Effects of protein reduction in lamb fattening concentrate on *in vivo* digestibility, nitrogen balance and meat quality.

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Effects of protein reduction in lamb fattening concentrate on *in vivo* digestibility, nitrogen balance and meat quality.

Determining the optimal crude protein (CP) content in small ruminant diets is crucial for reducing ammonia. This study assessed the feasibility of decreasing CP content in light lamb diets and its effect on apparent digestibility and meat quality. Isoenergetic fattening concentrates with different CP contents: 18% (Low) vs. 20% (Control) during the growing phase (14 to 19 kg body weight (BW)) and 17% (Low) vs. 19% (Control) during the finishing phase (19 to 25 kg BW) were tested in two experiments. The *in vivo* digestibility experiment (Experiment 1) involved 24 Rasa Aragonesa lambs, 12 per phase. Apparent nutrient digestibility, nitrogen balance and blood metabolites were determined. Nutrient digestibility was compared using total faecal collection and spot faecal samples with acid-insoluble ash (AIA) as internal marker. In Experiment 2, meat quality of *longissimus thoracis et lumborum* muscle of 24 Ripollesa lambs fed concentrates differing in CP was evaluated. The reduction of CP tended to decrease the acid detergent fibre digestibility (total faecal method) and decreased both organic and dry matter digestibility (AIA method) during the growing phase. Overall, digestibility coefficients were underestimated with AIA analysis compared to total faecal collection. Lambs of the Low group had lower plasmatic β-hydroxybutyrate in the finishing phase and results of N balance were similar between groups. Meat quality was minimally affected, although lower CP increased metmyoglobin after 6-day oxygen exposure and altered minor fatty acids. Reducing CP by 2% in lamb diets is advisable as it has limited impact on apparent digestibility and meat quality.

Key words: acid-insoluble ash; blood metabolites; fatty acid profile; lipid oxidation; meat colour.

Abbreviations: AIA, acid insoluble ash; ADF, acid detergent fibre, BHB, β-hydroxybutyrate; BW, body weight; CP, crude protein; DM, dry matter; DMb, deoxymyoglobin; DMI, dry matter intake; FA, fatty acid; FDM, faecal dry matter; IMF; intramuscular fat; LTL, *longissimus*

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thoracis et lumborum; MMb, metmyoglobin; MUFA, monounsaturated fatty acids; N, nitrogen; NDF, neutral detergent fibre; OMb, oxymyoglobin; OMI, organic matter intake; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

1. Introduction

Spain is the largest producer of lamb meat of the European Union (EUROSTAT, 2024), with fattened light lambs being the most common product sold. These lambs are typically fed concentrates and straw *ad libitum* (90:10 forage:concentrate ratio) from weaning (14-16 kg body weight (BW)) to slaughter (22-26 kg BW). The concentrates used are high in energy and crude protein (CP) content and often expensive, mainly due to the inclusion of protein-rich ingredients, which can jeopardise the profitability of farms under certain economic circumstances. In addition to economic considerations, optimizing dietary protein is also essential from environmental perspective, given the increasing concern about the impact associated with livestock production systems. Nitrogen (N) is an essential nutrient for animal production. However, ruminants are inefficient users of N, with low retention and high excretion rates (Cole and Todd, 2008). Therefore, surplus N in the diet is excreted in faeces and urine, acting as contaminating sources of both soil and water (Dijkstra et al., 2013). In addition, the deamination and subsequent excretion of excess N require substantial energy expenditure, thereby reducing the efficiency of nutrient utilization (Van Soest, 1994). Dietary protein restriction has emerged as a feasible strategy to reduce N excretion while optimizing protein use, as previous studies have shown in a wide range of ruminants, including lambs (Seoni et al., 2018), cattle (Dijkstra et al., 2011) and goats (Atti et al., 2004). Thus, it is essential to optimise the protein content in the diet to match the production requirements of the animals, improve farm profitability and reduce the environmental impact.

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The reduction in the CP content in the diet can affect the digestibility of nutrients, although findings in the literature remain inconsistent. Some studies observed with higher CP content an increase in dry matter (DM) digestibility, others an increase in the apparent CP digestibility (Kiran and Mutsvangwa, 2009; Muruz et al., 2017), whereas others no reported effect (Gao et al., 2016). Nevertheless, most of these studies have been conducted on heavy and old animals, whereas studies on growing lambs from small-frame autochthonous ovine breeds across different periods are scarce.

The relationship between dietary CP contents and carcass traits in lambs is complex, as it depends on several factors such as growth rate, fattening duration, and breed characteristics. Some studies indicate that high CP intake supports lean muscle deposition and reduces fat accumulation (Atti et al., 2004; Kioumarsi et al., 2008). The effect of CP on meat colour is still unclear in the literature, with some research reporting no influence (Cañeque et al., 2003; Ponnampalam et al., 2004), while others associate lower dietary CP contents with increased intramuscular fat, altering the yellow and red indexes of meat (Teye et al., 2006; Youssef and Barbut, 2009). Research on fatty acid (FA) composition has shown inconclusive results, with some studies reporting no differences (Arsenos et al., 2007; Ponnampalam et al., 2004), while others found variations in unsaturated FA content when dietary CP was modified (Cañeque et al., 2003; Seoni et al., 2018). These inconsistencies highlight the need for further research to clarify the influence of dietary protein on meat quality in light lamb production systems. Given this framework, we hypothesized that the range of adequate protein content in the diets of light fattening lambs may allow for reductions without negative implications for growth and meat quality. Therefore, the aim of this study was to evaluate the reduction of CP content of the diet in growing and finishing phase of fattening light lambs on digestibility, N balance, and meat quality.

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2. Materials and Methods

To assess the effect of CP content on apparent digestibility and on lamb meat quality two experiments were performed: i) The *in vivo* digestibility assay was carried out at the facilities of the Aragón Center for Agrifood Research and Technology (CITA) in Montañana (Zaragoza, Spain). The animals used were handled in accordance with the Spanish Animal Protection Regulations RD 53/2013, which complies with European Union Directive 2010/63 with regard to the protection of animals used for experimental and other scientific purposes (CEEA, protocol no. 2017–07); ii) The meat proceeds from the lambs slaughtered at 25 kg after being fed in the facilities of BonÀrea Agrupa company (Guissona, Lleida, Spain).

In both experiments, two CP contents (Control vs. Low) were evaluated in two phases during the lamb fattening period: growing (14 to 19 kg BW) and finishing (19 to 25 kg BW). Thus, four concentrates were formulated and assigned to treatments groups. On a DM basis, CP contents of the concentrates were 20 % (Control group) and 18 % CP (Low group) in the growing phase and 19% (Control group) and 17% of CP (Low group) in the finishing phase. All concentrates were isoenergetic (1 UFC/kg) and were formulated with the same ingredients and additives, modifying the percentage of inclusion of vegetable protein (Pelegrin-Valls et al., 2020). The chemical composition of the concentrates is presented in Table 1. The feed presentation was granulated with a pellet diameter of 3.5 mm and the granulation temperature was 60 °C.

2.1. Experiment 1. In vivo *digestibility assay*

Two *in vivo* digestibility trials were conducted, the first during the growing phase and the second during the finishing phase. Twelve different lambs were used in each trial. Initially, the lambs were penned in groups during 10 days to acclimatise to concentrate feeding. Then, they were individually housed in metabolic crates (120 × 50 × 90 cm; length × width × height) for 7 days: 2 days to adapt to the cages and 5 days to collect samples to estimate the apparent digestibility. The cages were equipped with a feeder, drinker and excreta collector with a mesh

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to allow for the separation of faeces and urine. Nose to nose contact between the lambs in adjacent crates was allowed during the entire study period. The lambs were fed *ad libitum* with the different concentrates according to the phase and CP treatment, with a refusal allowance of approximately 10%. Daily at 8:00 h, the amount of feed offered, refusals, faeces and urine were recorded, and composite samples were collected per animal and per phase. Urine was collected in a deposit with 50 ml of 10% (v/v) H_2SO_4 to reach a final pH below 3. Feed and faecal samples were dried in an oven at 60 °C for 48 h and then ground and sieved through a 1 mm screen. Additionally, a small part of these samples was sieved through a 0.2 mm screen. All samples were stored in total darkness until further analysis.

The total tract apparent digestibility of DM, CP, neutral and acid detergent fibre (NDF and ADF, respectively) was calculated using two methods. First, the percentages were calculated using daily feed intake and total faecal collection as:

$$Dig_{TF}\ (\%) = [(DMI \times Z_{diet}) - (FDM \times Z_{faeces})] / (DMI \times Z_{diet})$$

where DMI is the daily DM intake, FDM is the daily faecal DM excreted, and Z_{faeces} and Z_{diet} are the nutrient concentrations (%) in the faeces and in the diet, respectively.

In addition, the apparent tract digestibility was also estimated analysing the acid insoluble ash (AIA) content as internal marker in the diet and faeces as described by Pelegrin-Valls et al. (2020), with the following equation:

$$Dig_{AIA}\ (\%) = 100 - [100 \times (AIA_{diet}/AIA_{faeces}) \times (Z_{faeces}/Z_{diet})]$$

where AIA_{diet} and AIA_{faeces} are the AIA concentrations (%) in the faeces and in the diet, respectively.

A comparative study was conducted between digestibility percentages calculated with total faecal collection (Dig_{TF}) and those estimated from the concentrations of AIA in faeces and diet

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Dig $_{AIA}$ (%). The N retention was calculated by the difference of N consumed and the total N excreted (faecal and urinary).

Blood samples were collected from the jugular vein into tubes containing heparin or ethylenediaminetetraacetic acid (EDTA) before each morning meal supply at the beginning and at the end of each *in vivo* digestibility trial. Blood samples were immediately centrifuged (3000 *g* for 15 min at 4 °C) and plasma was harvested and stored at −20 °C until metabolite analyses.

2.2. Experiment 2. Meat quality study

Meat samples were collected from 24 Ripollesa male lambs belonging to a previous and larger trial (Pelegrin-Valls et al., 2020). Briefly, 120 weaned male lambs of 45-60 days of age and 15 ± 1.5 kg of BW were fattened during 42 days, divided into two phases: growing phase (15 to 19 kg BW) and finishing phase (19 to 25 kg BW). Half of the lambs were fed the Low CP concentrate in each phase, while the other half received the Control concentrate. The concentrates were the same as those previously described in Experiment 1 (Table 1), in addition lambs had access to barley straw.

The lambs were slaughtered when they reached 25 kg BW, and 24 of them were used to study meat quality. After slaughter, each cold carcass (24h post-mortem) was carefully split longitudinally into the two half carcasses and the *longissimus thoracis et lumborum* (LTL) muscles were collected to analyse colour using a Minolta CM−2006d spectrophotometer (Konica Minolta Holdings, Inc., Osaka, Japan) in the CIELAB space (Lobón et al., 2019), chemical composition, lipid oxidation, and FA profile.

The muscle between the 6th to 13th thoracic vertebrae were sliced. The pH of the LTL muscle was measured with a pH meter equipped with a Crison 507 penetrating electrode (Crison Instruments,S.A., Barcelona, Spain). The portions from the 4th to the 6th lumbar vertebrae were vacuum packed, frozen and stored for the proximate chemical composition and FA analyses. The

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remaining part of the muscles was sliced into 2.5-cm thick samples for colour and lipid oxidation determinations. Slices were randomly placed in trays wrapped with oxygen-permeable polyvinyl chloride film and kept in darkness at 4 °C until the colour was measured (0, 3, and 6 days of air exposure). The samples of day 0 were also allowed to bloom in darkness at 4 °C for 1 h before being measured. The percentages of haem pigments in the meat were estimated by the method of quantification without limit values (AMSA, 2012). Immediately after the colour measurements were conducted, the samples were vacuum packed and frozen at −20 °C until lipid oxidation analysis.

2.3. Chemical Analyses

The chemical composition analyses of feedstuffs, faeces and urine were run in duplicate, according to Lobón et al. (2020). Acid-insoluble ashes were estimated by the procedure described in Álvarez-Rodríguez et al. (2017), based on the method of Shrivastava and Talapatra (1962). For blood metabolites analysis, an automatic analyser (GernonStar, RAL/TRANSASIA, Dabhel, India) was used. Urea was determined using a spectrophotometric kit based on the GLHD UV urease kinetic method (RAL, Barcelona, Spain), creatinine using a spectrophotometric kit based on the enzymatic hydrolysis of endogenous creatine (RAL, Barcelona, Spain) and, finally, β-hydroxybutyrate (BHB) was determined using a spectrophotometric kit based on its enzymatic oxidation (Randox Laboratories Ltd., Antrim, UK).

To determine the chemical composition of meat, the samples were weighed before and after freeze-drying (DM content). The CP content was analysed following the Dumas procedure using a N analyser (Model NA 2100, CE Instruments, Thermoquest SA, Barcelona, Spain) and the intramuscular fat (IMF) content using an Ankom XT10 (AOCS, 2005). The lipid oxidation, measured as malondialdehyde, was determined following the procedure reported by Bertolín et al. (2019). The FA were determined using a GC (Bruker 436 Scion gas, Billerica, MA, USA) equipped with a cyanopropyl capillary column (BR-2560, 100 m × 0.25 mm ID × 0.20 μm thick,

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Bruker, Billerica, MA, USA) with a flame ionisation detector and Compass CDS software. Helium was used as carrier gas and the oven temperature was set at 70 °C for 1 min, then increased at 5 °C/min for 2 min up to 225 °C and held for 17 min, giving a total run time of 80 min. Injector and detector temperatures were set at 260 and 250 °C, respectively. The FA identification was performed using the GLC-532, GLC-401, GLC-643, GLC-642, GLC-463, C18:1 t11, C19:0 and C23:0 standard references (Nu-Chek-Prep Inc., Elysian MN, USA) and the relative retention times observed in the literature (Bravo-Lamas et al., 2016; Lee et al., 2012).

2.4. Statistical Analyses

Statistical analyses were performed using SAS 9.4 (SAS Inst. Inc., Cary, NC, USA). Data from the growing and finishing phase in Experiment 1 (intake, apparent digestibility, and N balance, metabolites) were analysed separately using the MIXED model based on Kenward-Roger′s adjusted degrees-of-freedom solution. For plasma metabolites, the mean of the two sampling times (at the beginning and at the end of each digestibility trial) was used as the representative value for each animal. The model considered the CP content (Low vs. Control) as fixed effect and the lamb as random effect and the initial BW was used as a covariate. For the comparative study of digestibility percentages calculated with total faecal collection and with dietary and faecal AIA contents, the same model was used, considering the digestibility estimation method (total faecal collection vs. AIA), the CP content and their interaction as fixed effects, and the lamb as random effect. In Experiment 2, the meat chemical composition and FA composition of the LTL muscle were analysed using a general linear model (GLM procedure) with the CP content as the fixed effect. The colour and lipid oxidation of the LTL muscle were analysed with a mixed model using repeated measurements. The CP content, the display time and their interaction were included as fixed effects, and the lamb was included as a random effect.

The experimental unit was the lamb for all traits. Multiple comparisons among treatments were performed using Tukey's method. The least-squares means and standard errors were

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obtained, and differences were considered significant when $P<0.05$. The trends were discussed when $0.10 < P \leq 0.05$.

3. Results

3.1. Experiment 1: In vivo *digestibility assay*

The daily nutrient feed intakes and apparent digestibility using total faecal collection and AIA contents in feed and faecal samples are presented in Table 2. No differences between treatments were observed in any phase ($P>0.05$), except for a trend in DMI and OMI during the finishing phase, with greater intake in the Low group ($P<0.10$). The reduction of CP content in the concentrate did not affect total tract digestibility using total faecal collection ($P>0.05$), except for a tendency towards a lower total tract ADF digestibility ($P=0.06$) in the Low group during the growing phase. Regarding the results using the AIA method, the total tract DM and OM digestibility was higher in the Control group in the growing phase ($P<0.05$), but no effect was found in the finishing phase ($P>0.05$).

The comparison of digestibility estimation methods by phase is presented in Figure 1. The digestibility coefficients were consistently higher when estimated using total faecal collection than when estimated using the AIA content in feed and faecal samples ($P<0.001$), regardless of the CP content.

The decrease in the CP content of concentrate had no effect on N balance in any phase ($P>0.05$; Table 3). Regarding the average plasma metabolite concentrations (Table 4), none of them (urea, creatinine and BHB) were affected by the CP content in any phase, except for the BHB in the finishing phase ($P>0.05$), which tended to be lower in the Low treatment ($P=0.09$).

3.2. Experiment 2: Meat quality study

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There were no differences in the pH values of meat at cutting from animals fed the Control and the Low protein diets (P>0.05; Table 5). The CP content in the concentrate did not affect the chemical composition of the meat and had minimal impact on most FA in the muscle. Focusing on the existing differences, the Low CP diets tended to increase the percentage of C18:1 c14 (P=0.05), reduced the percentage of C18:2 c9,t12 (P<0.05), and tended to decrease the percentages of C20:2n-6 (P=0.07), C20:4n-6 (P=0.06) and C22:0 (P=0.05). The observed effects on individual FA did not translate into differences in the major FA groups in meat or in PUFA/SFA and n-6/n-3 ratios (Table 5).

Regarding the colour of the LTL muscle, lightness (L*), redness (a*), and saturation (C*) were only affected by display time (Figure 2, P>0.05). The hue (h_{ab}) was influenced by the interaction between CP content and display time (P<0.xx). At the time of cutting (day 0), h_{ab} was higher in animals fed the Control diet (P<0.05), but this difference disappeared with increasing exposure time (P>0.05). Concerning the haem pigments (Figure 2), deoxymyoglobin (DMb) and oxymyoglobin (OMb) were only affected by oxygen exposure time (P<0.001), while metmyoglobin (MMb) tended to be influenced by the interaction between treatment and time (P=0.09). The MMb levels were higher in meat from lambs fed the Low protein diet at 3 days and 6 days of exposure (P=0.005). Meat lipid oxidation was only affected by oxygen exposure time (P<0.001), with no effect of the CP content in the concentrate (P>0.05; Figure 3).

4. Discussion

4.1. Effect of dietary CP on in vivo digestibility and N balance

The absence of effect of CP content on nutrient intake agrees with the results reported by Pelegrin-Valls et al. (2020) using the same concentrates but in a large-scale farm experiment during the whole fattening period, implying that the Low CP concentrate met the animal requirements. Similarly, other studies reported no effect on the intake when CP was reduced by

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10% in Hu sheep (Zhang et al., 2022) and in Anhui white goats (14.8 vs. 13.4% and 12% CP; Zhu et al., 2020). However, it is worth noting that in the finishing phase a trend towards higher daily DM and OM intake was observed in the Low group, which suggests a possible compensatory response, whereby animals fed a diet with lower protein concentration increased their overall feed intake to meet their nutritional requirements, particularly for protein since the diets were isoenergetic and nutritionally balanced. Nevertheless, the fact that the trial was conducted using metabolic cages, which guarantee accurate data collection but may prevent natural feeding behaviour, added to individual variation in feed intake, digestive efficiency and daily BW gains could have contributed to the lack of statistically detectable differences.

There is limited consensus in the literature regarding the effects of dietary CP contents on nutrient digestibility. Some studies evaluated diets differing not only in CP but also in energy content, making it difficult to isolate the effect of CP. The variability in digestibility results has often been related to the energy content of the diets (Sultan et al., 2010) and the feeding level (Andrews and Ørskov, 1970), with energy supply being a primary determinant of digestibility, and therefore assessing CP effects is most reliable when diets supply a similar net energy in pellet form. In the present study, where isoenergetic diets were used, a moderate CP reduction (2 percentage points) had no significant effect on the apparent digestibility of any of the nutrients studied. This finding is in line with previous studies in lambs (Gao et al., 2016) and goats (Dutta et al., 2009), which also found that moderate dietary CP reductions did not impair digestibility. In contrast, when larger differences in dietary CP contents are evaluated, several studies have reported that the diets with greater CP content resulted in increased CP digestibility without affecting the digestibility of other nutrients (Kiran and Mutsvangwa, 2009; Li et al., 2025; Muruz et al., 2017). These findings suggest that CP digestibility is partly influenced by the content of CP in the diet up to a threshold digestive efficiency.

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Our results demonstrate that the estimation method based on total faecal collection provides higher coefficients of apparent digestibility and substantially lower variability compared to the use of AIA as an internal marker. These results are consistent with the general understanding that total faecal collection is a more accurate and precise method for estimating digestibility, as it directly quantifies nutrient excretion without relying on marker recovery assumptions (Lee and Hristov, 2013). Despite its lower values, the AIA method may still offer a practical alternative for on-farm or comparative feed evaluation settings, especially in the finishing phase, where that the degree of underestimation ranged between 7% and 16% and showed the same lack of effect of CP content as the total collection method. This consistency allows for valid relative comparisons between diets, provided the limitations of the method are acknowledged. One potential reason for the reduced accuracy of the AIA method in this study may be related to the low AIA concentration in the diets used (0.51% on DM basis; data not shown). Previous research has indicated that the reliability of AIA as an internal marker improves when its dietary content exceeds 0.75% on DM (Thonney et al., 1985). Low concentrations of AIA can increase sampling error and marker recovery inconsistencies, thus contributing to the higher coefficients of variation observed herein.

Regarding the N balance, a high N retention was observed in both CP dietary treatments irrespectively of the phase, which may be attributed to the low urine and faecal N excretion. The lambs used here had been recently weaned at a young age, and therefore they were in a phase of rapid growth, during which a high N retention is expected. In this sense, Teixeira et al., (2023) also observed high N retention in finishing lambs of similar BW (21.5). However, references regarding N balance in lambs are not conclusive, likely due to the numerous factors influencing N metabolism. According to Hristov et al. (2019), several variables can affect the accurate measurement of faecal N excretion in *in vivo* trials, including the period of adaptation to the diet, duration of faecal collection, diet composition, losses of volatile N (e.g., ammonia, action of

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311 microbial ureases), and sampling and handling protocols for diet, urine and faeces. Any
312 unaccounted N losses during collection and processing are typically considered as N retained in
313 body tissues, potentially leading to overestimation of N retention (Owen, 1967; Spanghero and
314 Kowalski, 1997). Environmental conditions can also affect the N balance. For example, Queiroz
315 de Carvalho et al. (2024) found greater N retention in lambs housed under shaded conditions
316 compared to those exposed to direct sunlight. In the present study, the high N retention observed
317 may be partly attributed to the aforementioned factors, and to some extent to handling or
318 sampling errors, particularly particle precipitation during refrigeration or freezing, which may
319 have caused nitrogenous compounds to settle and thus be underestimated during analysis.

320 Although no differences were observed here in N excretion, diets with higher CP content often
321 lead to greater N excretion in urine, particularly when protein intake exceeds the animal
322 requirements (Zhou et al., 2019). In a large-scale trial using the same experimental diets as here,
323 Pelegrin-Valls et al. (2020) observed that lambs fed higher-CP concentrates excreted more
324 urinary N but had similar growth rates, reinforcing the idea that moderate reductions in CP can
325 reduce N losses without compromising performance.

326 Blood metabolites of each phase in Experiment 1 were consistent with the normal range for
327 growing lambs (Kaneko et al., 2008). Dietary CP contents correlate directly with plasma urea
328 concentrations in fattening lambs (Dabiri and Thonney, 2004; Rocha et al., 2004), however, in
329 the current study the CP content was not reflected in an increase in blood urea concentration in
330 any phase, probably due the similar CP intake reported.

331 *4.2. Effect of dietary CP on lamb meat quality*

332 The meat pH values observed in this study were consistent with those reported for crossbred
333 light lambs (Romane × Berberine × Ripollesa) and Rasa Aragonesa breeds slaughtered at similar
334 live weights (Bottegal et al., 2024; Lobón et al., 2017). These values were normal and suggest

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that there were no physiological or stress-related issues in the animals (Carrasco et al., 2009). Regarding the chemical composition of the meat, neither the protein content nor the fat content was affected by the level of protein in the diet. The CP content in meat is not consistently reported across studies, making direct comparisons challenging. This may be due to the fact that modifying the protein content of muscle tissue is inherently difficult, as it is largely regulated by genetic and developmental factors rather than by short-term dietary changes (Chang and Ma, 2025). For instance, Atti et al. (2004) found no significant effect of dietary protein level on muscle CP content in goat kids.

Intramuscular fat content is an important quality trait in meat, as it contributes to flavour, juiciness, and consumer acceptability. In the present study, the lack of effect of dietary CP content on IMF is consistent with the similar growth performance observed by Pelegrin-Valls et al. (2020) prior to slaughter, and aligns with previous studies in lambs that also reported no changes in IMF with different CP contents in the diet (Seoni et al., 2018; Yongjie Wang et al., 2021). In kids, however, Atti et al. (2004) reported a non-linear response, with intermediate CP contents reducing IMF compared with both low and high CP contents. The inconsistency observed in ruminants may be attributed to differences in the animal growth stages, breeds and feeding plane, as fat deposition patterns are closely linked to physiological maturity, energy partitioning and genetics (Schumacher et al., 2022). In contrast, studies in pigs have consistently shown that when the protein intake was inadequate there is an increase in fat deposition due to the limitation of protein synthesis and increases energy available for fat deposition (Pettigrew and Esnaola, 2001).

Fatty acid profile in meat is a key nutritional quality trait, increasingly valued by consumers due to its implications for human health. In the present study, dietary CP content induced only minor changes in individual FA, without altering any of the main FA groups or the PUFA/SFA and n-6/n-3 ratios, indicating a limited biological relevance. The minimal impact of dietary CP content on meat FA profile is likely attributable to the similar FA composition of the

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experimental diets. As widely reported, the FA profile of meat is strongly influenced by the FA profile of the diet (Wood et al., 2008), even in ruminants where biohydrogenation partially modifies dietary lipids, but dietary trends are still reflected in tissue composition. Additionally, the IMF content was similar across treatments, which may have further limited differences in FA proportions, as total fat content can influence the FA profile by diluting or concentrating specific FA groups (Wood et al., 2008). In contrast, Seoni et al. (2018) reported lower MUFA and higher PUFA levels in lambs fed a low-protein diet (15% vs. 20.2% CP). The authors attributed this to numerical differences in IMF content, although slight differences in dietary FA profiles may also explain their observed changes. Zhang et al. (2023) also found that reducing dietary CP by approximately 10% and adjusting soluble protein levels altered the FA profile in lamb meat, with a reduction in SFA and an increase in PUFA, particularly n-3 PUFA. Nevertheless, the lack of data on the FA composition of the diets and the IMF content in that study limits the interpretation of their results.

Meat colour is a key quality attribute influencing consumer purchasing decisions, as a bright red hue is commonly perceived as an indicator of freshness (Testa et al., 2021). In the present study, the level of CP in the diet had no effect on any colour parameters in LTL muscles, but h_{ab} at the time of cutting. All values fell within the normal range observed in light lambs reared under similar conditions and followed the expected changes due to post-mortem ageing (Bottegal et al., 2024; Lobón et al., 2017). These results are consistent with those reported by Wang et al. (2021b) which showed no significant effect of varying dietary CP contents on meat colour in lambs. However, findings in the literature remain inconsistent. For instance, in heavy lambs fed a low CP diet, Wang et al. (2021a) reported increases in L*, a*, b*, C* and h_{ab} values in lambs receiving 8% vs 12% CP in the diet, while Seoni et al. (2018) observed an effect only on a* when comparing 15% vs. 20.2% CP contents. Moreover, the dietary CP differences were greater in the abovementioned studies.

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Unexpectedly, MMb levels were higher in the Low CP group on days 3 and 6 of display. Although MMb accumulation is generally associated with brownish discolouration and reduced consumer acceptance (Sañudo et al., 2007), this increment did not translate into measurable changes in the instrumental colour traits assessed. This suggests that moderate CP reductions may not have a perceptible impact on meat appearance under the conditions studied. Nevertheless, very few studies have directly examined the relationship between dietary protein levels and pigment oxidation dynamics, limiting the scope for comparison. Further research is warranted in this area, as meat discolouration due to imbalances in MMb formation contributes to substantial economic losses in the meat industry (Nair et al., 2014).

Lipid oxidation is a key indicator of meat quality, as it is closely associated with the development of rancid flavours and a reduction in shelf-life. No differences in malondialdehyde levels were observed here between treatments at any of the evaluated time points. This lack of effect on lipid oxidation due to the CP content in the diet may be attributed to the absence of differences in intramuscular fat content and FA composition between the diets. The FA profile, particularly the degree of unsaturation, is strongly associated with lipid oxidation susceptibility, with PUFA being especially prone to oxidative degradation (Wood et al., 2008).

Overall, the few differences observed in the present study suggest that moderate dietary protein reduction does not substantially alter the FA composition of lamb meat when diets are isoenergetic, have similar lipid profiles, and result in similar fat deposition.

5. Conclusions

A reduction of 2% CP in the fattening concentrates (growing and finishing phases) can be recommended, as the nutrient digestibility is not affected. This indicates the interest of exploring further reductions of dietary CP to reduce the N emission, without impairing performances. In addition, the variation in dietary protein content influences certain aspects of meat composition,

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however these effects are relatively minor and do not significantly change the overall quality of the meat. This suggests that altering dietary CP within the studied range may not have practical implications for meat producers, retailers and consumers, since the intramuscular fat content was not affected.

CRediT authorship contribution statement

Sandra Lobón: Writing – original draft, Validation, Supervision, Methodology, Investigation, Conceptualization. Clàudia Baila: Writing – original draft, Formal analysis, Data curation. Javier Álvarez-Rodríguez Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition. Mireia Blanco: Writing – review & editing, Methodology, Investigation, Conceptualization. Isabel Casasús: Writing – review & editing, Methodology, Investigation, Conceptualization. Jonathan Pelegrin-Vall Formal analysis, Data curation. Margalida Joy: Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition.

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Declaration of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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623 **Table 1**

624 Chemical composition of the concentrates fed during the growth and finishing periods of light

625 lambs[1].

	Growing (14-19 kg of BW)		Finishing (19-25 kg of BW)	
	LOW (18% CP)	**CONTROL** (20% CP)	**LOW** (17% CP)	**CONTROL** (19% CP)
Dry matter, g /kg fresh matter	883±0.2	880±0.7	874±0.6	880±0.9
Crude protein, g/kg DM	181±1.6	204±3.1	174±0.7	192±0.7
Ether extract, g/kg DM	22.0±0.75	21.4±1.85	22.0±0.40	24.4±2.10
Neutral detergent fiber, g/kg DM	186±1.9	210±0.4	294±0,0	259±7.1
Acid detergent fiber, g/kg DM	70.5±2.85	82.0±2.25	91.1±3.50	83.4±0.60
Lignin (sa), g/kg DM	12.3±0.10	13.1±0.75	9.5±0.20	6.7±2.45
Starch, g/kg DM	449±4.3	419±6.3	444±3.0	434±3.0
Gross energy, MJ/kg DM	19.50±0.386	19.58±0.194	19.17±0.617	19.86±0.472
Fatty acids (FA), % of identified FA				
C12:0	0.14 ± 0.001	0.07 ± 0.015	0.22 ± 0.091	0.16 ± 0.021
C14:0	0.30 ± 0.010	0.26 ± 0.003	0.63 ± 0.403	0.32 ± 0.006
C16:0	18.65 ± 0.465	19.83 ± 0.003	19.10 ± 1.051	19.61 ± 0.389
C16:1n-9	0.25 ± 0.019	0.20 ± 0.002	0.20 ± 0.003	0.21 ± 0.013
C17:0	0.14 ± 0.018	0.12 ± 0.001	0.17 ± 0.044	0.12 ± 0.003
C18:0	4.84 ± 0.483	5.91 ± 0.030	4.97 ±0. 863	5.10 ± 0.504
C18:1 c9	22.20 ± 0.658	20.18 ± 0.010	20.69 ± 0.075	20.97 ± 0.221
C18:2n-6	48.49 ± 0.265	48.43 ± 0.010	49.34 ± 2.259	48.45 ± 0.590
C18:3n-3	3.26 ± 0.006	3.26 ± 0.016	3.09 ± 0.066	3.34 ± 0.052
C20:0	0.30 ± 0.005	0.29 ± 0.002	0.27 ± 0.002	0.30 ± 0.001
C22:0	0.13 ± 0.005	0.13 ± 0.001	0.12 ± 0.002	0.14 ± 0.002

626 [1]mean ± standard error

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628 **Table 2**

629 Effect of reduction of crude protein content in the concentrates on the intake and total apparent
630 digestibility coefficients calculated with total faecal collection and with feed and faecal AIA
631 contents during the growth and finishing periods in Experiment 1.

	Growing (14-19 kg of BW)				Finishing (19-25 kg of BW)			
	LOW (18% CP)	**CONTROL** (20% CP)	SE[1]	*P-value*	**LOW** (17% CP)	**CONTROL** (19% CP)	SE[1]	*P-value*
n	6	6			6	6		
Intake, g/d [2]								
DM	542.8	520.2	39.85	0.69	772.2	665.4	37.98	0.096
OM	504.3	480.5	36.88	0.66	714.5	611,0	37.07	0.098
CP	99.5	106.8	7.64	0.51	135.8	127.6	7.29	0.48
NDF	95.0	109.1	8.41	0.26	188.4	169.7	9.69	0.23
ADF	37.0	42.9	3.31	0.23	60.1	52.8	3.03	0.16
Apparent digestibility								
with total faecal collection, %								
DM	82.7	81.6	1.1	0.49	84.1	84.9	1.07	0.61
OM	84.1	82.9	1.02	0.43	85.4	85.8	1.06	0.80
CP	78.9	78.2	1.67	0.75	78.7	79.9	1.65	0.61
NDF	53.7	61.5	3.02	0.10	68.9	73.6	2.26	0.17
ADF	48.3	57.8	3.15	0.06	64.1	64	3.13	0.99
with feed and faecal AIA contents, %								
DM	62.6	68.2	1.51	0.02	77.9	74.0	1.53	0.13
OM	67.5	72.3	1.34	0.03	80.9	77.8	1.39	0.18
CP	58.2	64.6	2.61	0.12	72.8	67.0	2.74	0.19
NDF	27.5	27.7	2.83	0.96	64.7	61.4	1.71	0.23
ADF	22.0	17.2	3.13	0.31	53.4	54.2	1.81	0.79

632 [1]Standard error.

633 [2]DM: Dry matter, OM: organic matter, CP: crude protein, NDF: neutral detergent fibre, ADF:
634 acid detergent fibre

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636 **Table 3**

637 Effect of crude protein content of concentrate on the nitrogen (N) balance during the growing
638 and finishing phases in fattening light lambs in Experiment 1.

	Growing (14-19 kg of BW)[1]				**Finishing (19-25 kg of BW)[2]**			
	LOW (18% CP)	**CONTROL** (20% CP)	SE[1]	*P-value*	**LOW** (17% CP)	**CONTROL** (19% CP)	SE[1]	*P-value*
n	6	6			6	6		
Intake N, g/d	15.9	17.1	1.22	0.55	21.7	20.4	1.16	0.48
Urinary N, g/d	2.5	2.4	0.12	0.62	3.3	4.0	0.45	0.33
Faecal N, g/d	3.3	3.6	0.32	0.55	4.5	4.1	0.36	0.47
Total excreted N, g/d	5.9	6.1	0.30	0.66	7.8	8.1	0.27	0.50
Retained N, g/d	10.1	11.0	1.09	0.54	13.9	12.3	1.04	0.34
Retained N, %	62.8	63.8	2.23	0.76	64.1	59.9	1.86	0.17

639 [1]Standard error

640

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641 **Table 4**

642 Effect of crude protein content of concentrate on plasma metabolites during the growing and
643 finishing phases of the fattening light lambs in Experiment 1.

	Growing (14-19 kg of BW)				Finishing (19-25 kg of BW)			
	LOW (18% CP)	**CONTROL** (20% CP)	SE[2]	*P-value*	**LOW** (17% CP)	**CONTROL** (19% CP)	SE[2]	*P-value*
n	6	6			6	6		
BHB, mmol/l	0.16	0.21	0.05	0.55	0.25	0.36	0.04	0.09
Urea, mmol/l	3.4	3.64	0.42	0.70	4.58	5.19	0.44	0.35
Creatinine, µmol/l	38.01	43.61	6.33	0.55	60.92	63.21	4.62	0.73

644 [1]β- hydroxybutyrate

645 [2]Standard error

646

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647 **Table 5**

648 Effect of crude protein content of concentrate on pH, chemical composition and fatty acid (FA)

649 profile in the *longissimus thoracis et lumborum* muscle of light lambs in Experiment 2.

	LOW[1]	**CONTROL[2]**	SE[3]	*P-value*
n	12	12		
pH	5.58	5.59	0.05	0.85
Intramuscular fat, % DM	1.43	1.25	0.089	0.31
Crude protein, % DM	19.99	19.96	0.123	0.90
FA, % of identified FA				
C10:0	0.22	0.2	0.007	0.51
C12:0	0.23	0.2	0.013	0.27
C14:0	1.73	1.58	0.084	0.40
C15:0	0.47	0.50	0.020	0.46
C16:0	5.72	0.5	0.213	0.92
C16:1 c9	1.18	1.17	0.037	0.80
C17:0	1.55	1.70	0.089	0.42
C17:1 c5	1.03	1.12	0.091	0.62
17:1 c9	0.93	1.01	0.045	0.40
C18:0	11.54	11.45	0.253	0.86
C18:1 c6/c8	0.29	0.29	0.010	0.79
C18:1 c9	21.91	20.63	0.614	0.31
C18:1 c11	2.53	2.48	0.067	0.76
C18:1 c12	0.26	0.21	0.015	0.13
C18:1 c13	0.23	0.23	0.009	0.71
C18:1 c14	0.08	0.06	0.0053	0.05
C18:1 c15	0.100	0.085	0.0067	0.25
C18:1 t5	0.022	0.021	0.0028	0.82
C18:1 t6/t8	0.17	0.17	0.012	0.96
C18:1 t9	0.15	0.13	0.0084	0.35
C18:1 t10	3.50	4.02	0.297	0.39
C18:1 t11	0.56	0.35	0.072	0.16
C18:1 t12	0.17	0.16	0.005	0.78
CLA[4] t7,c9	0.017	0.015	0.0013	0.50
CLA[4] c9,t11	0.20	0.13	0.028	0.23

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CLA[4] t9,c11	0.12	0.13	0.005	0.18
CLA[4] t10,c12	0.008	0.008	0.0011	0.88
C18:2 c9,t12	0.01	0.02	0.003	0.04
C18:2n-6	11.40	12.22	0.426	0.35
C18:3n-3	0.43	0.38	0.018	0.19
C20:2n-6	0.08	0.1	0.006	0.07
C20:4n-6	3.74	4.27	0.134	0.06
C20:5n-3	0.28	0.28	0.019	0.96
C22:0	0.07	0.08	0.003	0.05
C22:5n-3	0.28	0.28	0.024	0.96
C22:5n-6	0.08	0.1	0.006	0.18
C22:6n-3	0.20	0.21	0.012	0.55
Σ Saturated FA (SFA)	45.59	45.25	0.273	0.53
Σ Monounsaturated FA	35.54	34.48	0.390	0.19
Σ Polyunsaturated FA (PUFA)	18.87	20.27	0.543	0.21
Σ CLA[4]	0.41	0.38	0.029	0.33
PUFA:SFA	0.41	0.45	0.014	0.22
Σ n-6 PUFA	16.15	17.59	0.558	0.21
Σ n-3 PUFA	1.49	1.5	0.060	0.94
n-6:n-3	11.16	11.93	0.490	0.42

[1]Low: Concentrate with 18% CP in the growing phase (14-19 kg of BW) and 17% CP during the finishing phase (19-25 kg of BW).

[2]Control: Concentrate with 20% CP during the growing phase and 19% CP during the finishing phase.

[3]Standard error

[4]Conjugated linoleic acid

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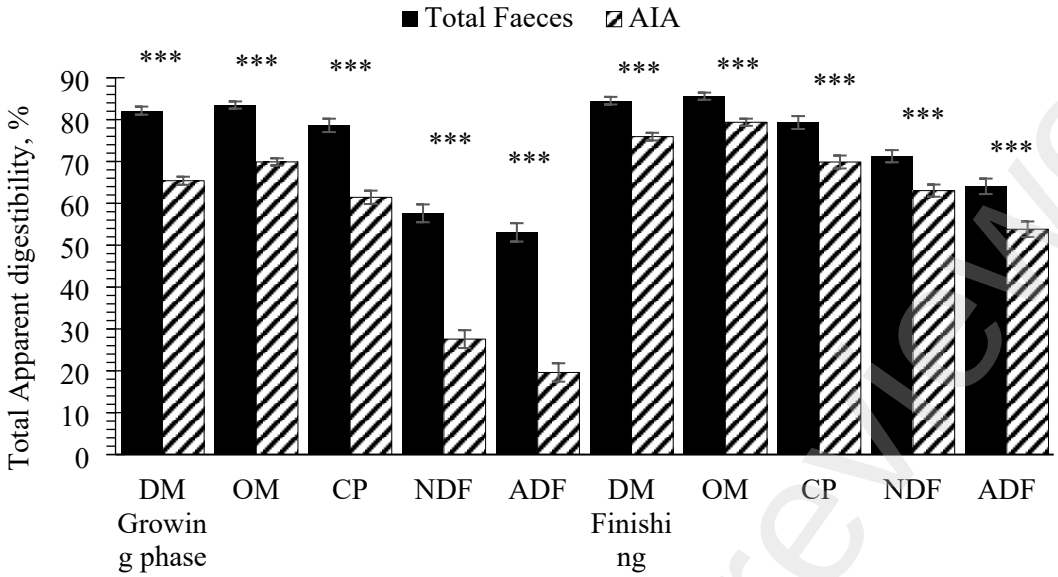

657

658 **Figure 1.** Comparison of total apparent digestibility of dry matter (DM), organic matter (OM),
659 crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF)
660 calculated with total faecal collection and with dietary and faecal AIA contents by phase in
661 Experiment 1. *** P<0.001

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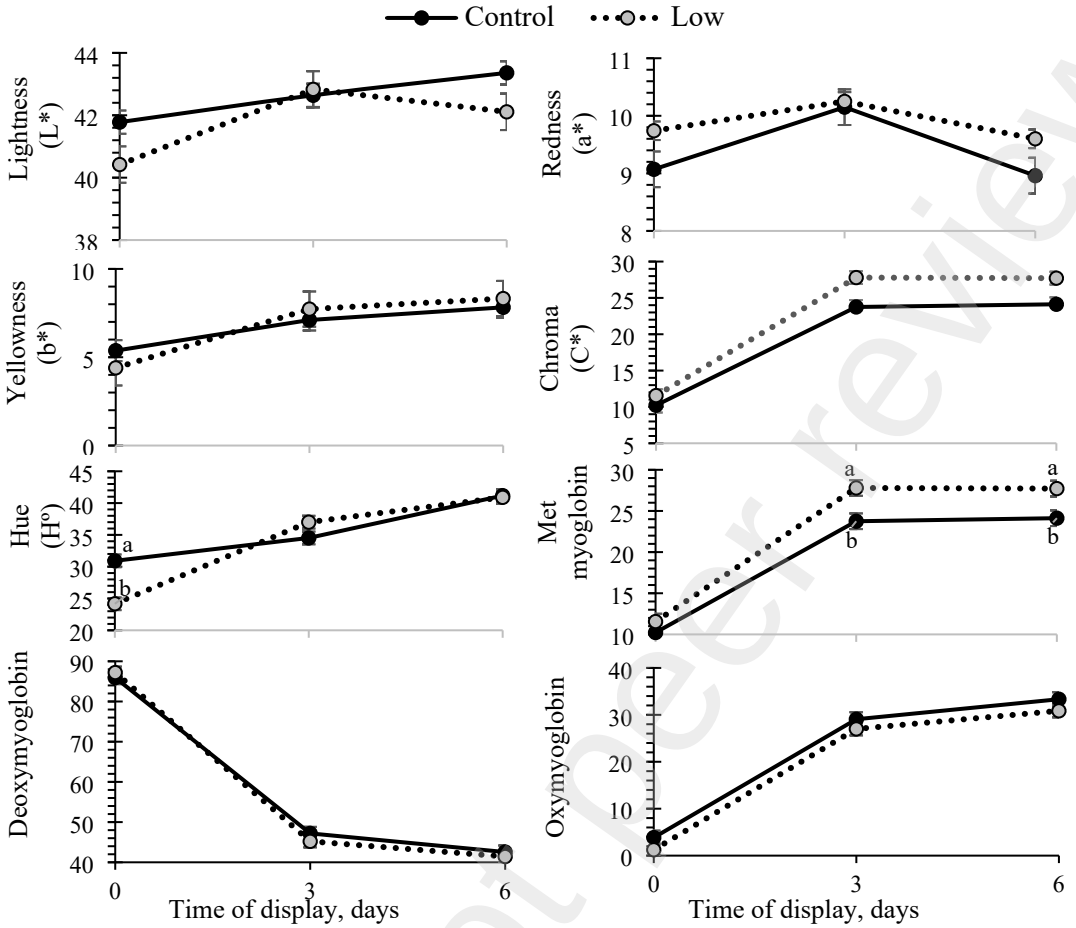

Figure 2. Colour traits in the *longissimus thoracis et lumborum* muscle of light lambs according to the crude protein content of their fattening concentrates and time of display in Experiment 2.

Control: Concentrate with 20% CP during the growing phase (14-19 kg of BW) and 19% CP during the finishing phase (19-25 kg of BW). Low: Concentrate with 18% CP in the growing phase and 17% CP during the finishing phase. For each trait and measurement day, different letters indicate significant differences ($P<0.05$).

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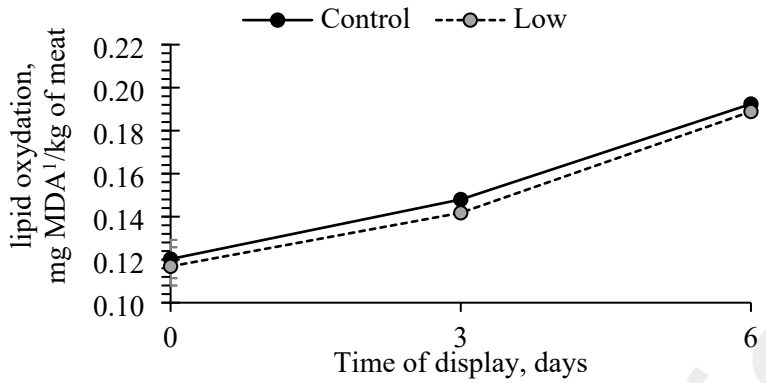

673

674 **Figure 3.** Lipid oxidation in the *longissimus thoracis et lumborum* muscle of light lambs
675 according to the crude protein content of their fattening concentrates and time of display in
676 Experiment 2. [1]malondialdehyde

677 Control: Concentrate with 20% CP during the growing phase (14-19 kg of BW) and 19% CP
678 during the finishing phase (19-25 kg of BW). Low: Concentrate with 18% CP in the growing
679 phase and 17% CP during the finishing phase.

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