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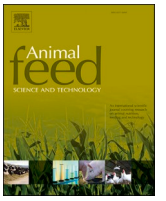

Effects of protein reduction in lamb fattening concentrate on *in vivo* digestibility, nitrogen balance and meat quality

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ABSTRACT

Determining the optimal crude protein (CP) content in light lambs' diets is crucial for reducing ammonia. This study assessed lowering CP in fattening concentrates and its effect on digestibility and meat quality (two experiments). Two phases were tested: growing (14–19 kg BW; 18 % vs. 20 % CP) and finishing (19–25 kg BW; 17 % vs. 19 % CP). Experiment 1 involved 24 Rasa Aragonesa lambs, 12 per phase (growing: 14.0 ± 0.87 kg BW; finishing: 18.9 ± 0.78 kg BW, n = 6 per treatment) to assess apparent digestibility, nitrogen balance, and blood metabolites. The adaptation period to the diets were 12 days, while the collection period was 5 days. Digestibility was measured by total faecal collection and acid-insoluble ash (AIA) as internal marker. Experiment 2 evaluated meat quality of 24 Ripollesa lambs, slaughtered at 24.6 ± 0.23 kg BW after 42 days of fattening. Lower CP tended to decrease the acid detergent fibre (ADF) digestibility using total faecal method ($P = 0.06$) and decreased organic and dry matter digestibility with AIA ($P = 0.03$) during the growing phase ($P < 0.05$). No differences were observed in the finishing phase ($P > 0.10$). Digestibility coefficients were consistently underestimated with AIA compared to total faecal collection ($P < 0.001$). Nitrogen balance was unaffected by CP level ($P > 0.05$). Plasma metabolites were similar between treatments, except for β-hydroxybutyrate, which tended to be lower in Low CP lambs during the finishing ($P = 0.09$). Meat quality was minimally affected, however, Low CP increased metmyoglobin after 6 days of oxygen exposure ($P = 0.005$) and modified minor fatty acids. A moderate reduction of 2 % in dietary CP of light lambs appears feasible and does not impair nutrient digestibility and meat quality.

Abbreviations: AIA, acid insoluble ash; ADFom, acid detergent fibre exclusive of residual ash; ANDFom, neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash; BHB, β-hydroxybutyrate; BW, body weight; CP, crude protein; CV, coefficient of variation; DM, dry matter; DMb, deoxymyoglobin; DMI, dry matter intake; FA, fatty acid; FDM, faecal dry matter; IMF, intramuscular fat; Lignin sa, lignin determined by solubilization of cellulose with sulphuric acid; LTL, *longissimus thoracis et lumborum*; MMb, metmyoglobin; MUFA, monounsaturated fatty acids; N, nitrogen; OMb, oxymyoglobin; OMI, organic matter intake; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

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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, the latter ranging between 19–21 % in growing and 16–20 % in finishing diets (Ferret et al., 2008). However, the use of protein-rich ingredients increases feed costs which can compromise overall farm profitability. Nitrogen (N) is an essential nutrient for animal production but ruminants are inefficient users of N, with low retention and high excretion rates (Cole and Todd, 2008). Excess dietary N is excreted in faeces and urine, becoming a source of contamination of both soil and water. It has been estimated that a 2-percentage-point reduction in CP may lower total N excretion by approximately 15–20 %, depending on diet formulation and animal efficiency (Dijkstra et al., 2013). Recent studies confirm that lowering dietary CP can significantly enhance nitrogen use efficiency and mitigate environmental impact (Sajid et al., 2023; Seoni et al., 2018, Zhang et al., 2022).

The reduction in the CP content in the diet can affect the digestibility of nutrients, although findings in the literature remain inconsistent. Some studies reported an increase in dry matter (DM) digestibility or CP digestibility with higher CP content whereas others did not show any effect (Gao et al., 2016; Kiran and Mutsvangwa, 2009; Muruz et al., 2017). 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. High CP intake is generally associated with lean muscle deposition and reduced fat accumulation (Atti et al., 2004). However, effects on meat colour and fatty acid composition remain unclear, with some studies reporting no influence and others suggesting changes linked to intramuscular fat or unsaturated FA content (Seoni et al., 2018; Youssef and Barbut, 2009).

These inconsistencies in the existing literature, especially regarding lamb production systems, highlight the need for further research to clarify the influence of dietary protein on performance and meat quality. This need is even more pronounced in light autochthonous breeds, for which published data are particularly scarce, despite their economic and regional relevance in Mediterranean production systems. Given this framework, we hypothesized that there is a scope for reducing protein content of the diets of light fattening lambs without negative implications for growth and meat quality. Therefore, the aim of this study was to evaluate the effects of a moderate reduction (2 %age units) in dietary CP on apparent digestibility, nitrogen balance, blood metabolites, and meat quality in light lambs.

2. Materials and methods

2.1. Experimental design and diets

To assess the effect of CP content on apparent digestibility and on lamb meat quality, two experiments were performed using a total

Table 1
Chemical composition of the concentrates fed during the growth and finishing periods of light lambs[a].

	Growing		Finishing	
	(14–19 kg of BW)		(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 fibre, g/kg DM	186 ± 1.9	210 ± 0.4	294 ± 0,0	259 ± 7.1
Acid detergent fibre, 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

[a] mean ± standard error

of 48 male lambs: 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 (14–19 kg BW) and finishing (19–25 kg BW). Thus, four concentrates were formulated to allow an average gain of lambs of 200–250 g/day 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. The 2 % reduction in dietary CP was selected to represent a moderate and practically achievable decrease within the range of CP levels commonly used in commercial lamb diets in Spain (15–21 % CP on a DM basis; Bello et al., 2016). Recommended dietary CP concentrations for local Spanish breeds generally range from 19 to 21 % in growing diets and 16–20 % in finishing diets, depending on growth potential and dietary energy density (Ferret et al., 2008). Therefore, a reduction of 2 %age points (from 20 % to 18 % CP during growth and from 19 % to 17 % during finishing) was considered sufficient to detect potential differences in performance and nitrogen utilization, while still remaining within the range of practical formulation limits. All concentrates were isoenergetic (1 unité fourragère, UF/kg, net energy for maintenance and growth; Nozière et al., 2018) and were formulated with the same ingredients and additives, modifying the percentage of inclusion of vegetable protein (Supplementary Table 1). 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.

2.2. Experiment 1. In vivo digestibility assay

Two *in vivo* digestibility trials were conducted using a total of 24 Rasa Aragonesa male lambs, the first during the growing phase and the second during the finishing phase. Twelve different lambs were used in each phase (i.e., growing and finishing phases), with six animals per treatment in each phase. 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 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. To ensure this pH and avoid N losses, the amount of H_2SO_4 added was calculated taking into account the urine excretion for each phase (i.e., 390 and 461 mL of urine on average during the growth and finishing periods, respectively) and the variability between days and between animals (mean coefficients of variation of 36.3 % and 54.5 %, respectively). 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 (aNDFom and ADFom, respectively) was calculated using two methods. First, the percentages were calculated using daily feed intake and total faecal collection as:

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

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:

$$\text{Dig}_{\text{AIA}}\ (\%) = 100 - [100 \times (\text{AIA}_{\text{diet}}/\text{AIA}_{\text{faeces}}) \times (Z_{\text{faeces}}/Z_{\text{diet}})]$$

where AIA_{diet} and $\text{AIA}_{\text{faeces}}$ 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 Dig_{AIA} (%). The N retention was calculated by the difference of N consumed and the total N excreted (faecal and urinary).

Blood samples from all lambs were collected from the jugular vein into tubes containing heparin or ethylenediaminetetraacetic acid (EDTA) before each morning meal was offered at the beginning and at the end of each *in vivo* digestibility trial. Lambs were not fasted because they were fed *ad libitum*; however, sampling was always performed at the same time relative to feed delivery. 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.

2.3. 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 (15–19 kg BW) and finishing phase (19–25 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 around 25 kg BW, and 24 of them, with 24.6 ± 0.23 kg BW, were used to study meat quality. For slaughtering, they were stunned by a captive bolt pistol and exsanguinated in the experimental abattoir of the Research Centre, using standard commercial procedures and according to Council Regulation (EC) Nº 1099/2009. After chilling the carcasses at 4 °C in total darkness each cold carcass (24 h 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 spectrophotometer (Konica Minolta Holdings, Inc., Osaka, Japan) with zero and white calibration (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 in the cold carcass 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 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 and haem pigments were 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.

2.4. Chemical analyses

The chemical composition analyses of feedstuffs, faeces and urine were run in duplicate, according to Baila et al. (2025). Briefly, DM, ash and CP were carried out according to AOAC (2000). Contents of neutral detergent fibre (aNDFom), acid detergent fibre (ADFom), and lignin determined by solubilization of cellulose with sulphuric acid (lignin (sa)) were determined using the Ankom 200/220 fibre analyser (Ankom Technology Corporation) and all values were corrected for ash–free content. The total starch of the concentrates was analysed following the amyloglucosidase/α-amylase method.

Acid-insoluble ashes were analysed according to a standard procedure (BOE, 1995). Briefly, 5 g of ground samples of feed or excreta were hydrolysed in a beaker with 75 mL of 3 *N* HCl and 25 mL of distilled water and boiled for 30 min. The sample was then filtered through ash free filter paper and washed the residue with 50 mL of hot distilled water. The filter with residue was put in a tared crucible, dried at 103 ºC for 24 h and ashed at 550 ºC for 3 h for crude ash determination. Afterwards, ashes were transferred again to a beaker with 75 mL of 3 *N* HCl and gently boiled for 15 min. The dilution was filtered again through an ash free filter and washed with hot distilled water until disappearance of acid reaction. Finally, the filter with residue was dried and ashed on a tared crucible at 550 ºC for 3 h. The crucible and its content were cooled in a desiccator to room temperature and weighed to calculate the AIA content.

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 (inter-assay coefficient of variation (CV) = 0.73−2.99 %; RAL, Barcelona, Spain), creatinine using a spectrophotometric kit based on the enzymatic hydrolysis of endogenous creatine (inter-assay CV = 2.77−3.09 %; RAL, Barcelona, Spain) and, finally, β-hydroxybutyrate (BHB) was determined using a spectrophotometric kit based on its enzymatic oxidation (inter-assay CV = 0.4−1 %; 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 total intramuscular fat (IMF) content was determined 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 gas chromatography (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, 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).

2.5. 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 included dietary CP content (Low vs. Control) as fixed effect, lamb as random effect, and initial BW as a covariate, and was expressed as:

$$Y_{ij} = \mu + CP_i + \beta\ (BW_{0j}) + u_j + \varepsilon_{ij}$$

where CP_i is the dietary crude protein level, BW_{0j} is the initial body weight, u_j is the random lamb effect, and ε_{ij} is the residual error.

For the comparative study of digestibility percentages calculated with total faecal collection and with dietary and faecal AIA

contents, the same model was used, including the digestibility estimation method (total faecal collection vs. AIA), the CP content and their interaction as fixed effects, and the lamb as random effect:

$$Y_{ijk} = \mu + CP_i + Method_j + (CP \times Method)_{ij} + u_k + \varepsilon_{ijk}$$

where Y_{ijk} is the digestibility response, CP_i is the dietary crude protein level, $Method_j$ is the digestibility estimation method, $(CP \times Method)_{ij}$ is their interaction, u_k is the random lamb effect, and ε_{ijk} is the residual error.

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: $Y_i = \mu + CP_i + \varepsilon_i$, where Y_i is the chemical or fatty acid composition variable, CP_i is the dietary crude protein level, and ε_i is the residual error.

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:

$$Y_{ijk} = \mu + CP_i + Time_j + (CP \times Time)_{ij} + u_k + \varepsilon_{ijk}$$

where Y_{ijk} is the measured colour or lipid oxidation parameter, CP_i is the dietary crude protein level, $Time_j$ is the display time, $(CP \times Time)_{ij}$ is their interaction, u_k is the random lamb effect, and ε_{ijk} is the residual error.

The experimental unit was the lamb in both experiments for all traits. Multiple comparisons among treatments were performed using Tukey's method. The least-squares means and standard errors were obtained, and differences were considered significant when $P < 0.05$. The tendencies 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 tendency for greater intake in the Low group, with DM intake being about 107 g/d higher ($P = 0.096$) and OM intake about 103 g/d higher ($P = 0.098$) in finishing phase. 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 lower total tract ADFom digestibility in the Low group during the growing phase (about 9.5 % lower, $P = 0.06$). Regarding the results using the AIA method, the total tract DM and OM digestibility was higher in the Control group in the growing phase, with DM digestibility about 5.6 % higher ($P = 0.02$) and OM digestibility about 4.8 % higher ($P = 0.03$), but no effect was found in the finishing phase ($P = 0.13$ and 0.18 for DM and OM digestibility, respectively). The recovery rate of AIA did not differ across dietary CP levels either in growing period (56.7 vs 63.2 ± 6.41 %, in Low and Control, respectively; $P = 0.73$) or in finishing period (78.4 vs. 76.3 ± 6.41 %, in Low and Control, respectively; $P = 0.73$).

Table 2
Effect of reduction of crude protein content in the concentrates on the intake and total apparent digestibility coefficients calculated with total faecal collection and with feed and faecal AIA 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[a]	*P-value*	**LOW** (17 % CP)	**CONTROL** (19 % CP)	SE[a]	*P-value*
n	6	6			6	6		
Intake, g/d[b]								
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
aNDFom	95.0	109.1	8.41	0.26	188.4	169.7	9.69	0.23
ADFom	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
aNDFom	53.7	61.5	3.02	0.10	68.9	73.6	2.26	0.17
ADFom	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
aNDFom	27.5	27.7	2.83	0.96	64.7	61.4	1.71	0.23
ADFom	22.0	17.2	3.13	0.31	53.4	54.2	1.81	0.79

[a] Standard error.
[b] DM: Dry matter, OM: organic matter, CP: crude protein, aNDFom: neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash, ADFom: acid detergent fibre exclusive of residual ash;

The comparison of digestibility estimation methods by phase is presented in Fig. 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. These differences are more pronounced during the growing phase, since the degree of underestimation ranged from 16 % in OM digestibility to 63 % in ADFom digestibility. In contrast, during the finishing phase, the degree of underestimation ranged from 7 % in OM digestibility to 16 % in ADFom digestibility.

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, which tended to be lower in the Low treatment ($P = 0.09$).

3.2. Experiment 2: meat quality study

There were no differences in the pH values of meat at cutting from animals fed the Control and the Low protein diets ($P = 0.85$; Table 5). Most of the FA in the lipid profile of LTL from light lambs are reported in Table 5, while those considered to be minor are presented in Supplementary Table 2. 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 ($P = 0.04$), 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*), yellowness (b*) and saturation (C*) were only affected by display time (Fig. 2; $P < 0.05$). The hue (h_{ab}) was influenced by the interaction between CP content and display time ($P < 0.01$). At the time of cutting (day 0), h_{ab} was higher in animals fed the Control diet ($P = 0.03$), but this difference disappeared with increasing exposure time ($P = 0.41$). Concerning the haem pigments (Fig. 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 ($P = 0.68$; Fig. 3).

4. Discussion

4.1. Feed intake and apparent digestibility

The absence of effect of CP content on nutrient intake agrees with previous studies in lambs and goats (Pelegrin-Valls et al., 2020, Zhang et al., 2022, Zhu et al., 2020), implying that the Low CP concentrate met the animal requirements. However, it is worth noting that in the finishing phase a tendency 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 evaluation of feed intake requires longer experimental periods (Osonowo et al., 2025), and the fact that the trial was conducted using metabolic cages, which guarantee accurate data collection but may prevent natural feeding behaviour, combined with individual variation in feed intake, digestive efficiency and daily BW gains could have contributed to the lack of statistically detectable differences.

Limited consensus exists in the literature regarding the effects of dietary CP contents on nutrient digestibility because many studies vary in energy content, making CP effects difficult to isolate. The variability in digestibility results has often been related to the energy

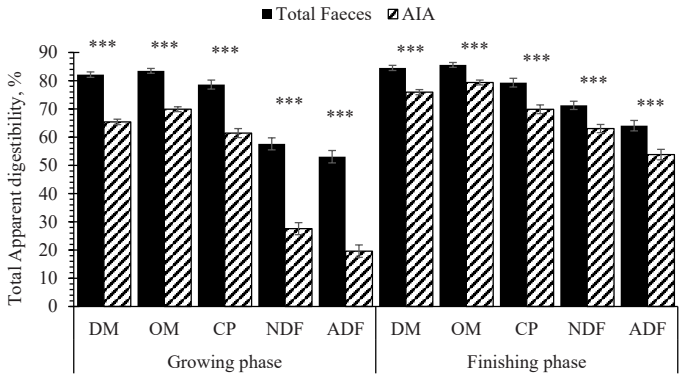

Fig. 1. Comparison of total apparent digestibility of dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fibre (aNDFom) and acid detergent fibre (ADFom) calculated with total faecal collection and with dietary and faecal AIA contents by phase in Experiment 1. *** $P < 0.001$.

Table 3
Effect of crude protein content of concentrate on the nitrogen (N) balance during the growing and finishing phases in 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[a]	*P-value*	**LOW** (17 % CP)	**CONTROL** (19 % CP)	SE[a]	*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

[a] Standard error

Table 4
Effect of crude protein content of concentrate on plasma metabolites during the growing and 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[1], 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

[1] β- hydroxybutyrate
[2] Standard error

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. 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 %age points) did not affect apparent digestibility which agrees with previous findings in lambs and goats (Gao et al., 2016; Dutta et al., 2009). 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.

Total faecal collection yielded higher and less variable digestibility coefficients than AIA, which consistently underestimated values, particularly during the growing phase. 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 underestimation, the AIA method could be a valuable tool to compare dietary CP level under commercial conditions, when only spot faecal and feed sampling are available, especially during the finishing phase. This consistency allows for valid relative comparisons between diets, provided the limitations of the method are acknowledged. The low recovery rate of AIA could be attributed to its low content in the concentrate feed (0.5 ± 0.08 % on DM basis; data not shown), since this internal marker shows accurate estimations when the dietary AIA content exceeded 0.75 % on a DM basis (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.

4.2. Nitrogen balance and blood metabolites

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

Table 5
Effect of crude protein content of concentrate on pH, chemical composition and fatty acid (FA) 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
C14:1 c9	0.065	0.062	0.0278	0.66
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
C16:1 t9	0.011	0.009	0.0141	0.24
C17:0	1.55	1.70	0.089	0.42
C17:1 c5	1.03	1.12	0.091	0.62
C17:1 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
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-6	0.15	0.15	0.034	0.75
C18:3n-3	0.43	0.38	0.018	0.19
C20:2n-6	0.08	0.1	0.006	0.07
C20:3n-6	0.35	0.36	0.045	0.85
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
C22:4n-6	0.34	0.38	0.012	0.22
C22:5n-3	0.58	0.62	0.014	0.36
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

Other minor FA of the *longissimus thoracis et lumborum* muscle of light lambs in Experiment 2 are presented as Supplementary Material (Supplementary Table 2).

[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

may have caused nitrogenous compounds to settle and thus be underestimated during analysis.

In the present digestibility trial, no effect of moderate CP reduction on nitrogen excretion was observed. However, Pelegrin-Valls et al. (2020), using the same dietary treatments in a large-scale study with more animals, reported a reduction in urinary N excretion without compromising growth performance. These complementary findings highlight that while our controlled trial did not detect

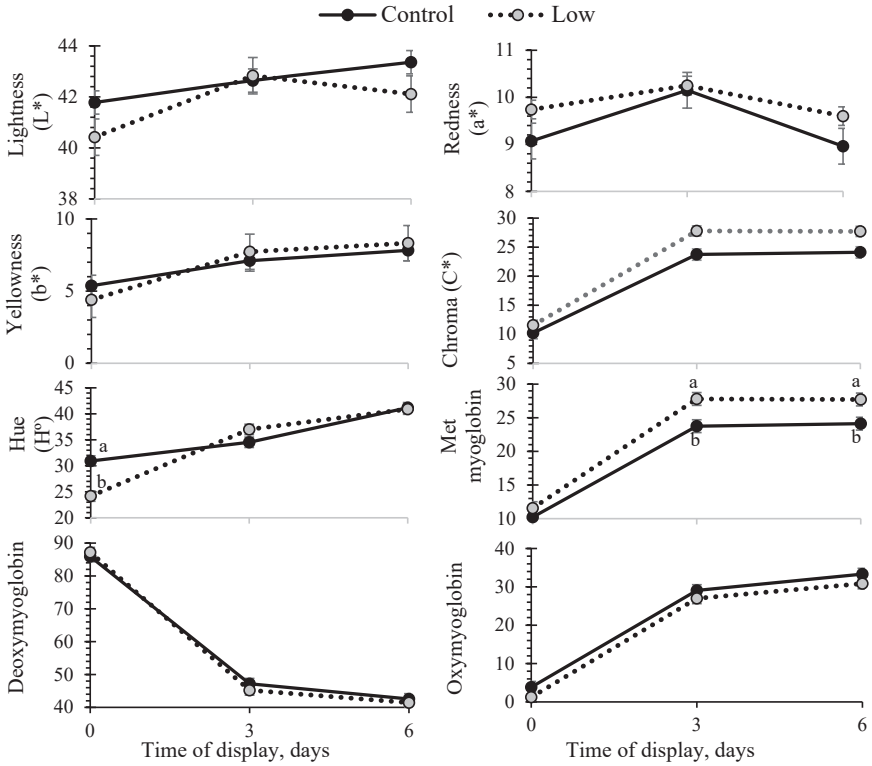

Fig. 2. Colour traits in the *longissimus thoracis et lumborum* muscle of light lambs according to the crude protein content of their fattening concentrates and time of display in Experiment 2.
Control: Concentrate with 20 % CP during the growing phase (14–19 kg of BW) and 19 % CP during the finishing 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 ($P < 0.05$).

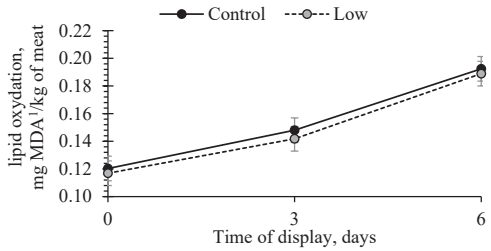

Fig. 3. Lipid oxidation in the *longissimus thoracis et lumborum* muscle of light lambs according to the crude protein content of their fattening concentrates and time of display in Experiment 2. [1]malondialdehyde. Control: Concentrate with 20 % CP during the growing phase (14–19 kg of BW) and 19 % 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.

changes in N excretion, evidence from commercial conditions suggests that CP reduction may influence N losses at a broader scale.

Blood metabolites of each phase in Experiment 1 were consistent with the normal range for growing lambs (Kaneko et al., 2008). Dietary CP contents correlate directly with plasma urea concentrations in fattening lambs (Dabiri and Thonney, 2004; Rocha et al., 2004), however, in the current study the CP content was not reflected in an increase in blood urea concentration in any phase, probably due the similar CP intake reported. Therefore, the lack of effect suggests that lambs' protein requirements were adequately met even at the lower CP level.

4.3. Meat quality and oxidative stability

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

normal and suggest 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. 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 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 tendencies 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 (+16 %) 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.

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. From a physiological perspective, changes in protein supply can modulate oxidative metabolism and systemic redox balance (e.g., reflected by changes in circulating ketone bodies such as BHB in ruminants), which could secondarily influence myoglobin redox cycling and pigment stability (Celi et al., 2015). Although these mechanisms were not directly assessed in the lambs used for MMb determination in the present study, they provide a plausible biological framework for interpreting dietary protein effects on colour stability. 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

Reducing dietary crude protein by 2 % during the growing and finishing phases did not impair nutrient digestibility or nitrogen balance and only caused minor changes in meat composition. These findings suggest that moderate reductions in protein may be feasible without compromising carcass quality or consumer acceptance. Future research should investigate whether more substantial reductions in dietary crude protein could enhance nitrogen efficiency without compromising animal performance or carcass quality.

CRediT authorship contribution statement

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

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Declaration of Competing 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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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.anifeedsci.2026.116670.

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