents of lipoprotein that delivers triglyceride to
430 the mammary gland (Bauchard et al., 1998). However, methionine is also involved through
431 transulfuration pathway into the synthesis of cysteine, taurine and glutathione synthesis in the
432 liver (Martinov et al., 2010). In association with the greater use of methionine for milk protein
433 synthesis, it may be suggested that most of the available supplemental methionine was
434 partitioned to mammary protein synthesis, glutathion and taurine synthesis rather than liver

lipoprotein synthesis in the mid-lactating goats used in our trial. Consequently, no supplemental lipoprotein was available to deliver TG to the mammary gland after RPM supplementation. ~~This is on line with the lack of change in the proportion milk fat proportion of stearic acid and oleic acids (data not shown), that are the 2 main FA transported by the liver VLDL.~~

To our knowledge, there is no data reporting the effect of RPM supplementation on plasma parameters of oxidative status in dairy goats. Supplementation with RPM increased plasma methionine concentration in our study, confirming the bioavailability observed in cows (Graulet et al., 2005). This may explain the increase in hepatic glutathione production (Zhou et al., 2016b; Batistel et al. 2018), that is the most abundant thiol compounds against oxidative stress (Osorio et al., 2014). Methionine may also have been used for taurine synthesis, another potent antioxidant (Martinov et al., 2010), as its plasma concentration tended to increase with RPM supplementation. However, increase in plasma total glutathione concentration and the numerically higher plasma taurine concentration with RPM supplementation were not reflected in lower plasma concentration of MDA and PC that reflects lipid peroxidation and protein damage respectively, nor in increase in SOD or catalase. Similar increase in plasma GSH, taurine and cysteine concentrations have been also observed in lactating cows supplemented with RPM during the transition period, thus increasing antioxidant and β-oxidation capacities (Osorio et al., 2014; Zhou et al., 2016a).

4.4. Effect of genetic variant for S1-casein on milk performance and composition, and plasma parameters.

Similarly to previous studies (De La Tore et al. 2008; Schmidely et al. 2002) we observed higher MPY and MPC associated with a higher milk casein concentration, and a higher PDI gross efficiency in High CNS1 block goats compared to those in Low CNS1

block. Goats in High CNS1 block expressing a majority of Ab, Ac Ad and B alleles has been shown to induce a higher secretion of S1-casein relatively to those expressing the intermediate variant (Grosclaude et al., 1987), which was the E allele in that study. This was surprisingly associated with an increase in plasma concentration of EAA (except for Lys and Hist), and in some case with the increase in plasma concentration of some NEAA. Polymorphism of CNS1 has been shown to be involved in the cellular mechanisms of transport and secretion of protein in the mammary gland, with a perturbed intracellular transport of those casein in the defective or low variants (Neveu et al., 2002; Le Parc et al., 2010). Paradoxically, despite a lower protein yield, this could be due to a higher AA requirement in the Low CNS1 block, associated with a lower efficiency use of AA than in the High CNS1 block as we observed in the present study. This could also explain the interaction between the CNS1 blocks and supplementation with RPM for MPY, MPC, milk casein content, and PDI efficiencies in line with our second hypothesis that RPM could be more efficient in goats exhibiting high protein synthesis. This is also in line with the data of Jacobsen (2015) who reported a weak response to RPM supplementation of dairy goats exhibiting low secretion of CNS1 in relation with low Met requirement.

5. Conclusions

Goats fed high CP diet had similar milk production, milk protein content and milk protein yield, efficiency for feed, energy and PDI use than those fed low CP diet, and this was associated with a lack of response in DMI to dietary CP contents, which suggests that Net Energy for lactation was a limiting factor in our experimental diets. Goats fed rumen protected methionine had higher intake of Methionine, but similar intake of Net Energy for lactation and similar energy efficiency, that was reflected into the lack of response of ~~raw~~ milk yield and protein yield to rumen-protected methionine. However, with methionine

485 supplementation, milk protein content was increased in goats fed high CP diet, but not in
486 those fed low CP diet that had higher milk yield, suggesting changes in metabolic partition of
487 nutrient between milk secretion vs milk protein synthesis according to the level of limitation
488 of methionine in diet. Simultaneously, oxidative status was improved in goats fed rumen
489 protected methionine, as reflected by the increase in plasma glutathion and taurine
490 concentrations. In conclusion, our data suggest that further research are needed to determine if
491 specific dietary requirements for methionine can be quantified in goats exhibiting high protein
492 secretion to optimize milk performance response to methionine supplementation.

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671

672 **Table 1.**

673 Ingredients, chemical composition, and predicted nutritive values of the s experimental diets.

Experimental diet [a]	LP	HP
Ingredients, % of DM		
Grass hay	15.0	10.0
Dehydrated alfalfa	18.0	23.0
Pressed beet pulp	40.0	35.0
Soybean meal	5.0	10.0
Barley,	5.0	5.0
Concentrate [b]	15.0	15.0
Mineral and vitamins [c]	2.0	2.0
Chemical composition, % DM [d]		
CP	13.9	15.8
NDF	47.5	44.8
ADF	26.0	25.3
Crude Cellulose	23.0	21.9
Starch	6.60	6.25
EE	2.20	2.25
Lysine	0.64	0.76
Methionine	0.22	0.23
Histidine	0.32	0.38
Leucine	0.93	1.10
Predicted nutritive value [e]		
PDIA, g/kg DM	37.0	53.0
PDIE, g/kg DM	100.0	115.0
PDIN, g/kg DM	92.0	109.0
NE_L, Mcal/kg of DM	1.55	1.52
PDIE/NE_L, g/Mcal	64.5	75.7

674 [a] LP = diet with 14% CP; HP = diet with 16% CP.

675 [b] Concentrate contained 30% soybean meal, 15% sunflower meal, 10% wheat, 10% rapeseed
676 meal, 9% dehydrated alfalfa, 7% soybean seed, 5% corn, 4% beet pulp, 4% wheat bran, 3%
677 molasses, 2% linseed, 1% sodium bicarbonate.

678 [c] Mineral contained 30% dicalcium phosphate, 20% sodium bicarbonate, 20% trace-
679 mineralized salt, 20% limestone, 4% $CaSO_4$, 4% MgO_3, 2% urea, 650,000 IU/kg of vitamin
680 A, 350,000 IU/kg of vitamin D, and 4000 IU/kg of vitamin E.

681 [d] OM, Organic matter; CP, Crude protein; EE, Ether extract; NDF, Neutral detergent fiber;
682 ADF, Acid detergent fiber.

683 [e] PDIA = dietary CP undegraded in the rumen but truly digestible in the small intestine; PDIN
684 = protein digested in the small intestine supplied by dietary RUP and by microbial protein

685 from rumen NH_3; PDIE = protein digested in the small intestine supplied by dietary RUP and
686 by microbial protein from rumen-fermented OM (INRA, 2007).

Table 2.

Body weight, dry matter intake, Net energy of lactation, and selected nutrient intake in alpine goats differing by the genetic variant for CNS1 casein (High vs Low) and fed low (LP) vs high (HP) CP diet supplemented (+) or not (-) with rumen protected Methionine (RPM).

	Treatment								RMSE	P					
										RPM	Diet	CNS1	RPM x Diet	RPM x CNS1	Diet x CNS1
CNS1	Low				High										
Diet CP	LP		HP		LP		HP								
RPM	-	+	-	+	-	+	-	+							
Body weight, kg	66.8	65.2	64.3	64.2	64.9	64.0	64.2	63.4	1.43	0.10	0.50	0.57	0.10	0.94	0.75
DMI, kg DM/d	3.20	3.19	3.10	3.36	3.36	3.31	3.59	3.39	0.83	0.96	0.55	0.20	0.55	0.006	0.55
NEl, Mcal/d	5.01	4.93	4.80	5.07	5.27	5.10	5.54	5.15	0.08	0.38	0.79	0.21	0.77	0.10	0.68
Starch, g/d	204.6	*199.6*	188.4	200.0	216.1	208.8	223.7	203.2	15.0	0.34	0.76	0.20	0.88	0.14	0.69
Fat, g/d	68.4	66.8	68.6	72.6	72.2	69.8	80.7	73.9	5.1	0.36	0.24	0.20	0.88	0.14	0.68
CP, g/d	448.5	439.9	486.2	514.2	473.1	456.9	570.7	523.3	36.9	0.42	0.02	0.20	0.92	0.14	0.61
PDIE, g/d	315.5	308.4	355.1	374.8	331.6	321.8	414.8	381.8	22.3	0.36	0.005	0.20	0.91	0.11	0.61
LysDi, g/d	23.4	23.3	24.2	26.2	24.6	24.2	28.0	26.5	1.4	0.38	0.001	0.20	0.90	0.10	0.59
MetDi[a], g/d	6.4	8.5	6.4	9.2	6.7	8.8	7.4	9.2	0.61	0.001	0.10	0.20	0.91	0.17	0.63
HistDi, g/d	7.6	7.5	7.9	8.6	7.9	7.8	9.2	8.7	0.72	0.36	0.002	0.20	0.90	0.10	0.60
LeuDi, g/d	27.2	27.1	28.9	31.3	28.6	28.2	34.5	33.6	2.3	0.39	0.003	0.20	0.94	0.12	0.60

[a] MetDi intake: digestible Met including supplementation in RPM (2.4 g/d). LysDi, HistDi, LeuDi = lysine, histidine, leucine, digestible in the intestine, respectively.

693 **Table 3**

694 Milk yield and composition, ~~body weight~~, and efficiencies in alpine goats differing by the genetic variant for CNS1 casein (High vs Low) and fed

695 low (LP) vs high (HP) CP diet supplemented (+) or not (-) with rumen protected Methionine (RPM).

	Treatment								RMSE	P					
										RPM	Diet	CNS1	RPM x Diet	RPM x CNS1	Diet x CNS1
CNS1	Low				High										
Diet CP	LP		HP		LP		HP								
RPM	-	+	-	+	-	+	-	+							
Milk yield, kg/d	3.86	3.98	4.21	4.04	4.10	4.17	4.39	4.29	8.4	0.75	0.22	0.18	0.02	0.92	0.98
Protein															
Yield, g/d	109.5	111.9	127.7	127.2	130.9	132.8	142.4	142.0	8.21	0.59	0.13	0.05	0.40	0.96	0.72
Content, %[a]	2.85	2.85	2.98	3.12	3.21	3.21	3.23	3.29	0.77	0.008	0.26	0.02	0.008	0.05	0.42
Fat															
Yield, g/d	114.6	126.6	118.6	121.35	120.3	126.5	125.9	120.4	13.8	0.12	0.92	0.46	0.05	0.16	0.98
Content, %	3.11	3.08	2.83	2.92	2.93	2.98	2.98	2.93	0.62	0.74	0.19	0.71	0.84	0.67	0.22
Lactose															
Yield, g/d	174.9	179.9	190.7	184.9	180.2	186.3	197.1	193.3	12.0	0.88	0.21	0.45	0.02	0.74	0.93
Content, %	4.50	4.47	4.51	4.51	4.46	4.50	4.51	4.51	0.26	0.90	0.21	0.87	0.82	0.31	0.84
Casein, g/L[a]	21.1	24.7	24.6	24.0	27.1	26.9	26.3	27.3	0.22	0.14	0.67	0.02	0.22	0.36	0.48
Fat content / protein content, g/g	1.09	1.08	0.93	0.91	0.92	0.94	0.95	0.93	0.06	0.68	0.07	0.10	0.19	0.75	0.05
SCC, log	6.38	6.45	6.61	6.82	6.44	6.46	6.44	6.49	0.42	0.30	0.36	0.52	0.59	0.49	0.40
Milk urea, g/L	0.47	0.51	0.53	0.55	0.44	0.47	0.55	0.56	0.06	0.06	0.01	0.80	0.40	0.68	0.38
Feed efficiency, kg milk/kg DM intake[b]	1.17	1.18	1.33	1.22	1.23	1.26	1.26	1.33	0.09	0.31	0.20	0.64	**0.04**	**0.001**	0.56

Energy efficiency, Mcal in milk/Mcal intake[b]	0.52	0.52	0.57	0.54	0.53	0.57	0.57	0.59	0.04	0.25	0.12	0.67	**0.003**	**0.001**	0.61
PDI gross efficiency[c]	0.33	0.33	0.36	0.33	0.40	0.40	0.36	0.38	0.02	0.95	0.62	0.03	0.22	0.001	0.30
PDI metabolic efficiency[c]	0.39	0.39	0.42	0.38	0.47	0.48	0.41	0.43	0.03	0.96	0.26	0.05	0.23	0.001	0.20

[a] Interaction RPM x dietary CP x Genetic variant of CNS1: $P < 0.11$ for milk protein content, and $P < 0.04$ for milk casein content.

[b]. Interaction RPM x dietary CP x Genetic variant of CNS1: $P < 0.001$ for feed efficiency, and $P < 0.02$ for energy efficiency

[c] Interaction RPM x dietary CP x Genetic variant for CNS1: $P < 0.001$ for PDI gross efficiency and $P < 0.002$ for PDI metabolic efficiency.

699 **Table 4**

700 Plasma characteristics, antioxidant enzymes activities, malondialdehyde, total and oxidized glutathion, MDA and protein carbonyls

701 concentrations in alpine goats differing by the genetic variant for CNS1 casein (High vs Low) and fed low (LP) vs high (HP) CP diet

702 supplemented (+) or not (-) with rumen protected Methionine (RPM)[a].

	Treatment								RMSE	P					
										RPM	Diet	CNS1	RPM x Diet	RPM x CNS1	Diet x CNS1
CNS1	Low				High										
Diet CP	LP		HP		LP		HP								
RPM	-	+	-	+	-	+	-	+							
Glucose, g/L	0.59	0.59	0.60	0.62	0.62	0.63	0.61	0.61	0.02	0.32	0.80	0.11	0.54	0.41	0.14
NEFA, µM/L	136	150	137	148	189	149	138	162	61.9	0.83	0.61	0.36	0.16	0.35	0.61
BHB. mM/L	0.64	0.71	0.58	0.57	0.54	0.60	0.61	0.63	0.11	0.05	0.75	0.66	0.12	0.66	0.32
Urea, g/L	0.48	0.53	0.59	0.60	0.46	0.48	0.64	0.67	0.10	0.07	0.006	0.79	0.61	0.77	0.33
Insulin, pM/L	56.0	48.0	54.0	72.0	78.0	74.0	77.0	102.1	20.0	0.12	0.60	0.31	0.006	0.80	0.92
SOD, U/mL	198.3	205.9	201.6	198.0	215.25	221.8	233.8	236.2	16.9	0.28	0.68	0.14	0.21	0.69	0.59
Catalase, U/L	23.2	21.9	24.3	24.7	25.0	24.2	27.7	27.1	4.8	0.49	0.23	0.21	0.53	0.89	0.83
MDA, µM/L	7.8	8.1	8.2	8.2	8.8	8.8	8.5	8.8	0.51	0.07	0.90	0.02	0.92	0.94	0.32
GSH total, pM/L	77.6	78.1	77.3	78.4	79.0	83.3	89.0	90.3	5.8	0.09	0.42	0.15	0.57	0.35	0.42
GSH oxidized, pM/L	14.7	14.0	15.8	16.7	12.1	13.6	16.5	15.4	3.4	0.84	0.03	0.44	0.73	0.85	0.62
GSH peroxidase, U/mL	84.7	81.3	78.6	83.0	82.9	84.7	82.3	8.2	7.5	0.63	0.65	0.81	0.29	0.91	0.96
PCs, nM/mg protein	20.1	22.5	20.4	21.8	23.7	24.2	24.5	25.3	2.9	0.27	0.24	0.001	0.72	0.84	0.70

703 [a] SOD = superoxide dismutase; MDA = Malondialdehyde; GSH = glutathion; PCs = protein carbonyls.

704

705 **Table 5**

706 Plasma free AA concentrations (µM/L) in alpine goats differing by the genetic variant for CNS1 casein (High vs Low) and fed low (LP) vs high

707 (HP) CP diet supplemented (+) or not (-) with rumen protected Methionine (RPM).

	Treatment								RMSE	*P*-value					
										RPM	Diet	CNS1	RPM x Diet	RPM x CNS1	Diet x CNS1
CNS1	Low				High										
Diet CP	LP		HP		LP		HP								
RPM	-	+	-	+	-	+	-	+							
Essential Amino-Acids															
Arg	175.8	167.1	189.9	189.1	183.4	192.9	224.9	218.8	39.8	0.88	0.05	0.06	0.84	0.75	0.53
His	56.0	54.1	64.4	59.8	64.4	66.5	72.7	65.7	11.8	0.34	0.35	0.13	0.32	0.90	0.78
Ile	140.6	128.3	148.2	142.9	169.4	177.3	169.5	177.2	25.5	0.93	0.50	0.002	0.79	0.20	0.50
Leu	149.3	126.6	166.3	154.8	192.9	192.0	191.3	190.6	22.3	0.21	0.33	0.003	0.69	0.26	0.27
Lys	176.41	141.9	141.6	161.7	137.3	131.4	154.7	160.7	34.9	0.69	0.43	0.35	0.06	0.68	0.12
Met	29.2	31.3	29.2	31.0	37.5	43.0	36.4	40.2	8.2	0.10	0.67	0.001	0.81	0.53	0.71
Phe	56.4	48.7	59.1	48.4	71.1	74.1	76.4	70.5	13.3	0.12	0.79	0.001	0.38	0.26	0.97
Thr	76.0	72.0	101.6	89.5	105.5	111.6	112.1	103.3	22.5	0.41	0.20	0.006	0.31	0.56	0.17
Trp	52.8	49.3	63.8	55.1	63.9	67.2	69.3	63.2	11.2	0.18	0.27	0.001	0.20	0.41	0.35
Val	259.8	227.9	311.2	314.1	353.4	361.8	356.0	355.5	44.5	0.64	0.20	0.004	0.57	0.42	0.17
Non-essential amino-acids															
Asn	60.8	54.9	69.1	63.5	74.1	80.1	86.2	76.2	18.8	0.41	0.20	0.001	0.41	0.69	0.65
Asp	5.8	5.90	13.9	14.8	18.0	18.4	16.2	16.0	4.8	0.90	0.25	0.001	0.91	0.98	0.06
Ala	201.9	182.2	198.5	206.3	223.8	231.3	209.28	201.3	26.8	0.66	0.49	0.03	0.69	0.69	0.06

Gln	249.4	240.1	273.6	267.6	301.4	307.0	286.6	284.0	38.5	0.76	0.88	0.10	0.91	0.64	0.31
Glu	56.8	51.8	46.6	46.8	62.4	63.4	53.9	57.2	6.4	0.95	0.20	0.15	0.26	0.17	0.98
Gly	731	702	751	733	829	793	719	664	94	0.15	0.50	0.76	0.93	0.64	0.29
Pro	133.9	116.5	161.7	165.2	179.4	170.2	177.9	165.3	21.2	0.10	0.13	0.02	0.41	0.71	0.07
Ser	129.9	121.6	116.7	96.5	130.3	128.7	138.5	113.0	24.2	0.03	0.34	0.34	0.16	0.95	0.53
Tyr	65.7	56.1	65.4	54.7	79.6	77.7	90.2	77.9	15.6	0.04	0.69	0.001	0.47	0.70	0.60
Total Amino-Acids	3848	3584	4100	4021	4389	4459	4575	4419	532	0.43	0.23	0.002	0.92	0.64	0.44

708

Table 6

Plasma non-proteinogenic AA and AA derivatives concentrations (µM/L) in alpine goats differing by the genetic variant for CNS1 casein (High vs Low) and fed low (LP) vs high (HP) CP diet supplemented (+) or not (-) with rumen protected Methionine (RPM).

	Treatment								RMSE	*P*-value					
										RPM	Diet	CNS1	RPM x Diet	RPM x CNS1	Diet x CNS1
CNS1	Low				High										
Diet CP	LP		HP		LP		HP								
RPM	-	+	-	+	-	+	-	+							
AA and derivatives															
1-CH3 histidine	68.7	70.2	71.3	59.2	65.1	67.8	68.7	62.9	10.1	0.19	0.79	0.90	0.04	0.46	0.85
3-CH3 histidine	5.3	5.1	5.2	4.8	6.0	6.2	5.5	5.2	0.8	0.31	0.19	0.09	0.37	0.70	0.37
α-aminoadipic acid	5.9	5.6	5.2	5.0	6.7	6.5	5.3	4.8	0.4	0.02	0.003	0.27	0.73	0.67	0.27
α-aminobutyric acid	9.2	9.2	7.2	8.5	8.6	9.9	6.5	7.7	1.2	0.003	0.05	0.70	0.33	0.30	0.65
β-alanine	5.5	5.3	6.3	5.5	5.9	6.2	5.2	5.9	0.9	0.94	0.99	0.77	0.77	0.03	0.27
γ-aminobutyric acid	5.1	5.7	5.5	5.1	5.9	5.9	6.6	6.0	0.9	0.73	0.93	0.55	0.09	0.40	0.82
Carnosine	44.0	42.5	47.0	46.1	50.1	51.9	56.4	47.1	9.21	0.29	0.67	0.17	0.27	0.58	0.79
Citrulline	181.1	188.6	199.5	200.7	181.7	180.7	222.4	221.2	38.1	0.87	0.21	0.68	0.87	0.78	0.56
OH-Proline	13.1	14.5	14.5	15.5	18.0	26.0	17.2	17.0	6.3	0.12	0.53	0.09	0.19	0.39	0.30
Sulfur containing compounds															
Cystine	2.8	3.4	3.0	3.6	3.4	4.1	3.5	3.9	0.6	0.001	0.88	0.18	0.62	0.99	0.83
Cysteine	25.1	26.0	33.0	33.8	36.7	37.8	38.3	34.9	6.8	0.93	0.23	0.02	0.50	0.58	0.17
Taurine	72.3	81.7	106.8	115.8	107.1	135.9	130.1	172.8	57.4	0.13	0.24	0.12	0.82	0.36	0.94

712 **Figure 1.**

713 Description of the experimental design.

Genotype of milk CNS1[a]	Adaptation period (Week 1-Week 2)	Experimental period (Week 3 – Week 11)		
		Period 1 (Week 3 – Week 6)	WO[b] (Week 7)	Period 2 (Week 8 –Week 11)
	CP content of the diet (% DM)	Experimental treatment[c]		
High (n=2) vs Low (n=2)	13 -> 14	RPM	CTL	CTL
High (n=2) vs Low (n=2)	13 -> 14	CTL	CTL	RPM
High (n=2) vs Low (n=2)	13 -> 16	RPM	CTL	CTL
High (n=2) vs Low (n=2)	13 -> 16	CTL	CTL	RPM

714 [a] High vs Low = genetic variant with high vs low secretion of casein S1 in milk with n=
715 number of goats.

716 [b] WO = washout period.

717 [c] Experimental treatment: supplemented (RPM) or not (CTL) with rumen protected
718 Methionine.