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Nutritive value of traditionally water-debittered white lupin grains for ruminants

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Abstract. White lupin (*Lupinus albus* L.) grains, locally known in Anatolia as *termiye*, is traditionally boiled and soaked under running water for 7–10 days to remove quinolizidine alkaloids. However, little is known about its nutritive value for ruminants after this processing. In this study, water-debittered *L. albus* from five smallholder production lots (L1–L5) was characterised for chemical composition, Cornell Net Carbohydrate and Protein System (CNCPS) protein fractions, *in situ* degradability, *in vitro* fermentation kinetics, and predicted methane production. *In situ* degradability was determined in two rumen-cannulated dry Holstein cows over 2–48 h. *In vitro* fermentation was assessed using a semi-automatic gas production system. Rumen pH, ammonia nitrogen (NH₃-N) and volatile fatty acids (VFA) were measured at 24 and 48 h, and methane production was predicted from VFA molar proportions. Crude protein (CP) content ranged from 35.8 to 40.2% of dry matter (DM) and neutral detergent fibre (NDF) content from 35.3 to 36.9% of DM. CNCPS fraction B accounted for 88.0–93.2% of CP and rumen-undegradable protein (RUP) at $k = 0.02/\text{h}$ was 30.6–32.9% of CP. Twenty-four-hour gas production ranged from 58 to 68 ml/460 mg DM, end-point pH 7.07–7.22, NH₃-N 9.2–10.0 mM, total VFA 125–132 mmol/l, acetate 55.9–58.2 mol%, propionate 26.2–

27.1 mol%, and the (acetate + butyrate)/propionate ratio 2.54–2.69. Predicted methane ranged from 17.7 to 18.5 ml/460 mg DM. Water-debittered *L. albus* had high CP content, a true-protein-dominated nitrogen profile, RUP comparable to soybean meal, and an acetate-rich fermentation profile. Composition and fermentation characteristics were largely consistent between lots, indicating that traditional aqueous debittering results in a relatively uniform protein feed. However, confirmation of its feeding value requires *in vivo* evaluation.

Key words: CNCPS protein fractions, predicted methane production, quinolizidine alkaloids, rumen-undegradable protein, soybean meal alternative, volatile fatty acids

Introduction

Turkey imports between 4 and 5 mln t of soybean meal each year to support its dairy and beef sectors (FAO, 2022). The economic pressure associated with these imports, together with consumer concern regarding genetically modified soybean, has increased interest in locally grown legume seeds as alternative protein sources.

White lupin (*Lupinus albus* L.) is a promising protein source for ruminants because its seeds contain 32–42% crude protein (CP) on a dry matter (DM) basis (Sujak et al., 2006; Gresta et al., 2023); it grows on poor soils, fixes atmospheric nitrogen, and tolerates the semi-arid conditions of Central Anatolia. In Turkey, white lupin is grown in the Konya plain, the Lakes Region and parts of the western Black Sea coast. The grain is traditionally boiled and soaked in running water for 7–10 days to remove bitter quinolizidine alkaloids before sale. The end product, known locally as *termiye*, is sold for both human consumption and animal feed.

Research on lupin nutrition for ruminants has focused mainly on Australian sweet varieties of *L. angustifolius* (Morsy et al., 2024). Information on *L. albus* from Anatolia is more limited, and few studies have evaluated its nutritive value from chemical composition and *in situ* degradability to *in vitro* fermentation and methane production.

In the present study, we characterised debittered *L. albus* grain from five production lots in Konya and Isparta provinces. The study aimed to characterise the nutritive value of *termiye* in terms relevant to ruminant rations, assess variation in fermentation characteristics between production lots, and compare the results with soybean meal and published data for other lupin

species. We measured chemical composition, Cornell Net Carbohydrate and Protein System (CNCPS) protein fractions, *in situ* DM and neutral detergent fibre (NDF) degradability, *in vitro* DM digestibility (IVDMD), gas production kinetics, end-point pH, ammonia nitrogen (NH₃-N), volatile fatty acids (VFA) and predicted methane production. Because all measurements were conducted *in situ* or *in vitro*, the study is intended as a screening-level assessment rather than as a substitute for an *in vivo* feeding trial.

Material and methods

All experimental procedures involving animals were reviewed and approved by the Local Animal Experiments Ethics Committee of Selçuk University, Faculty of Veterinary Medicine (Approval No. 2024/137, dated September 5, 2024). The two rumen-cannulated dry Holstein cows used for *in situ* incubations and rumen-fluid collection were housed at the research farm of the Faculty of Veterinary Medicine, Selçuk University (Konya, Turkey) and fed a maintenance diet consisting of 60% forage and 40% concentrate, in accordance with national and institutional regulations for the care and use of experimental animals.

Experimental design

The study followed a completely randomised design, with production lot as the single experimental factor at five levels (L1–L5). The lots corresponded to five independent smallholder production units and were not experimentally assigned treatments; therefore, comparisons between lots were observational with respect to growing area and processing history, and lot was treated as a fixed effect. All laboratory and incubation work was carried out using the same five ground and homogenised subsamples, thus the same materials were compared in every assay.

Because the experimental unit differed between assays, it was defined separately for each assay. For chemical composition and CNCPS fractionation, the experimental unit was the analytical replicate within a lot ($n = 2$ per lot). For *in situ* incubation, the experimental unit was

the nylon bag, with three bags per cow at incubation time in each of the two donor cows ($n = 6$ per lot per time point). For IVDMD, the experimental unit was the filter bag ($n = 3$ per lot per time point). For *in vitro* gas production, the experimental unit was the serum bottle, with 14 bottles per lot distributed between two independent incubation runs performed on separate days ($n = 14$ per lot). For end-point pH and VFA, the experimental unit was the incubation bottle ($n = 9$ per lot), whereas for $\text{NH}_3\text{-N}$, it was the analytical replicate ($n = 2$ per lot). Each *in vitro* run also included five substrate-free bottles containing buffered rumen fluid, which were used to correct gas volumes for blank fermentation.

Two features of this design should be considered when interpreting the results. First, rumen fluid for all incubations was obtained from two donor cows, thus replicate bottles or bags within a run used the same inoculum and were in fact subsamples with respect to animal. The two independent runs therefore provided the only replication at the level of the inoculum batch. Second, the number of analytical replicates for chemical composition and $\text{NH}_3\text{-N}$ ($n = 2$) was low, resulting in limited statistical power for these comparisons. The consequences of both constraints are considered in the Discussion.

Source area and sample collection

Lupinus albus is the only species of this genus cultivated commercially in Turkey, with an annual national production of approximately 663 t from a total area of approx. 635 ha, of which 45% is concentrated in the Doğanhisar district of Konya province and the surrounding villages. Production is restricted to the Lakes Region (Göller Bölgesi) of central-southern Anatolia, a semi-arid transitional zone between the Mediterranean and continental climates, where rainfed legume cultivation is integrated with smallholder ruminant production. In this region, white lupin grain is processed by households and small cooperatives. Seeds are boiled in water and then soaked under running spring or stream water for 7–10 days to leach out the bitter quinolizidine alkaloids. The resulting grain is sold for human consumption as a salted snack and, increasingly, as a ruminant feed ingredient.

To capture the range of grain available to local feed buyers, samples were obtained during the 2023 harvest from five smallholder production lots in the Lakes Region. Four lots originated from the Hüyük, Seydişehir, Doğanhisar and Ilgın districts of Konya province (37.91° N, 31.58° E; 37.49° N, 31.95° E; 38.19° N, 31.67° E; 38.26° N, 31.95° E) and one from the Şarkikaraağaç district of Isparta province (38.02° N, 31.35° E). The sampling sites covered the main commercial production area of Turkish white lupin (Doğanhisar and its catchment in Konya), together with four additional smallholder operations spanning an east–west distance of approx. 50 km and an elevational range from about 1000 to 1200 m a.s.l. The five lots are referred to as L1–L5.

At each source, 3 kg of debittered grain was purchased directly from the producer or from a producer cooperative. The producers confirmed that the grain had been processed using traditional 7–10-day aqueous debittering protocol described above, with no additional thermal or mechanical treatment. In the laboratory, the samples were dried at 60 °C for 48 h in a forced-air oven, ground to pass through a 1 mm screen, and stored at a room temperature in polyethylene bags until analysis.

Chemical composition

DM (AOAC 934.01), ash (AOAC 942.05), crude protein (CP; Kjeldahl, AOAC 984.13) and ether extract (Soxhlet, AOAC 920.39) were determined according to standard procedures (AOAC, 2005). NDF, acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined by sequential analysis using an ANKOM 200 fibre analyser (Van Soest et al., 1991). NDF was determined with the addition of heat-stable α -amylase and sodium sulphite and is reported inclusive of residual ash (aNDF). ADL was determined by subsequent treatment of the ADF residue with 72% sulphuric acid. Neutral detergent insoluble CP (NDICP) and acid detergent insoluble CP (ADICP) were determined using procedures described by Licitra et al. (1996). Non-fibre carbohydrates (NFC) were calculated as:

$$100 - (\text{CP} + \text{EE} + \text{ash} + \text{NDF}) \text{ (NRC, 2001),}$$

where: CP – crude protein, EE – ether extract, NDF – neutral detergent fibre.

Each lot was analysed in duplicate.

CNCPS protein fractions and energy estimates

Nitrogen fractions A, B and C were determined according to Sniffen et al. (1992) and Licitra et al. (1996). Rumen-undegradable protein (RUP) was estimated at ruminal passage rates of 2 and 4% per h. Total digestible nutrients (TDN), metabolisable energy (ME), net energy for lactation (NEL), net energy for maintenance (NEM) and net energy for gain (NEG) were estimated from chemical composition using the prediction equations in NRC (2001).

***In situ* degradability**

Two dry Holstein cows (650 ± 25 kg body weight) with permanent rumen cannulas were used at the research farm of the Faculty of Veterinary Medicine, Selçuk University. The animals were fed a maintenance diet consisting of 60% forage and 40% concentrate and had been adapted to this diet for at least three weeks before the first incubation period to minimise variation in inoculum characteristics. Two donor animals were used because this was the minimum number generally accepted for pooled-inoculum *in situ* trials and the number of permanently cannulated cows available at the institutional facility under the approved ethics protocol. No additional cannulated animals could be used without a new surgical procedure, which was considered unjustifiable by the Ethics Committee for a feed-characterisation study under the 3R principle of reduction. To partly compensate for the limited biological replication, three bag replicates were incubated per animal at each time point, giving six bags per lot at each time point. All five lots were incubated simultaneously in both animals, so that lot comparisons were made within rather than between animals. The implications of this limited animal replication for interpretation of the results are addressed in the Discussion section.

Approximately 5 g of ground sample (DM basis) was placed in 5 × 10 cm nylon bags with a pore size of 50 µm (Ankom R510). The bags were incubated in the rumen for 2, 4, 6, 8, 12, 24, 36 and 48 h, with three bag replicates per animal at each time point. After incubation, the bags were washed under cold running water, oven-dried at 60 °C for 48 h and weighed. *In situ* DM degradability $P(t)$ was fitted to the Ørskov and McDonald (1979) model (Equation 1), and effective degradability (ED) was calculated at ruminal outflow rates $k = 0.02, 0.05$ and $0.08/h$ (Equation 2):

$$P(t) = a + b \times (1 - e^{-c \cdot t}) \quad (1)$$

$$ED = a + (b \times c) / (c + k) \quad (2)$$

where: $P(t)$ – proportion of DM degraded at time t (h), a – rapidly soluble fraction (%), b – potentially degradable but insoluble fraction (%), c – fractional degradation rate of b (per h) and k – assumed ruminal outflow rate (per h).

NDF residues were measured in the bag contents as described above (Van Soest et al., 1991). The 8 and 36 h NDF data points were excluded from the analysis owing to insufficient sample replication.

***In vitro* DM digestibility**

IVDMD was assessed using a Daisy II incubator (ANKOM Technology, Macedon, NY, USA) with a modification of the two-stage method of Tilley and Terry (1963). The general principles, standardisation requirements and known limitations of this method, and the batch gas production procedure described below, were reviewed by Chávez-Solis et al. (2026). Briefly, approx. 0.5 g of ground sample (DM basis) was weighed into pre-weighed ANKOM F57 filter bags (25 µm pore size, 5.0 × 5.5 cm), which were heat-sealed and randomly distributed in the digestion jars. Media were prepared and dispensed according to the standard ANKOM Daisy II protocol. Each digestion jar contained 1 330 ml of McDougall buffer, 266 ml of reducing solution and 400 ml of filtered fresh rumen fluid, resulting in a buffer-to-inoculum

ratio of approximately 4:1 and a total incubation volume of about 2 l. The headspace was flushed with CO₂ directly before the jar was sealed. Rumen fluid was collected before the morning feeding from the same two cannulated cows, strained through four layers of cheesecloth under a continuous CO₂ flow and kept at 39 °C until use. The jars were incubated at 39 °C under continuous rotation, and bags were removed after 2, 4, 6, 8, 12, 24 and 48 h, with three bag replicates per lot at each time point. Retrieved bags were rinsed under cold running water until the effluent ran clear, oven-dried at 60 °C for 48 h and weighed. IVDMD was calculated as follows (Equation 3):

$$\text{IVDMD (\%)} = [(\text{sample DM} - \text{residue DM}) / \text{sample DM}] \times 100 \quad (3)$$

***In vitro* gas production and fermentation kinetics**

Each lupin lot was incubated in 14 replicate bottles, and the full procedure was repeated on different days. Five additional bottles per run contained substrate-free buffered rumen fluid and were used for blank correction. Approx. 460 mg of ground, oven-dried grain was placed in 120-ml serum bottles. Each bottle was filled with 40 ml of pre-warmed buffer adjusted to pH 6.8 and 20 ml of strained rumen fluid, while CO₂ was flushed through the headspace during filling. The measuring system followed López et al. (2007), and the incubation conditions described below were in accordance with the general recommendations for batch *in vitro* rumen fermentation summarised by Chávez-Solis et al. (2026). Rumen fluid was collected before the morning feeding from the same two cannulated cows and strained through four layers of cheesecloth under a continuous CO₂ flow.

The buffer described by Menke and Steingass (1988) was prepared using distilled water and five separately prepared stock solutions. The macromineral stock contained 5.7 g Na₂HPO₄, 6.2 g KH₂PO₄ and 0.6 g MgSO₄ per l of bi-distilled water. The bicarbonate buffer contained 35 g NaHCO₃ and 4 g NH₄HCO₃ per l of bi-distilled water. The trace mineral solution was prepared from a concentrated stock in 100 ml distilled water: 13.2 g CaCl₂·2H₂O, 10 g MnCl₂·4H₂O, 1 g CoCl₂·6H₂O and 0.8 g FeCl₃·6H₂O. Resazurin (0.1 g/100 ml) was used as an

indicator of the redox state of the medium. The reducing solution was freshly prepared from 285 mg $\text{Na}_2\text{S} \cdot 7\text{H}_2\text{O}$ and 4 ml of 1 N NaOH, and made up to 100 ml with distilled water. The sealed bottles were incubated at 39 °C for 48 h. The bottles were manually swirled at 1, 3, 5, 7, 15, 23, 33 and 47 h to keep contents homogeneous.

Gas production was monitored using an automated modular system in which a pressure transducer and a temperature probe, both mounted in the bottle cap, recorded measurements every 5 min. Up to 50 bottles could be run in parallel, and the accompanying software converted the raw pressure signals into cumulative gas volumes and stored the data in real time. Gas volumes at 6, 12, 24 and 48 h were extracted from the time series and fitted to a logistic curve using the curve subtraction approach of Schofield et al. (1994) to derive the kinetic descriptors. A Gompertz equation (Equation 4) was used to estimate lag time:

$$y = A + C \times \exp\{-\exp[-B(X - M)]\} \quad (4)$$

where: A – y-intercept, B – gas production rate (ml/h), C – asymptotic gas volume (ml/g DM), X – cumulative incubation time (h) and M – time (h) at which the maximum gas production rate was reached.

ME and NEL of the lupin substrates were estimated based on the 24-h net gas production using the equations of Menke and Steingass (1988) (Equations 5 and 6):

$$\text{ME (MJ/kg DM)} = 0.157 \times \text{GP} + 0.0084 \times \text{CP} + 0.022 \times \text{EE} - 0.0081 \times \text{ash} + 1.06 \quad (5)$$

$$\text{NEL (MJ/kg DM)} = 0.115 \times \text{GP} + 0.0054 \times \text{CP} + 0.014 \times \text{EE} - 0.0054 \times \text{ash} - 0.36 \quad (6)$$

Where: GP – 24-h net gas production, CP – crude protein and EE – ether extract. Gas production was measured using a 460 mg DM sample and is reported throughout this article as ml/460 mg DM. For use in Equations 5 and 6, GP was converted to ml/200 mg DM, which was

the basis used to calibrate the equations of Menke and Steingass (1988). CP, EE and ash are expressed in g/kg DM.

Microbial biomass production at 24 and 48 h (MBP, mg/g DM) was estimated from the relationship reported by Blümmel et al. (1997) (Equation 7):

$$\text{MBP (mg/g DM)} = \text{IVDMD} - (\text{GP}_{24,48} \times 2.2) \quad (7)$$

Where: $\text{GP}_{24,48}$ – net gas production (ml/g DM) at the corresponding time point and IVDMD – *in vitro* DM digestibility measured at the same time.

Rumen pH, $\text{NH}_3\text{-N}$ and volatile fatty acids

At the end of incubation, pH was measured using a digital pH meter (Hanna HI 8314; Hanna Instruments, Woonsocket, RI, USA). $\text{NH}_3\text{-N}$ was determined by the phenol–hypochlorite method of Weatherburn (1967) at 625 nm on a Shimadzu UV-1900i spectrophotometer (Shimadzu Corp., Kyoto, Japan) at 24 and 48 h. For VFA analysis, 5 ml of rumen fluid was deproteinised with 1 ml of 25% (w/v) meta-phosphoric acid and centrifuged at 5000 rpm for 10 min. The supernatant was stored at -20°C until analysis. VFA (acetate, propionate, butyrate, valerate) were measured using an Agilent 6890N gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionisation detector (FID) and a DB-FFAP (free fatty acid phase, nitroterephthalic acid-modified polyethylene glycol) capillary column ($30\text{ m} \times 320\text{ }\mu\text{m} \times 1.00\text{ }\mu\text{m}$). The injection temperature was 250°C , the oven was programmed from 100 to 200°C at $10^\circ\text{C}/\text{min}$, and the detector temperature was 280°C . Standards were prepared from a Supelco VFA mix (Sigma-Aldrich, Steinheim, Germany).

Predicted methane production

Methane production was predicted from the VFA molar proportions according to the stoichiometric fermentation balance of Wolin (1960) (Equation 8):

$$\text{pCH}_4 \propto (0.5 \times \text{C}_2 - 0.25 \times \text{C}_3 + 0.5 \times \text{C}_4 - 0.25 \times \text{C}_5) \quad (8)$$

where: C2, C3, C4 and C5 – molar proportions (mol%) of acetate, propionate, butyrate and valerate, with C5 calculated as $100 - (C2 + C3 + C4)$. The proportional value was scaled using the measured total VFA concentration and incubation volume, and predicted methane is reported as ml CH₄/460 mg DM, using the same substrate basis as the gas production data. Because methane was derived indirectly from the VFA profile rather than measured directly, the values represent stoichiometric predictions and are treated as such throughout the article.

Statistical analysis

All analyses were performed in SPSS 25.0 (IBM Corp., Armonk, NY, USA) and in Python 3.12 using the SciPy and Statsmodels libraries. Residual normality was assessed using the Shapiro–Wilk test and normal quantile–quantile plots, whereas homogeneity of variance was evaluated using Levene’s test. All variables satisfied both assumptions at $P > 0.05$, and no transformation was required.

Chemical composition, CNCPS fractions, IVDMD at each time point, gas production kinetic parameters, end-point pH, NH₃-N, individual VFA molar proportions, total VFA, the (acetate + butyrate)/propionate ratio, and predicted methane production were analysed by one-way analysis of variance (ANOVA) with lot as the fixed effect (Equation 9):

$$Y_{ij} = \mu + L_i + \varepsilon_{ij} \quad (9)$$

where: Y_{ij} – observation made on the j th experimental unit of the i th lot, μ – overall mean, L_i – fixed effect of the lot ($i = 1-5$) and ε_{ij} – residual error. For the *in vitro* assays, incubation run was first included as a random block effect. As it accounted for a negligible proportion of the total variance and did not change the ranking of lots, the reduced model in Equation 9 was used for the final analysis.

In situ DM degradability was analysed by two-way analysis of variance with lot, incubation time and their interaction as fixed effects (Equation 10):

$$Y_{ijk} = \mu + L_i + T_j + (LT)_{ij} + \varepsilon_{ijk} \quad (10)$$

where: T_j – fixed effect of incubation time ($j = 2, 4, 6, 8, 12, 24, 36$ and 48 h) and $(LT)_{ij}$ – lot $\times$ time interaction. Because the interaction was significant, the effect of lot was then examined separately within each incubation time. *In situ* NDF degradability was analysed using the same model after exclusion of the 8 and 36 h time points.

Where the F-test for lot was significant, pairwise comparisons between the five lots were made with Bonferroni adjustment across the ten possible contrasts. Tukey's honestly significant difference test was applied as a secondary, less conservative check, and the two occasions on which the two procedures disagreed are identified explicitly in the text and in the table footnotes. Parameters of the Ørskov and McDonald (1979) model were estimated for each bag replicate separately by non-linear least squares using the Levenberg–Marquardt algorithm (`scipy.optimize.curve_fit`), and the resulting parameter estimates were then subjected to ANOVA described above. Goodness of fit was summarised by the coefficient of determination. Results are presented as means $\pm$ standard deviation together with the pooled standard error of the mean. Significance was declared at $P < 0.05$ and a tendency at $0.05 \leq P < 0.10$.

Results

Chemical composition and predicted energy values

Chemical composition and predicted energy values are given in Table 1. Native DM was determined from a single bulk subsample per lot and ranged from 31.95 (L3) to 34.73% (L2). Of the parameters analysed in duplicate, ash showed a significant overall lot effect ($P < 0.001$) with L3 (1.84%) higher than L4 (1.35%) after Bonferroni correction, whereas no significant differences were detected between the other lots. CP varied between 35.81% (L3) and 40.17% (L4), and the overall ANOVA results were significant ($P = 0.027$), but no individual comparison remained significant after Bonferroni correction. EE (5.26–5.70%), NDF (35.31–36.89%), ADF (30.86–32.70%), ADL (0.53–0.56%) and NFC did not differ between lots

($P > 0.05$). Predicted TDN ranged from 67.28 to 70.69%, ME 2.66–2.83 Mcal/kg and NE_L - 3×1.69 –1.81 Mcal/kg.

CNCPS protein fractions

Nitrogen fraction B was dominant (87.95–93.15% of CP), while fraction A accounted for 2.03–5.86% and fraction C for 4.82–6.69%. RUP at $k = 0.02/h$ ranged from 30.64 to 32.88% of CP, and RUP digestibility 61.84–64.72% (Table 2).

***In Situ* DM degradability and Ørskov–McDonald parameters**

In situ DM degradability and kinetic parameters are shown in Tables 3 and 4, and the fitted curves are presented in Figure 1. Degradability increased from approx. 9% at 2 h to over 84% at 48 h in all lots. The two-way ANOVA showed a strong effect of incubation time ($F = 1240.822$, $P < 0.001$), a marginal lot effect ($F = 2.545$, $P = 0.041$) and a significant lot $\times$ time interaction ($F = 2.933$, $P < 0.001$). At individual incubation times, differences between lots were observed only at 6 h: L1 (34.3%) was higher than L3, L4 and L5, while L2 (32.4%) was higher than L4 and L5; L3 (24.2%) was intermediate and overlapped with both extremes.

Fitted parameters were: $a = -6.4$ to -1.6% , $b = 83.6$ –89.9%, $c = 0.072$ –0.080/h, ED ($k = 0.05/h$) = 47.1–49.5%. None of the kinetic parameters differed between lots ($P > 0.05$).

***In situ* NDF degradability**

NDF degradability values are summarised in Table 5 and Figure 2. NDF degradability increased from approx. 30% at 2 h to 60–73% at 48 h. The 8 and 36 h time points were excluded because of insufficient sample replicates. The overall lot effect was significant at 6 h ($P = 0.032$), with L2 (34.22%) higher than L5 (31.17%) after Bonferroni correction; L4 had the numerically highest value (37.40%) but did not differ significantly from any specific lot. At 24 h, the lot effect was also significant ($P = 0.009$), with L1 (46.10%) higher than L2 (40.16%) after Bonferroni correction, while the remaining lots had intermediate values.

***In vitro* DM digestibility**

IVDMD is reported in Table 6 and Figure 3 for 4 and 24 h. These were the two incubation times at which dispersion among the three bag replicates was acceptable in all five lots: the coefficient of variation did not exceed 25% at 4 h, and at 24 h it ranged from 2 to 14% in four of the five lots. At the remaining incubation times (2, 6, 8, 12 and 48 h), the coefficient of variation reached 56–120% in at least one lot, the standard deviation equalling or exceeding the mean in several instances. These data are not reported because they do not support a meaningful comparison. IVDMD increased from 4.69–8.19% at 4 h to 26.73–30.90% at 24 h. No lot effect was detected at either time ($P = 0.059$ at 4 h, $P = 0.967$ at 24 h), although the 4 h comparison approached a tendency threshold. One value within the reported set still showed high dispersion: L3 at 24 h ($26.73 \pm 16.66\%$, coefficient of variation 62%), and is flagged in Table 6 for cautious interpretation. Even within the reported time points, IVDMD at 24 h (26.73–30.90%) was approx. 50% of the *in situ* DM degradability measured at the same time point (60.57–62.48%). This systematic difference between the two techniques is addressed in the Discussion.

***In vitro* gas production kinetics**

Gas production parameters are given in Table 7 and Figure 4. Gas production at 24 h ranged from 57.97 ml/460 mg DM (L5) to 67.63 ml/460 mg DM (L1) and did not differ between lots ($P = 0.27$). MBP ranged from 109.5 to 123.4 mg and PF from 4.04 to 4.23, with neither parameter showing a lot effect. The only parameter that differed between lots was early gas production (0–3 h): L3 (13.24 ml) was higher than L2 (9.84 ml) and L5 (9.41 ml) after Bonferroni correction ($P = 0.016$), while L1 and L4 reached intermediate values and did not differ from any other lot. The logistic model returned asymptotic gas volumes (VF) of 75–82 ml/460 mg DM. No distinct lag phase was detected in any lot: measurable gas had accumulated in every bottle within the first 3 h of incubation (9.41–13.24 ml), indicating that fermentation began at or close to the start of incubation. The time to maximum fermentation

rate occurred within the first half of the incubation period in all lots. ME and NEL calculated from 24-h net gas production (Equations 5 and 6) were 9.34–9.94 and 5.31–5.75 MJ/kg DM, respectively (Table 7). These gas-derived values were consistently lower (by 12–18%) than the corresponding values predicted from chemical composition using the NRC (2001) equations and reported in Table 1 (11.13–11.84 MJ ME/kg DM). The gas-derived ME was lower in all lots, and this discrepancy is addressed in the Discussion.

pH, NH₃-N, VFA profile and predicted methane production

At the end of incubation, pH was 7.07–7.22, with no lot effect observed ($P = 0.64$). NH₃-N at 24 h ranged from 9.20 to 10.01 mM and did not differ between lots (Table 8, Figure 6).

Acetate dominated the VFA profile (55.85–58.23 mol%), followed by propionate (26.15–27.14 mol%) and butyrate (9.87–14.24 mol%). The full profile and the (acetate + butyrate)/propionate ratio are shown in Figure 5. Butyric acid was the only VFA showing a significant lot effect ($P = 0.046$), with L3 (14.24 mol%) and L4 (13.94 mol%) reaching higher values than L2 (11.70 mol%) after Bonferroni correction. Total VFA ranged from 125.0 to 131.6 mmol/l, the (acetate + butyrate)/propionate ratio 2.54–2.69, and predicted methane 17.73–18.52 ml/460 mg DM. None of these variables differed between lots ($P > 0.05$; Table 8).

Discussion

Composition and growing-area variation

The CP content of the present water-debittered white lupin grain samples (35.81–40.17% DM) was within the 32–42% range reported for *L. albus* from other regions (Aufrère et al., 1991; Sujak et al., 2006; Gresta et al., 2023) and was slightly higher than the 32.2% CP measured by Uzun et al. (2007) in another set of Turkish-grown white lupin.

White lupin has slightly lower CP than soybean meal (44–48% DM), but higher crude fat content (5–10% versus 1–2%). Consequently, its energy density is similar to that of soybean

meal (Gresta et al., 2023). The EE values (5.26–5.70%) were at the lower end of the reported range, which was consistent with the lipid loss reported by Chamone et al. (2023) during traditional aqueous debittering.

Fibre composition showed little variation between lots, with NDF (35.31–36.89%), ADF (30.86–32.70%) and ADL (0.53–0.56%) all within the typical range reported for *L. albus* (Gresta et al., 2023). The low ADL content indicates a low degree of lignification, which is consistent with the high *in situ* degradability observed in the present study. Annicchiarico et al. (2014) reported a similar pattern in *L. albus* collections from Italy and Poland, with CP varying with growing area, and NDF showing much less variation.

Two practical implications can be drawn from these findings. CP was the only compositional variable to show a significant overall lot effect was CP ($P = 0.027$), although no pairwise comparison remained significant after Bonferroni correction, and its content ranged from 35.81 to 40.17% of DM. A difference of approximately four percentage points is small enough that a single tabulated value could be used for ration formulation without substantial error, but at the same time sufficiently large to justify analysing incoming batches for protein content rather than assuming a fixed value, particularly when traditionally water-debittered white lupin grain is intended to replace a defined amount of soybean meal.

Ash was the one variable that differed significantly between lots ($P < 0.001$; L3 1.84% versus L4 1.35%), and is also the variable most likely to reflect the debittering process rather than the growing site. Prolonged soaking in running spring or stream water can leach soluble minerals from the grain while simultaneously exposing it to suspended silt, thus the net effect may depend on water quality and on how the grain is drained and dried afterwards. The low values observed in all five lots (1.35–1.84% of DM) indicate that soil contamination was not a significant issue in the sampled material. This is relevant because visible sediment can be a concern with traditionally processed feed ingredients. Together with the limited variation in

fibre fractions, these findings indicate that the compositional outcome of the processing method was relatively uniform despite the smallholder and traditional nature of the production process.

CNCPS fractions and bypass protein

For all lots, fraction B accounted for 88–93% of CP, indicating that most of the nitrogen was present in the true protein. Fraction A remained low (2–6% of CP), which was consistent with the possibility that non-protein nitrogen and small peptides, being water-soluble, were partly removed during prolonged aqueous soaking (Chamone et al., 2023). Fraction C accounted for only 5–7%, suggesting that the boiling and soaking step did not result in extensive binding of protein in Maillard reaction products.

RUP at $k = 0.02/\text{h}$ was 30.64–32.88% of CP, close to the value commonly cited for soybean meal (approx. 35%) (NRC, 2001). In feeding terms, traditionally water-debittered white lupin grain could supply a useful proportion of metabolisable protein when used to replace soybean meal in concentrate mixtures; Joch and Kudrna (2020) showed that white lupin could replace 30–50% of soybean meal in dairy cow diets without reducing milk yield. Heat processing such as extrusion results in a greater proportion of nitrogen being allocated to the bypass fraction (Titze et al., 2019), but traditional debittering does not.

NDICP (1.00–2.29%) and ADICP (1.75–2.61%) values were low and similar to those reported by Titze et al. (2019). The low proportion of fibre-bound protein may partly explain the high *in situ b* values observed in the present study. Taken together, the fraction profile has practical implications for the use of this material as a protein feed. A feedstuff in which most nitrogen is in fraction B, with relatively little fractions A and C, would be expected to provide a more gradual supply of ruminally available nitrogen than a feed with a large fraction A, while the undegraded fraction may provide protein to the small intestine. The RUP digestibility estimated here (61.8–64.7%) is consistent with this interpretation. This profile is relevant when a locally produced legume is considered as a partial substitute for soybean meal in concentrate mixtures. However, CNCPS fractions are chemical partitions defined by solubility in

a sequence of buffers and detergents rather than direct measurements of ruminal protein behaviour, and RUP estimates depend on the assumed passage rate. The values reported here should therefore be regarded as model-based estimates that depend on these assumptions rather than as direct measurements of ruminal protein escape.

***In situ* degradation kinetics**

R^2 values for the Ørskov–McDonald fit were generally above 0.95, and none of the kinetic parameters differed between lots ($P > 0.05$). For a feed mill blending water-debittered white lupin grain from several smallholders, this suggests that variation in *in situ* degradability between batches is unlikely to be a major practical concern.

The fractional rate ($c = 0.072$ – 0.080 /h) was within the range reported by Gresta et al. (2023) and Aufrère et al. (1991) for *L. albus* (0.06 – 0.09 /h) and slightly higher than that reported for soybean meal (0.06 – 0.08 /h). The b fraction (83.6 – 89.9%) was also in the upper part of the range reported for lupin. ED at $k = 0.05$ /h (47.1 – 49.5%) was within the broad range observed by Titze et al. (2019) for 15 batches and nine genotypes, including different outflow rates and thermal treatments. Extrusion of *L. albus* markedly reduces degradability under heat processing (Titze et al., 2019), whereas traditional debittering does not involve the same degree of thermal treatment and therefore leaves a moderate proportion of protein available as RUP.

One feature of the fitted parameters deserves comment because it could easily be misinterpreted. The a estimate, the intercept of the Ørskov–McDonald model, was negative in every lot (-6.44 to -1.63%). A negative intercept has no biological meaning as a soluble fraction, and its occurrence reflects the shape of the observed curve rather than a failure of the fit. DM disappearance at 2 h was only approx. 9%, followed by a marked increase in degradation, giving the curve a sigmoidal onset that a single-exponential model can accommodate only by displacing its intercept below zero. Thus, the negative a effectively accounts for a lag phase that is not represented as an explicit term in the model.

The negative a estimate has two implications. First, a should not be interpreted as a washout fraction for this substrate, and the $a + b$ sum (81.62–85.06%) is a more meaningful description of potential degradability than either parameter considered separately. Second, the small immediately soluble fraction implied by the negative intercept is consistent with the low CNCPS fraction A measured in the same material (2.03–5.86% of CP), and with the possibility that prolonged aqueous soaking removes readily soluble nitrogen before the grain reached the rumen. These two lines of evidence are consistent with the interpretation that the debittering process removes soluble components while leaving most of the true protein intact.

ED is the operative measure for ration formulation, and its dependence on outflow rate has practical implications. ED decreased from 63.98–66.07% at $k = 0.02/\text{h}$ to 47.14–49.53% at $k = 0.05/\text{h}$ and 36.95–39.56% at $k = 0.08/\text{h}$. Therefore, the proportion of protein escaping ruminal degradation depends substantially on intake level and passage rate. The same batch of water-debittered white lupin can be expected to supply more RUP to a high-producing cow on a high-intake ration than to a dry cow fed at maintenance. This dependence is not specific to lupin, but it means that a single RUP value is of limited use unless the assumed passage rate is also specified. For this reason, ED was reported at three outflow rates rather than at a single one.

Gas production kinetics and methodological context

Gas production at 24 h was 58–68 ml per 460 mg DM. This was within the 110–160 ml/g range reported for *L. albus* by Aufrère et al. (1991) and Gresta et al. (2023). Morsy et al. (2024) reported 115–130 ml/g DM for *L. angustifolius* using the Hohenheim gas test. Absolute gas volumes are, however, strongly dependent on the experimental system. Pressure-transducer systems such as the one used here (López et al., 2007) differ from Hohenheim syringes in headspace volume, venting regime and the extent to which CO₂ remains dissolved in the medium. Chávez-Solis et al. (2026) identified these factors among the main reasons why gas volumes are not directly comparable between laboratories. Numerical comparisons between

techniques should therefore be interpreted as indicating the same order of magnitude rather than directly comparable values.

The single-pool exponential model fitted the gas curves adequately ($R^2 > 0.95$), although multiphasic models have been proposed as a better description of substrates with mixed starch, protein and lipid composition. Alternative kinetic models were not tested in the present study. Asymptotic gas (VF = 75–82 ml) was within the range reported for *L. albus* and higher than the 30–50 ml typically reported for forages, which is consistent with a readily fermentable substrate.

The absence of a lot effect on 24-h gas production ($P = 0.271$) is the most relevant result in this section from a practical perspective. Gas production at 24 h reflects the fermentation of all substrate components and is the variable used in the Menke and Steingass (1988) energy equations. The lack of a significant difference between the five independently produced lots indicates that the energy contribution of water-debittered white lupin grain to a ration may be relatively consistent, whereas individual chemical components alone may provide a less reliable indication. The standard deviations of gas production at 24 h were also substantial, representing approximately 17–30% of the corresponding means. Therefore, the absence of a significant lot effect indicates that no difference was detected under the conditions of this experiment, rather than that gas production showed little variability. Modest differences between lots may not have been detected with 14 bottles per lot.

The energy estimates require separate consideration because the two prediction methods used in this study gave different results. ME calculated from 24-h gas production (9.34–9.94 MJ/kg DM) was 12–18% lower than that predicted from chemical composition using the NRC (2001) equations (11.13–11.84 MJ/kg DM) and was lower in all five lots. This consistent difference between the two methods is unlikely to reflect random measurement variation and should therefore be considered when interpreting the energy estimates.

The difference between the two estimates can be explained by the different contribution of carbohydrates, protein and lipids to gas production during fermentation. Gas is generated during carbohydrate fermentation to VFA, with CO₂ released both directly and from the buffering of the resulting acids. Protein fermentation produces considerably less gas per unit of organic matter, whereas lipids produce little gas, because fatty acids are not fermented but undergo biohydrogenation in the rumen. Traditionally water-debittered white lupin grain, with CP accounting for 35.8–40.2% and EE for 5.3–5.7% of DM, therefore contains a substantial proportion of its energy in fractions that are nutritionally valuable but produce relatively little gas. The Menke and Steingass (1988) equations include CP and EE to account for this, but these equations were calibrated largely using forages and mixed rations rather than protein- and oil-rich legume seeds. The present data suggest that correction may be insufficient for a substrate with the composition analysed here.

These findings have two practical implications. First, for a feedstuff of this type, the gas-derived ME should be interpreted with caution, and the value predicted from chemical composition appears more suitable for ration formulation. Accordingly, the NRC-based values have been retained in Table 1 as the primary energy estimates, while the gas-derived values are presented alongside them for comparison rather than as replacements. Second, the magnitude of the difference indicates that a single gas-derived ME value for a high-protein concentrate should not be reported without specifying the method used, since a difference of approximately 15% could affect ration formulation based on ME. Determining which approach gives the more appropriate estimate for traditionally debittered *L. albus* would require an *in vivo* digestibility trial, providing a further rationale for the experiment proposed below.

Early fermentation rate and microbial adaptation

Early gas production (0–3 h) was the only variable that differed clearly between lots, ranging from 9.41 ml in L5 to 13.24 ml in L3 ($P = 0.016$). Although the absolute difference was small, it may indicate differences in the rate at which rumen microbes colonised the

substrate during the first few hours of incubation. Schofield et al. (1994) reported that the early phase of gas curves is particularly sensitive part to physical structure and inhibitory compounds.

Two explanations may account for this variation. The traditional boiling and soaking process may leave small amounts of quinolizidine alkaloids (lupanin, sparteine), which have antimicrobial effects and could delay microbial colonisation. A more disrupted seed coat resulting from longer boiling may also allow faster microbial access to the cotyledon. Residual alkaloids were not measured in the present study, so their possible contribution to the observed differences remains uncertain and should be investigated in future work.

Despite the difference in early gas production, cumulative gas production at 20 and 24 h was similar between lots, indicating that any differences in the short-term fermentation response were greater than those in the overall extent of fermentation. The practical significance of this difference is limited but may still be relevant: a difference confined to the first 3 h of incubation would, in a fed animal, correspond to the period of first few hours after concentrate feeding, when the rate of fermentation may be more important than its overall extent in determining the risk of a rapid decline in rumen pH. Since the overall extent of fermentation was similar between lots, the five lots would be expected to have similar nutritive value for practical ration formulation. Any difference in the immediate post-feeding response would probably be small and cannot be quantified from the present data.

pH, NH₃-N and microbial protein synthesis

Final pH was 7.07–7.22 in all lots. These values are higher than the 6.2–6.8 usually recorded at the end of batch incubations, and this difference requires explanation. Three features of the present system may account for it. First, the buffer-to-inoculum ratio was 2:1 (40 ml of buffer to 20 ml of rumen fluid) and the medium contained 35 g/l NaHCO₃ and 4 g/l NH₄HCO₃, resulting in a high bicarbonate buffering capacity relative to the amount of acid generated. Second, the substrate load was relatively low (460 mg per 60 ml, approx. 7.7 g/l), thus although VFA concentration reached 125–132 mmol/l, total acid production remained within that

buffering capacity of the medium. Third, and most importantly, pH was measured after the bottles had been opened and vented at the end of the incubation. Depressurisation allows dissolved CO₂ to escape, altering the bicarbonate–carbonic acid equilibrium and increasing the measured pH relative to the value inside the sealed bottle.

The consequence is that end-point pH reflects the buffering conditions of the incubation medium rather than ruminal pH in a fed animal, and it should not be compared directly with *in vivo* reference values. This distinction is important because end-point pH from batch cultures is often reported alongside *in vivo* thresholds, which can be potentially misleading. The present pH data support comparison between lots, which showed no significant differences ($P = 0.636$), and indicated that the incubation medium did not become sufficiently acidic to inhibit fermentation or confound the comparison. Accordingly, the earlier statement that pH remained above the threshold at which cellulolytic activity declines (Russell and Wilson, 1996) should be interpreted as evidence that the assay conditions were adequate, rather than as evidence that traditionally water-debittered white lupin grain protects against ruminal acidosis *in vivo*. The lower values reported by Yáñez-Ruiz et al. (2004) for lupin substrates (6.80–7.05) are more likely to reflect differences in the buffer system and measurement protocol than differences in the substrate.

NH₃-N at 24 h was 9.20–10.01 mM between lots. These values were above the 3.5 mM minimum for microbial growth reported by Satter and Slyter (1974) and within the 5–8 mM range considered optimal by Schwab and Broderick (2017). They were also below the 15–20 mM range at which ammonia accumulation may indicate inefficient nitrogen capture. These results suggest that protein degradation was sufficient to support microbial protein synthesis without excessive ammonia accumulation and, consequently, without a substantial need for hepatic urea synthesis.

VFA profile, milk fat and methanogenesis

The VFA profile (55.85–58.23 mol% acetate, 26.15–27.14 mol% propionate, 9.87–14.24 mol% butyrate) was consistent with that expected from a legume seed fermented alone, and the limited lot effects, with butyrate being the only VFA that differed between lots, indicate that the five production lots provided a similar fermentable substrate to the microbial population. The (C2 + C4)/C3 ratio of 2.54–2.69 was above the 2.2 threshold associated with milk-fat depression risk by Yang and Beauchemin (2007) and within the 2.5–2.8 range reported by Morsy et al. (2024) for *L. angustifolius*.

This comparison should be interpreted narrowly. Milk fat depression is a multifactorial condition mainly associated with the formation of specific ruminal biohydrogenation intermediates, particularly trans-10, cis-12 conjugated linoleic acid, and the acetate-to-propionate balance is at most an indirect indicator. Moreover, the 2.2 value was derived from lactating cows fed complete rations, whereas the ratio reported here describes a single feedstuff fermented alone in a buffered batch system with a fixed inoculum. The present data therefore indicate that traditionally water-debittered white lupin grain does not, when fermented alone, produce the low-acetate, high-propionate fermentation pattern associated with milk fat depression. They do not predict the milk-fat response of cows fed a ration containing this feedstuff and cannot be used to claim a protective effect. That question can only be addressed in a lactation trial in which milk fat yield and milk fatty acid composition are measured directly.

The mechanistic link between the VFA ratio and methane emission is well established: acetate and butyrate production releases hydrogen, which methanogens use to reduce CO₂ to methane, while propionate production consumes hydrogen (Janssen, 2010). The ratios observed here are consistent with the moderate predicted methane values we report.

Total VFA concentration (125–132 mmol/l) was higher than the 75–100 mmol/l reported by Yáñez-Ruiz et al. (2004). Differences in donor diet, substrate-to-inoculum ratio and incubation time are possible explanations for this difference, making direct comparison between studies difficult. The relevant finding within the present study is that total VFA did not differ

between lots ($P = 0.439$), indicating that the amount fermentable organic matter available to the microbial population was similar between production units.

Predicted methane production and mitigation context

Predicted methane production was 17.73–18.52 ml/460 mg DM, corresponding to approx. 36–40 ml/g DM. These values were within the ranges reported by Morsy et al. (2024) for *L. angustifolius* (32–38 ml/g DM) and by Yáñez-Ruiz et al. (2004) for *L. albus* (30–42 ml/g DM at 24 h). Morsy et al. (2024) also found that adding *L. angustifolius* seeds to a TMR at 0.5–2.0% DM reduced methane emission by 15–22% of total biogas, which was attributed to the effects of monoterpenes acting on the protozoa–methanogen complex. This finding cannot be directly applied to the present material. Morsy et al. (2024) used a different species, incubated as a low-level supplement in a complete mixed ration and compared with an unsupplemented control, whereas the present lots were incubated as the sole substrate, at a single inclusion level and without a comparator ration. In addition, *L. albus* and *L. angustifolius* differ in their alkaloid and secondary-metabolite profile. The present data support the narrower conclusion that traditionally debittered *L. albus* does not generate an unusually high predicted methane yield compared with published values for lupin, and that predicted methane production did not differ between production lots ($P = 0.932$). Establishing a methane-mitigation effect would require dose–response incubations with a defined control ration and, ultimately, direct measurement in animals.

The Wolin (1960) equation predicts methane production indirectly from VFA stoichiometry, and direct measurements typically differ by 10–20%. The equation also assumes a closed hydrogen balance in which all reducing equivalents not captured in propionate are directed to methanogenesis. It therefore does not account for other hydrogen sinks, such as microbial biomass synthesis, reductive acetogenesis or biohydrogenation of unsaturated fatty acids. The latter may be relevant for a substrate containing 5.3–5.7% EE. Predicted methane production in this study should accordingly be interpreted as a stoichiometric index that allows

comparison between lots rather than as a direct estimate of methane yield. Comparison with a Hohenheim test in which CH₄ is measured directly, or with *in vivo* measurements using respiration chambers or the SF₆ tracer technique, would provide a stronger basis for evaluating methane production.

Microbial biomass production and partitioning factor

The partitioning factor (4.04–4.23 at 24 h) was in the upper half of the 2.75–4.50 range considered by Blümmel et al. (1997) to indicate efficient microbial protein synthesis. A high PF means that a larger proportion of the fermented substrate is incorporated into microbial biomass and a smaller proportion is associated with gas production. This interpretation requires caution in the present case. PF is calculated from IVDMD, and, as described above, the IVDMD values had large standard deviations and were low relative to the *in situ* degradability of the same material. Hence, any error in IVDMD affects both MBP and PF. The PF values reported here are consequently better regarded as indicating that microbial biomass production was not anomalously low rather than as precise estimates of microbial efficiency. The expectation that traditionally water-debittered white lupin grain would result in greater microbial protein flow to the duodenum is plausible but was not demonstrated in this study.

Considered together with the gas production data, PF also provides an indirect indication of the internal consistency of the incubations. PF varied over a very narrow range (4.04–4.23, $P = 0.183$), although it was calculated from two measurements, gas volume and IVDMD, both of which showed substantial variation. Greater stability of the ratio than of its individual components would be expected if the dominant source of variation between bottles resulted from differences in the amount of substrate fermented, which would affect components simultaneously, rather than from systematic difference between lots. This does not remove the limitations of the IVDMD measurements discussed below, but it does suggest that the variation was largely random rather than systematic.

Practical implications and study limitations

Several practical implications can be drawn from these findings. The consistency between lots suggests that a feed buyer sourcing *termiye* from different smallholders should not expect differences in nutritive value between batches. Protein content, CNCPS fractions and RUP were sufficiently similar to those of soybean meal to support partial replacement of soybean meal at 30–50% inclusion levels (Joch and Kudrna, 2020). This conclusion is based on the feeding trial reported by Joch and Kudrna (2020), rather than on the present data, which describe the feedstuff and not complete rations. The present study demonstrates that traditionally water-debittered *L. albus* has chemical composition and fermentation characteristics similar to the material used in such feeding trials. The predicted methane production values were within the range reported for lupin seed and did not indicate an unfavourable fermentation profile. However, for the reasons discussed above, these values do not by themselves support any conclusion regarding the sustainability of this feedstuff.

The most important constraint of this data set is the small number of donor animals. Rumen fluid for both the *in situ* incubations and the *in vitro* runs was obtained from two cannulated cows. Between-animal differences in ruminal microbial composition and activity is a well-recognised source of variance in both techniques. With only two donor animals, however, this component of variation cannot be estimated reliably or distinguished from residual error. The results therefore cannot be confidently generalised to the wider cow population. Three bag replicates per animal at each time point and 14 bottle replicates per lot provide replication within animals and incubation runs, but do not increase the number of independent donor animals. The absolute values reported here, including degradation rates, gas volumes, VFA concentrations and predicted methane production, should therefore be interpreted in relation to the inoculum used rather than as estimates applicable to the wider cow population. Comparisons between lots remain informative because all lots were exposed to the same inoculum within each run and

were incubated simultaneously. These comparisons assess differences between lots under a common inoculum. For this reason, the near-absence of lot differences indicates that they were not distinguishable under these experimental conditions but does not demonstrate that they are biologically equivalent.

The reasons for this variation are relevant to the design of comparable studies. Three bags per lot at each time point provide limited replication for estimating variance, and several sources of bag-to-bag variation may affect this particular substrate: position within the rotating digestion jar, differences in the effective sample-to-surface ratio within the sealed bag, incomplete rinsing of adherent microbial material and, most importantly for a finely ground, high-protein, low-lignin seed, the loss of small particles through the 25 μm pores of the F57 bag. Loss of undegraded fine particles can increase apparent digestibility, while incomplete penetration of the medium into a compacted bag reduce it, with these effects occurring in opposite directions between bags. Such variation is likely to be greatest at incubation times when the residue is smallest and most fragmented, consistent with the greatest variation observed at 6, 12 and 48 h. A study reproducing this work should therefore include more bags per time point and consider a smaller pore size or a coarser grind.

A second limitation concerns the precision of the IVDMD measurements. The series originally included seven incubation times, but at five of them the variation between the three bag replicates was too large for meaningful interpretation: the coefficient of variation reached 56–120% in at least one lot, and at 6, 12 and 48 h, the standard deviation equalled or exceeded the mean in individual lots. These time points were therefore excluded, and the analysis was restricted to 4 and 24 h, where variation was acceptable in all five lots. This approach avoids reporting means with high variability that would make comparisons of digestibility difficult to interpret.

A systematic difference between the two degradability techniques was also observed within the reported window. At 24 h, *in situ* DM degradability was 60.57–62.48%, whereas IVDMD

was 26.73–30.90% for the same lots, corresponding to approximately 43–51% of the *in situ* values. A difference of this magnitude is unlikely to be attributable to the substrate alone and may reflect differences between the two procedures. The *in situ* method used a 5 g sample in a 50 µm nylon bag exposed to the rumen environment, with fermentation products being continuously removed and the microbial population being replenished. In contrast, the *in vitro* method used a 0.5 g sample in a 25 µm filter bag in a closed jar with a fixed inoculum volume, where substrate depletion, accumulation of fermentation products and changes in microbial activity may limit degradation. The *in situ* series, with six replicates per lot at each time point and a relatively low residual variation, therefore provides a more reliable assessment of degradability in the present study. The IVDMD data are better regarded as supporting information, with their main role being to provide the IVDMD value required for the MBP and PF calculations at 24 h.

Chemical composition and NH₃-N were based on two analytical replicates per lot, so the marginal CP effect ($P = 0.027$), for which no pairwise comparison remained significant after Bonferroni correction, should be considered in light of the limited replication. Residual alkaloid content was not measured; given the proposed relationship between early fermentation rate and alkaloid load, this is an important limitation and should be addressed in future studies. Methane production was predicted from VFA stoichiometry rather than measured directly. Finally, the entire study was conducted *in situ* or *in vitro*. *In vivo* experiments measuring intake, milk yield, milk composition, and enteric methane directly would be required before feeding recommendation could be made. The current results should be regarded as a screening-level assessment that supports the need for such studies rather than as a substitute for them. The small number of analytical replicates for chemical composition and NH₃-N ($n = 2$ per lot), together with the three bags per lot per time point used for IVDMD, also resulted in low statistical power for these comparisons. The corresponding P -values should therefore be interpreted as indicative, and non-significant results should not be taken as evidence of equivalence.

Conclusions

Traditionally water-debittered *Lupinus albus* grain is a high-protein feedstuff whose nitrogen is predominantly present as true protein and whose rumen-undegradable protein fraction is similar to that of soybean meal, supporting its evaluation as a partial substitute for imported protein concentrates in ruminant rations. Traditional aqueous debittering is a relatively mild processing method that preserves the protein characteristics of the grain and results in a uniform chemical composition across independent smallholder production lots. However, the feeding value indicated by the *in situ* and *in vitro* results remains to be confirmed in *in vivo* trials.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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Table 1. Chemical composition and predicted energy values of debittered *Lupinus albus* grain from five lots (% of DM unless stated; n = 2 per lot), %

Item	L1	L2	L3	L4	L5	SEM	P-value
Native DM	32.55	34.73	31.95	33.74	33.44	—	n.t.
CP	38.36 ^{ab}	38.05 ^{ab}	35.81 ^b	40.17 ^a	39.17 ^{ab}	0.525	0.027*
EE	5.33	5.62	5.43	5.70	5.26	0.162	0.947 ^{ns}
Ash	1.68 ^{ab}	1.74 ^{ab}	1.84 ^a	1.35 ^b	1.50 ^{ab}	0.060	<0.001***
NDF	36.47	35.31	36.89	35.77	36.49	0.490	0.918 ^{ns}
ADF	30.86	31.59	31.19	31.19	32.70	0.259	0.163 ^{ns}
ADL	0.53	0.55	0.54	0.56	0.56	0.006	0.375 ^{ns}
NFC	18.15	19.29	20.03	17.01	17.58	0.590	0.571 ^{ns}
TDN	68.32	68.95	67.82	70.69	67.28	—	—
ME, Mcal/kg	2.71	2.73	2.66	2.83	2.69	—	—
NEL-3×, Mcal/kg	1.73	1.74	1.69	1.81	1.71	—	—
NEM-3×, Mcal/kg	1.84	1.86	1.80	1.95	1.82	—	—
NEG-3×, Mcal/kg	1.20	1.21	1.16	1.29	1.18	—	—

DM – dry matter, CP – crude protein, EE – ether extract, NDF – neutral detergent fibre, ADF – acid detergent fibre, ADL – acid detergent lignin, NFC – non-fibre carbohydrates, TDN – total digestible nutrients, ME – metabolizable energy, NEL – net energy for lactation, NEM – net energy for maintenance, NEG – net energy for gain; * $P < 0.05$, *** $P < 0.001$; ns – not significant, n.t. – not tested; SEM – standard error of the mean; means in a row with different superscripts are significantly different at $P < 0.05$. For Ash, only the L3↔L4 contrast survived Bonferroni adjustment (adjusted $P = 0.010$). For CP, Tukey HSD separated L3 (lowest, 35.81%) from L4 (highest, 40.17%) at $P = 0.020$. For Native DM, a single bulk subsample was processed per lot so no inferential test was performed (n.t.). Energy values were calculated from chemical composition using the NRC (2001) prediction equations and were not subjected to inferential testing.

Table 2. CNCPS nitrogen fractions and rumen-undegradable protein (RUP) of *Lupinus albus* grain

Item	L1	L2	L3	L4	L5
Fraction A (NPN), % CP	5.86	5.36	2.03	4.08	4.29
Fraction B (true protein), % CP	88.54	87.95	93.15	90.66	90.23
Fraction C (bound protein), % CP	5.61	6.69	4.82	5.26	5.47
RUP ($k = 2\%/h$), % CP	30.64	31.34	31.67	32.88	31.80
RUP digestibility, %	63.03	61.84	64.72	63.35	63.09
NDICP, % DM	1.43	1.64	1.00	2.29	1.28
ADICP, % DM	2.20	2.61	1.75	2.16	2.23

NPN – non-protein nitrogen, CP – crude protein, DM – dry matter, NDICP – neutral detergent insoluble crude protein, ADICP – acid detergent insoluble crude protein, k = assumed ruminal outflow (passage) rate. Calculated according to Sniffen et al. (1992) and Licitra et al. (1996). L1–L5 – production lots of traditionally water-debittered *L. albus* grain: L1 – Hüyük (Konya), L2 – Seydişehir (Konya), L3 – Doğanhisar (Konya), L4 – Şarkikaraağaç (Isparta), L5 – Ilgın (Konya)

Table 3. *In situ* DM degradability (%) of *Lupinus albus* by lot and incubation time (mean $\pm$ SD, n = 6)

Time, h	L1	L2	L3	L4	L5	SEM	P-value
2	9.37 $\pm$ 1.56	9.38 $\pm$ 0.86	9.55 $\pm$ 1.07	9.38 $\pm$ 1.28	9.38 $\pm$ 0.93	0.20	0.998 ^{ns}
4	15.24 $\pm$ 2.58	15.39 $\pm$ 2.42	15.03 $\pm$ 2.70	14.04 $\pm$ 2.72	15.06 $\pm$ 1.37	0.42	0.880 ^{ns}
6	34.29 $\pm$ 2.72 ^a	32.41 $\pm$ 3.12 ^{ab}	24.17 $\pm$ 5.76 ^{bc}	19.14 $\pm$ 4.15 ^c	19.18 $\pm$ 4.22 ^c	1.38	<0.001 ^{***}
8	38.43 $\pm$ 2.26	36.75 $\pm$ 3.00	40.13 $\pm$ 9.49	42.65 $\pm$ 6.25	38.59 $\pm$ 9.47	1.21	0.644 ^{ns}
12	53.15 $\pm$ 3.41	51.99 $\pm$ 2.22	49.07 $\pm$ 11.65	55.58 $\pm$ 3.12	54.79 $\pm$ 2.91	1.08	0.352 ^{ns}
24	61.86 $\pm$ 4.53	61.56 $\pm$ 3.85	62.48 $\pm$ 2.93	61.97 $\pm$ 2.76	60.57 $\pm$ 2.68	0.59	0.903 ^{ns}
36	74.45 $\pm$ 3.09	72.66 $\pm$ 2.95	69.93 $\pm$ 5.55	73.29 $\pm$ 2.04	69.76 $\pm$ 6.47	0.82	0.272 ^{ns}
48	87.82 $\pm$ 2.91	84.21 $\pm$ 3.74	85.11 $\pm$ 4.83	86.48 $\pm$ 2.86	88.00 $\pm$ 2.54	0.65	0.274 ^{ns}

DM – dry matter, SD – standard deviation; L1–L5 – production lots of traditionally water-debittered *L. albus* grain: L1 – Hüyük (Konya), L2 – Seydişehir (Konya), L3 – Doğanhisar (Konya), L4 – Şarkikaraağaç (Isparta), L5 – Ilgın (Konya). Means in a row with different superscripts are significantly different (Bonferroni-adjusted $P < 0.05$); time effect was highly significant within every lot ($P < 0.001$). SEM – standard error of the mean; *** $P < 0.001$; ns – not significant

Table 4. Ørskov–McDonald kinetic parameters for *in situ* DM degradability of *Lupinus albus* (mean $\pm$ SD, n = 6)

Parameter	L1	L2	L3	L4	L5	SEM	P-value
a, %	-1.63 $\pm$ 5.37	-1.95 $\pm$ 3.14	-2.63 $\pm$ 6.69	-6.44 $\pm$ 2.97	-4.95 $\pm$ 3.19	0.84	0.308 ^{ns}
b, %	86.69 $\pm$ 3.07	83.57 $\pm$ 1.66	87.23 $\pm$ 2.14	89.16 $\pm$ 3.39	89.94 $\pm$ 7.74	0.82	0.110 ^{ns}
c, 1/h	0.0740 $\pm$ 0.0177	0.0758 $\pm$ 0.0106	0.0721 $\pm$ 0.0304	0.0795 $\pm$ 0.0107	0.0751 $\pm$ 0.0264	0.0036	0.980 ^{ns}
a + b, %	85.06 $\pm$ 4.73	81.62 $\pm$ 3.86	84.60 $\pm$ 7.37	82.73 $\pm$ 1.67	84.99 $\pm$ 9.98	1.09	0.828 ^{ns}
ED ($k = 0.02$), %	66.07 $\pm$ 1.72	63.98 $\pm$ 1.82	64.03 $\pm$ 2.42	64.66 $\pm$ 1.71	64.43 $\pm$ 2.93	0.39	0.466 ^{ns}
ED ($k = 0.05$), %	49.53 $\pm$ 1.12	48.19 $\pm$ 0.96	47.14 $\pm$ 2.67	48.14 $\pm$ 2.19	47.17 $\pm$ 1.68	0.35	0.183 ^{ns}
ED ($k = 0.08$), %	39.56 $\pm$ 0.97	38.52 $\pm$ 0.82	37.27 $\pm$ 2.86	37.88 $\pm$ 2.26	36.95 $\pm$ 2.35	0.38	0.206 ^{ns}

DM – dry matter, SD – standard deviation; a – rapidly soluble fraction (%), b – potentially degradable but insoluble fraction (%), c – fractional degradation rate of b (per h), k = assumed ruminal outflow rate (per h), ED – effective degradability; Model: $P(t) = a + b \times (1 - \exp(-c \times t))$; effective degradability $ED = a + (b \times c) / (c + k)$. L1–L5 – production lots of traditionally water-debittered *L. albus* grain: L1 – Hüyük (Konya), L2 – Seydişehir (Konya), L3 – Doğanhisar (Konya), L4 – Şarkikaraağaç (Isparta), L5 – Ilgın (Konya); SEM – standard error of the mean; $P > 0.05$ (no statistically significant); ns – not significant; SD – standard deviation

Table 5. *In situ* NDF degradability (%) of *Lupinus albus* grain (mean $\pm$ SD)

Time, h	L1	L2	L3	L4	L5	SEM	P-value
2	31.73 $\pm$ 5.83	32.03 $\pm$ 5.46	37.53 $\pm$ 1.00	30.58 $\pm$ 5.55	30.59 $\pm$ 5.42	1.15	0.324 ^{ns}
4	45.98 $\pm$ 9.18	39.15 $\pm$ 6.19	34.59 $\pm$ 5.70	37.77 $\pm$ 11.31	42.51 $\pm$ 5.33	1.67	0.256 ^{ns}
6	35.25 $\pm$ 2.13 ^{ab}	34.22 $\pm$ 1.07 ^a	34.87 $\pm$ 2.88 ^{ab}	37.40 $\pm$ 4.40 ^{ab}	31.17 $\pm$ 1.11 ^b	0.59	0.032 [*]
12	40.75 $\pm$ 2.59	39.38 $\pm$ 5.26	44.59 $\pm$ 5.29	47.43 $\pm$ 1.01	41.77 $\pm$ 3.91	1.05	0.196 ^{ns}
24	46.10 $\pm$ 1.58 ^a	40.16 $\pm$ 2.34 ^b	46.13 $\pm$ 3.02 ^{ab}	42.53 $\pm$ 0.38 ^{ab}	38.22 $\pm$ 5.62 ^{ab}	0.98	0.009 ^{**}
48	63.03 $\pm$ 6.47	61.75 $\pm$ 2.87	63.69 $\pm$ 8.57	72.51 $\pm$ 5.85	59.74 $\pm$ 2.97	1.34	0.152 ^{ns}

NDF – neutral detergent fibre, DM – dry matter; L1–L5 – production lots of traditionally water-debittered *L. albus* grain: L1 – Hüyük (Konya), L2 – Seydişehir (Konya), L3 – Doğanhisar (Konya), L4 – Şarkikaraağaç (Isparta), L5 – Ilgın (Konya); values are means $\pm$ SD; SD – standard deviation. Means in a row with different superscripts are significantly different (Bonferroni-adjusted $P < 0.05$); at 6 h only the L2 $\leftrightarrow$ L5 contrast and at 24 h only the L1 $\leftrightarrow$ L2 contrast survived Bonferroni adjustment. Time effect was significant within every lot ($P < 0.001$). NDF was measured according to Van Soest et al. (1991) in an ANKOM 200 analyser; the 8 h and 36 h time points were excluded. SEM – standard error of the mean. * $P < 0.05$; ** $P < 0.01$ means that the data is significantly different; ns – not significant

Table 6. *In vitro* DM digestibility (IVDMD, %) of *Lupinus albus* grain at 4 and 24 h, the two incubation times at which replicate dispersion was acceptable in all five lots (mean $\pm$ SD, n = 3)

Time, h	L1	L2	L3	L4	L5	SEM	P-value
4	7.02 $\pm$ 1.79	4.69 $\pm$ 0.27	8.19 $\pm$ 1.24	6.90 $\pm$ 1.25	5.98 $\pm$ 1.23	0.42	0.059 ^{ns}
24	28.29 $\pm$ 1.15	30.90 $\pm$ 0.70	26.73 $\pm$ 16.66 [†]	29.26 $\pm$ 4.14	30.15 $\pm$ 2.30	1.74	0.967 ^{ns}

DM – dry matter, IVDMD – *in vitro* dry matter digestibility [IVDMD = (sample DM – residue DM) / sample DM $\times$ 100; modified Tilley and Terry (1963) two-stage technique (Daisy II incubator, ANKOM F57 filter bags, 25 μ m pore size)]; L1–L5 – production lots of traditionally water-debittered *L. albus* grain: L1 – Hüyük (Konya), L2 – Seydişehir (Konya), L3 – Doğanhisar (Konya), L4 – Şarkikaraağaç (Isparta), L5 – Ilgın (Konya); SEM – standard error of the mean; $P > 0.05$ (no statistically significant); ns – not significant; SD – standard deviation. [†] L3 at 24 h retains a coefficient of variation of 62% and is interpreted with caution. The series is restricted to the two incubation times at which the coefficient of variation among the three bag replicates was acceptable in every lot: 4 h ($\leq 25\%$ in all lots) and 24 h (2–14% in L1, L2, L4 and L5). Time points at which the coefficient of variation reached 56–120% in one or more lots (2, 6, 8, 12 and 48 h) are not reported

Table 7. *In vitro* gas production kinetic parameters for *Lupinus albus* grain (n = 14 per lot; semi-automatic pressure system following López et al. (2007))

Parameter	L1	L2	L3	L4	L5	SEM	P-value
0–3 h gas, ml	10.68 ± 3.86 ^{ab}	9.84 ± 2.84 ^b	13.24 ± 2.34 ^a	11.66 ± 2.44 ^{ab}	9.41 ± 3.93 ^b	0.40	0.016*
3–20 h gas, ml	47.59 ± 8.19	42.89 ± 10.45	45.38 ± 7.98	40.44 ± 10.56	40.04 ± 13.87	1.26	0.264 ^{ns}
20 h gas, ml	58.27 ± 11.12	52.73 ± 12.15	58.62 ± 9.54	52.10 ± 12.27	49.45 ± 16.15	1.51	0.220 ^{ns}
24 h gas, ml	67.63 ± 11.29	61.82 ± 12.76	66.62 ± 10.07	60.58 ± 13.52	57.97 ± 17.72	1.61	0.271 ^{ns}
MBP 24 h, ml	123.39 ± 14.09	115.94 ± 16.17	122.20 ± 17.92	116.45 ± 18.42	109.54 ± 25.27	2.26	0.307 ^{ns}
PF 24 h	4.04 ± 0.10	4.10 ± 0.14	4.05 ± 0.10	4.14 ± 0.22	4.23 ± 0.41	0.03	0.183 ^{ns}
ME from gas, MJ/kg DM	9.94	9.57	9.66	9.71	9.34	—	n.t.
NEL from gas, MJ/kg DM	5.75	5.48	5.57	5.56	5.31	—	n.t.

MBP – microbial biomass production, PF – partitioning factor, ME – metabolizable energy, NEL – net energy for lactation, DM – dry matter, SD – standard deviation, L1–L5 – production lots of traditionally water-debittered *L. albus* grain: L1 – Hüyük (Konya), L2 – Seydişehir (Konya), L3 – Doğanhisar (Konya), L4 – Şarkikaraağaç (Isparta), L5 – Iğın (Konya). Means in a row with different superscripts are significantly different (Bonferroni-adjusted $P < 0.05$). MBP – stoichiometric microbial biomass production (Blümmel et al., 1997); PF – partitioning factor (MBP/gas). SEM – standard error of the mean; * $P < 0.05$ means that the data is significantly different; ns – not significant. ME and NEL were calculated from 24 h net gas production using the Menke and Steingass (1988) equations (Equations 5 and 6) and, like the chemical-composition energy estimates in Table 1, were not subjected to inferential testing (n.t.); VF – asymptotic gas volume from the logistic fit. Gas volumes are expressed per 460 mg DM; for the energy equations they were normalised to the 200 mg DM basis on which those equations were calibrated; n.t. – not tested

Table 8. *In vitro* fermentation parameters at 24 h for *Lupinus albus* grain (VFA-related variables n = 9 per lot; NH₃-N n = 2; pH n = 9)

Parameter	L1	L2	L3	L4	L5	SEM	P-value
pH (HI 8314)	7.172 ± 0.296	7.067 ± 0.063	7.072 ± 0.192	7.218 ± 0.260	7.117 ± 0.320	0.036	0.636 ^{ns}
NH ₃ -N 24 h, mM	9.20 ± 0.93	10.01 ± 0.72	9.93 ± 0.33	9.89 ± 0.12	9.83 ± 1.20	0.205	0.828 ^{ns}
Acetic (C2), mol%	58.23 ± 2.59	57.47 ± 0.92	55.85 ± 1.21	56.34 ± 1.36	56.75 ± 4.22	0.363	0.208 ^{ns}
Propionic (C3), mol%	26.44 ± 1.08	26.98 ± 0.74	26.32 ± 1.39	26.15 ± 1.10	27.14 ± 1.54	0.180	0.308 ^{ns}
Butyric (C4), mol%	9.87 ± 4.94 ^{ab}	11.70 ± 0.61 ^b	14.24 ± 0.92 ^a	13.94 ± 0.85 ^a	12.53 ± 5.49 ^{ab}	0.546	0.046*
(C2 + C4)/C3	2.54 ± 0.10	2.57 ± 0.10	2.67 ± 0.19	2.69 ± 0.15	2.56 ± 0.19	0.024	0.367 ^{ns}
Total VFA, mmol/l	131.63 ± 11.10	125.23 ± 10.27	127.59 ± 1.57	125.00 ± 3.92	127.62 ± 11.59	1.292	0.439 ^{ns}
Predicted CH ₄ , ml/460 mg DM	17.92 ± 3.50	17.73 ± 1.72	18.52 ± 0.74	18.22 ± 0.71	18.15 ± 2.16	0.294	0.932 ^{ns}

NH₃-N – ammonia nitrogen, VFA – volatile fatty acids, GC-FID – gas chromatography with flame ionisation detection, C2 – acetate, C3 – propionate, C4 – butyrate, C5 – valerate, CH₄ – methane, DM – dry matter; L1–L5 – production lots of traditionally water-debittered *L. albus* grain: L1 – Hüyük (Konya), L2 – Seydişehir (Konya), L3 – Doğanhisar (Konya), L4 – Şarkikaraağaç (Isparta), L5 – Iğın (Konya); SD – standard deviation; SEM – standard error of the mean; * $P < 0.05$; ns – not significant. Values are means ± SD. Means in a row with different superscripts differ (Bonferroni-adjusted $P < 0.05$); for butyric acid, the L2↔L3 and L2↔L4 contrasts survived Bonferroni adjustment. pH measured with Hanna HI 8314 (n = 9). NH₃-N by the phenol–hypochlorite method of Weatherburn (1967) at 625 nm (n = 2); the Satter and Slyter (1974) threshold for microbial protein synthesis is approximately 3.5 mM. VFA measured by GC-FID (Agilent 6890N; DB-FFAP capillary column, 30 m × 320 µm ×

1.00 μm) after deproteinisation with meta-phosphoric acid ($n = 9$). Predicted methane (Wolin, 1960): $p\text{CH}_4 \propto (0.5 \times \text{C}_2 - 0.25 \times \text{C}_3 + 0.5 \times \text{C}_4 - 0.25 \times \text{C}_5) \times \text{total VFA}$, with C_5 calculated as $100 - (\text{C}_2 + \text{C}_3 + \text{C}_4)$

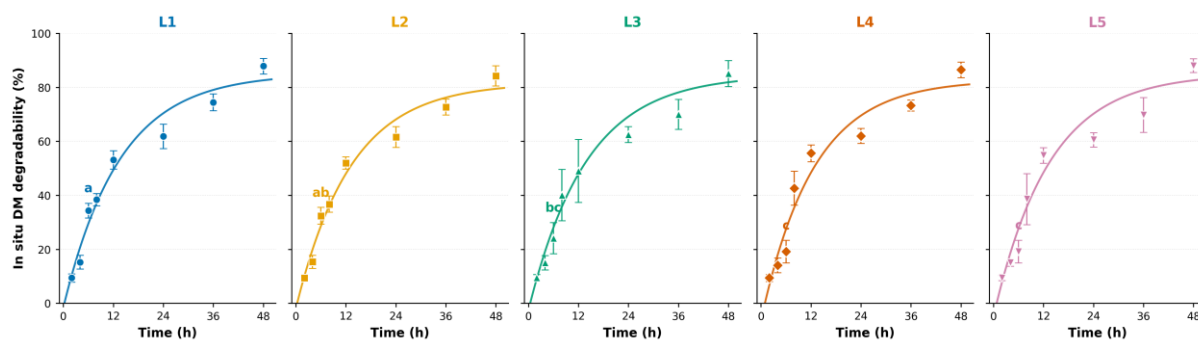


Figure 1. Fitted *in situ* DM degradability curves (Ørskov–McDonald model) for debittered *Lupinus albus* from five lots (one panel per lot)

DM – dry matter; L1–L5 – production lots of traditionally water-debittered *L. albus* grain: L1 – Hüyük (Konya), L2 – Seydişehir (Konya), L3 – Doğanhisar (Konya), L4 – Şarkikaraağaç (Isparta), L5 – Ilgın (Konya); data points are means $\pm$ SD ($n = 6$), SD – standard deviation. Letters at the 6 h time point denote Bonferroni-adjusted lot differences ($P < 0.05$)

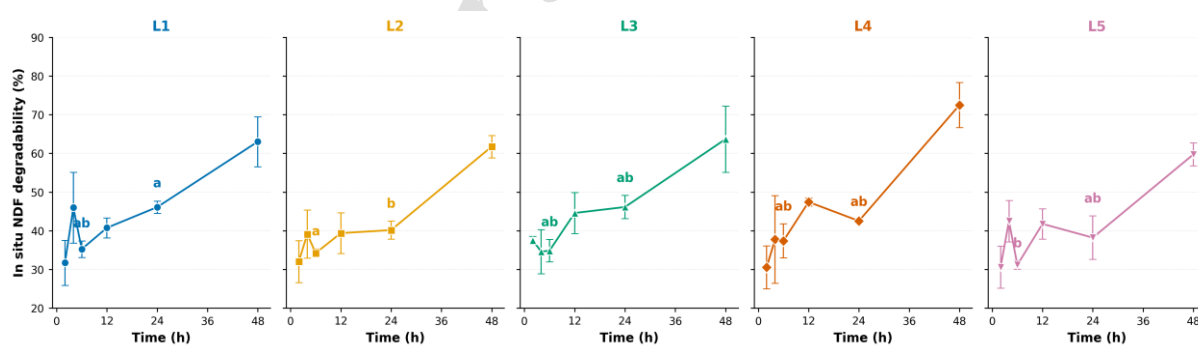


Figure 2. *In situ* NDF degradability curves for *Lupinus albus* from five lots (one panel per lot; 8 h and 36 h time points excluded)

L1–L5 – production lots of traditionally water-debittered *L. albus* grain: L1 – Hüyük (Konya), L2 – Seydişehir (Konya), L3 – Doğanhisar (Konya), L4 – Şarkikaraağaç (Isparta), L5 – Ilgın (Konya); data points are means $\pm$ SD ($n = 6$), SD – standard deviation. Letters at 6 h and 24 h denote Bonferroni-adjusted lot differences ($P < 0.05$)

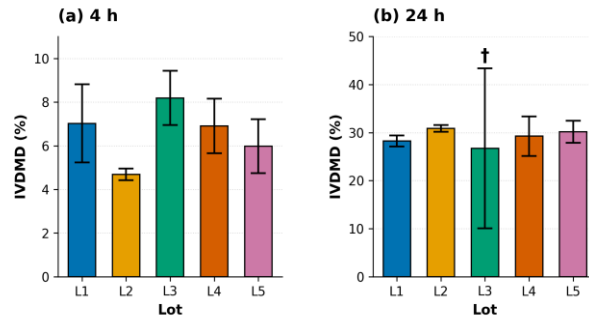


Figure 3. *In vitro* dry matter digestibility (IVDMD, %) of *Lupinus albus* grain from five lots at (a) 4 h and (b) 24 h, the two incubation times at which replicate dispersion was acceptable in all five lots

L1–L5 – production lots of traditionally water-debittered *L. albus* grain: L1 – Hüyük (Konya), L2 – Seydişehir (Konya), L3 – Doğanhisar (Konya), L4 – Şarkikaraağaç (Isparta), L5 – Ilgın (Konya); bars are means $\pm$ SD ($n = 3$); SD – standard deviation.

No lot effect at either time ($P = 0.059$ at 4 h, $P = 0.967$ at 24 h); † L3 at 24 h retains a coefficient of variation of 62%

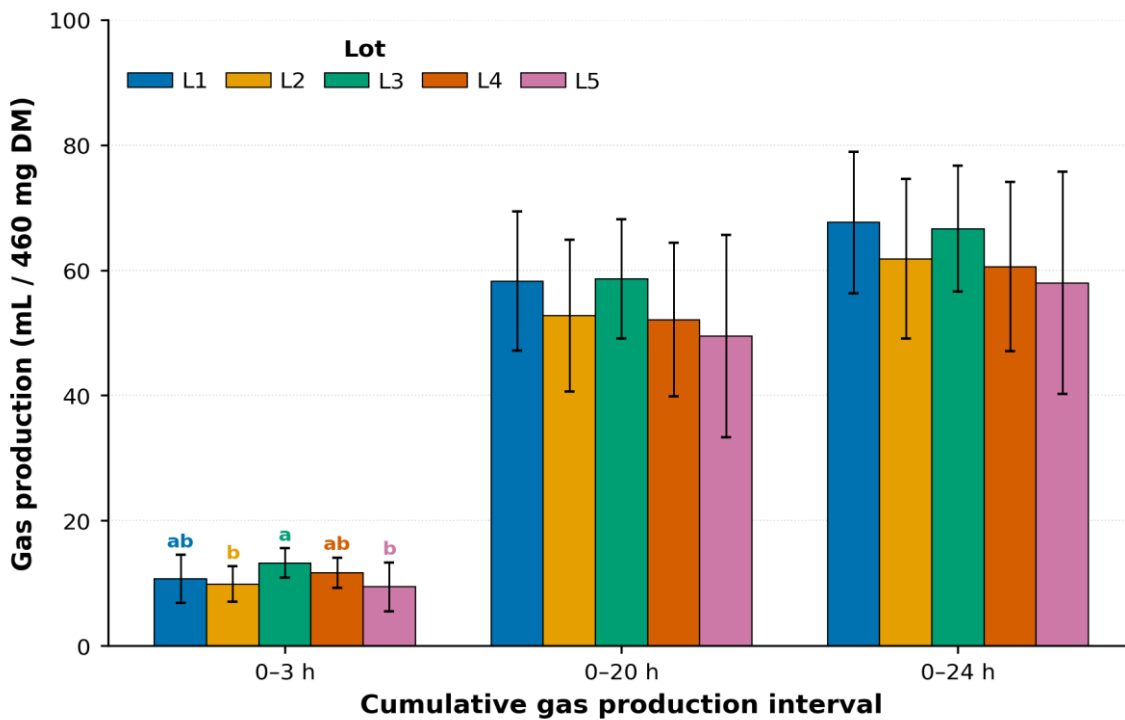


Figure 4. *In vitro* gas production from *Lupinus albus* grain at 0–3, 20 and 24 h (mean $\pm$ SD, $n = 14$)

L1–L5 – production lots of traditionally water-debittered *L. albus* grain: L1 – Hüyük (Konya), L2 – Seydişehir (Konya), L3 – Doğanhisar (Konya), L4 – Şarkikaraağaç (Isparta), L5 – Ilgın (Konya); DM – dry matter; SD – standard deviation; ^{ab} – means within a bar with different superscripts are significantly different at $P < 0.05$

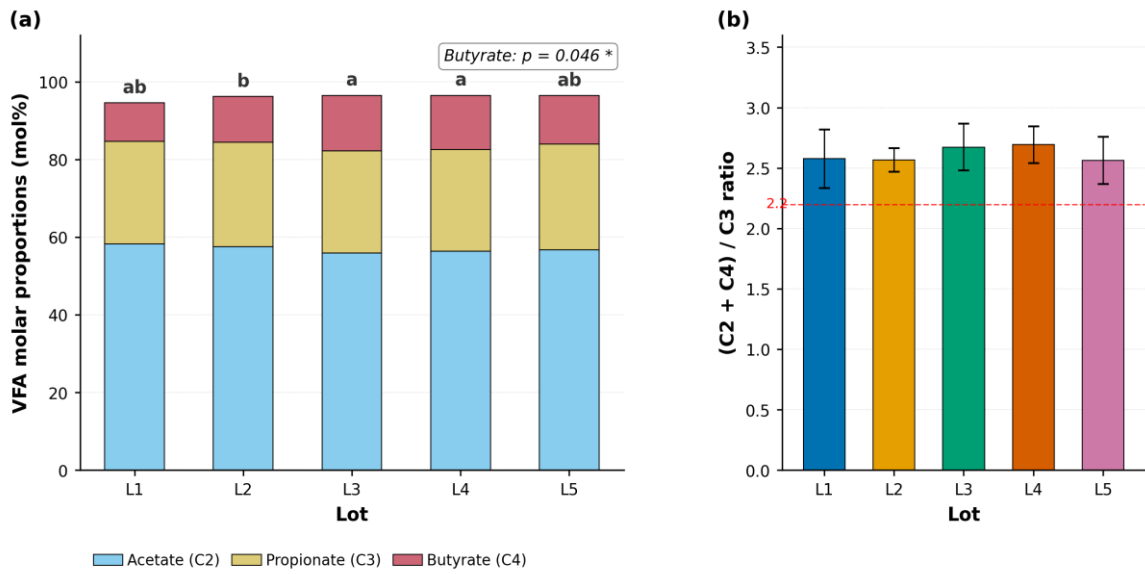


Figure 5. Volatile fatty acids (VFA) profile of *in vitro* fermentation of *Lupinus albus*: (a) molar proportions of acetate (C2), propionate (C3) and butyrate (C4); letters above bars indicate Bonferroni-adjusted lot effect for butyrate ($P = 0.046$). (b) (C2 + C4)/C3 ratio at 24 h; the dashed line marks the 2.2 reference value reported for lactating cows fed complete rations (Yang and Beauchemin, 2007), shown for orientation only and not as a threshold applicable to single-substrate batch incubations. Means $\pm$ SD, $n = 9$ per lot. SD – standard deviation. ^{ab} – means within a bar with different superscripts are significantly different at $P < 0.05$; C2 – acetate, C3 – propionate, C4 – butyrate; L1–L5 – production lots of traditionally water-debittered *L. albus* grain: L1 – Hüyük (Konya), L2 – Seydişehir (Konya), L3 – Doğanhisar (Konya), L4 – Şarkikaraağaç (Isparta), L5 – Ilgın (Konya)

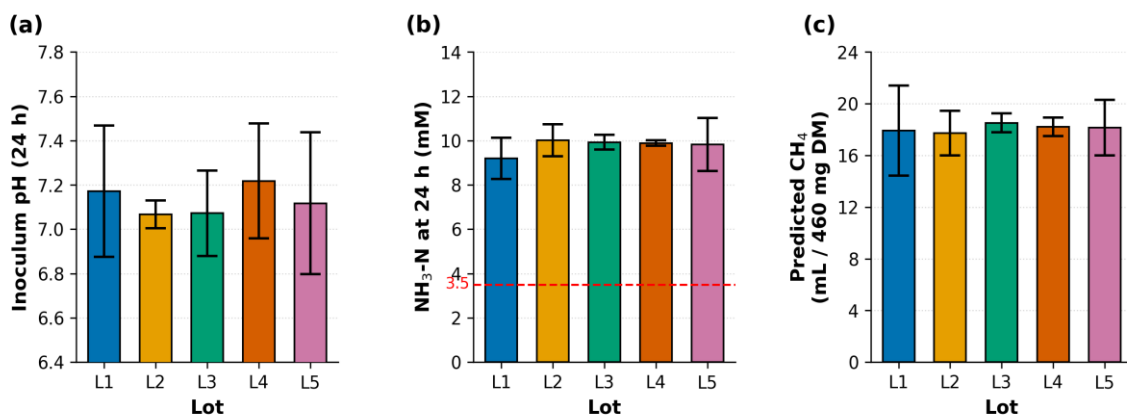


Figure 6. *In vitro* fermentation parameters: (a) pH; (b) NH₃-N at 24 h, mM; (c) predicted methane, ml/460 mg DM

DM – dry matter. Dashed lines indicate published reference thresholds (3.5 mM for microbial protein synthesis (Satter and Slyter, 1974)). L1–L5 – production lots of traditionally water-debittered *L. albus* grain: L1 – Hüyük (Konya), L2 – Seydişehir (Konya), L3 – Doğanhisar (Konya), L4 – Şarkikaraağaç (Isparta), L5 – Ilgın (Konya)