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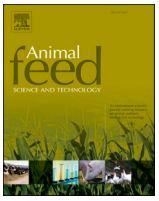

Sainfoin can be included up to 40% in the concentrate of finishing lambs without impairing their performance, rumen fermentation, and carcass quality

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ABSTRACT

Sainfoin (*Onobrychis viciifolia*) is an excellent forage legume to be included in sheep diets as fresh forage, but its inclusion in concentrates fed to indoor lambs reared has been scarcely studied. This study evaluated the effects of including different levels of dehydrated sainfoin in the concentrates fed to light lambs during the finishing period on animal performance, ruminal fermentation, and carcass traits. Twenty–six weaned male Rasa Aragonesa lambs (14.0 ± 0.49 kg body weight) were randomly grouped and individually fed *ad libitum* with isoproteic and isoenergetic pelleted concentrates containing 0% (0SF; n=9), 20% (20SF; n=9) or 40% sainfoin (40SF; n=8) for 40 days, from weaning to slaughter. In addition, an *in vitro* assay was carried out to evaluate the concentrates. The 40SF lambs had a higher dry matter intake ($P < 0.01$) and tended to show an improvement in average daily gain ($P < 0.10$). The diet had no effect on carcass weight, dressing percentage, *rectus abdominis* color or subcutaneous caudal fat color ($P > 0.05$). Regarding the rumen study, the diet did not affect most ruminal fermentation parameters ($P > 0.05$), except for pH, which was greater in 40SF lambs than in 20SF lambs ($P < 0.05$), and the proportion of acetic acid and the acetic:propionic ratio, both of which were higher in 40SF and 20SF lambs than in 0SF lambs ($P < 0.01$). The results from the *in vitro* assay showed that the 40SF diet decreased the *in vitro* dry matter degradability, increased propionic, and decreased butyric proportion compared to 0SF concentrate ($P < 0.05$), but no effect was obtained for gas, methane, total volatile fatty acids, and ammonia formation among diets ($P > 0.05$). The lack of detrimental effects on lamb performance and carcass traits suggests that the inclusion of up to 40% sainfoin in the concentrate of light lambs reared indoors would be advisable to promote the use of local forages.

Abbreviations: A, potential gas production; ABTS, 2,2-azinobis-3- ethylbensothiazoline-6-sulfonic acid; ADFom, acid detergent fiber exclusive of residual ash; ADG, average daily gain; BW, body weight; *c*, rate of gas production; C_2:C_3, acetic:propionic acid ratio; CCW, cold carcass weight; CH_4, methane; DM, dry matter; DMI, dry matter intake; FID, flame ionization detector; HCW, hot carcass weight; IVDMD, *in vitro* dry matter digestibility; Lignin sa, lignin determined by solubilization of cellulose with sulfuric acid; MDA, malondialdehyde; NEFA, nonesterified fatty acids; NDFom, neutral detergent fiber exclusive of residual ash; NH_3-N, ammonia; OM, organic matter; *P*, cumulative gas production; PAC, proanthocyanidins; S.e. m., standard error of the mean; VFA, volatile fatty acids.

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1. Introduction

Lamb production in Mediterranean regions, particularly Southern Europe, is predominantly based on intensive systems. Light lambs, weighing between 22 and 28 kg at slaughter, are reared indoors without grazing and fed *ad libitum* on cereal-based concentrate plus straw. This is done to ensure a uniform product and lamb size growth. However, this intensive system is facing some socio–economic challenges which are pushing towards some changes in production models. Global economic instability is forcing farmers to advocate for a system of local sourcing that provides greater self–sufficiency and less environmental impact, making it a good alternative to the growing concern about the contribution of livestock farming to climate change (IPCC, 2022). Besides, there is also an increasing demand for healthier products by consumers, which is one of the most important current goals in animal production.

The inclusion of locally–produced forages has been widely studied as one of the strategies to simultaneously achieve greater sustainability and self–sufficiency and provide added value and higher quality to edible ruminant products (Buccioni et al., 2015; Huyen et al., 2020; Moorby and Fraser, 2021; Santos-Silva et al., 2023). In this sense, sainfoin (*Onobrichis viciifolia*) is a rustic forage legume, well adapted to cold and water scarcity, with high yields and quality in the first spring cut. All these characteristics along with the need to preserve the excess of production made this crop an attractive ingredient to be introduced in the concentrate of lambs reared under intensive systems.

Compared to lambs fed cereal-concentrate, it is known that the inclusion of forages in lamb diets can reduce the carcass fatness, which could be detrimental to meat quality (Priolo et al., 2002). In addition, meat and carcass color can also be affected by some secondary compounds found in forages at different extent depending on the tissue and the type of forage (Ponnampalam et al., 2017), which could lead to rejection by consumers. However, when the forage is preserved, the content of those secondary compounds can be reduced (Rufino-Moya et al., 2022), decreasing their potential effect on carcass color. In view of the above, we hypothesize that the dehydrated sainfoin could be a good alternative for intensive light lamb production. Therefore, the objective of the present study was to evaluate the effects of the inclusion of dehydrated sainfoin in the pelleted concentrate (0%, 20%, and 40% of sainfoin) fed to light lambs during the finishing period on ruminal fermentation, performance, metabolic, antioxidant and blood status, and carcass traits of light lambs.

Table 1
Ingredients, chemical composition, total carotenoids, polyphenols, proanthocyanidins (PAC), and their fractions of the diets[a].

	0SF	20SF	40SF
n	18	18	18
Dry matter (DM) (g/kg)	905	904	903
Ingredients (g/kg DM)			
Barley	310	252	50
Corn	250	189	250
Wheat	50	50	102
Gluten feed	60	60	130
Soybean meal 47%	173	138	159
Bran	25	81	0
Palm oil	10	10	15
Calcium carbonate	15	13	4
Sodium chloride	5	5	5
Premix vitamin 0.2%	2	2	2
Sainfoin pellet	0	200	400
Straw	100	0	0
Chemical composition[b]			
Crude protein (g/kg DM)	174 ± 4.3	175 ± 6.5	173 ± 5.2
Ether extract (g/kg DM)	32.6 ± 3.25	35.7 ± 3.60	38.0 ± 3.44
Ash (g/kg DM)	75.2 ± 2.51	70.5 ± 2.06	78.5 ± 5.48
Starch (g/kg DM)	426 ± 6.9	360 ± 13.8	296 ± 9.6
Neutral detergent fiber (g/kg DM)	263 ± 20.9	292 ± 12.1	355 ± 16.4
Acid detergent fiber (g/kg DM)	129 ± 9.1	168 ± 6.5	249 ± 10.4
Lignin (sa) (g/kg DM)	17.0 ± 3.25	34.2 ± 3.17	59.6 ± 4.37
Gross energy (MJ/kg DM)	18.1 ± 1.37	18.4 ± 1.25	18.4 ± 0.94
Carotenoids (mg/kg DM)	7.72 ± 1.044	17.3 ± 1.355	29.9 ± 3.355
Polyphenols[c]	7.85 ± 0.710	12.07 ± 0.586	16.83 ± 0.960
Proanthocyanidins (PAC)[d]			
Total PAC	1.32 ± 0.527	3.04 ± 0.448	5.23 ± 0.550
Extractable PAC	0.41 ± 0.152	0.50 ± 0.169	0.75 ± 0.132
Protein-bound PAC	0.77 ± 0.529	2.07 ± 0.373	3.67 ± 0.508
Fiber-bound PAC	0.15 ± 0.115	0.47 ± 0.138	0.80 ± 0.118

[a] 0SF- 0% sainfoin; 20SF- 20% sainfoin; 40SF- 40% sainfoin in the diet.
[b] mean ± standard deviation
[c] g eq. tannic acid/kg DM
[d] g eq. sainfoin PAC/kg DM

2. Material and methods

All the experimental procedures were accomplished according to the international guidelines of the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for experimental purposes and were supervised and approved by the Animal Ethics Committee of the Centro de Investigación y Tecnología Agroalimentaria (CITA) de Aragón (CEEA, 2017–07).

2.1. Animal management and experimental design

The studies were carried out at the facilities of the CITA in Zaragoza (Spain, 41°43' N, 0°47' W; 216 m above sea level). Concentrates with different inclusion of sainfoin: a commercial cereal–based concentrate without sainfoin (0SF), concentrate with 20% sainfoin (20SF) and with 40% sainfoin (40SF) were evaluated by *in vivo* and *in vitro* assays. The sainfoin used was cut at flowering stage in the spring and pelleted. Concerning the pelleting process, the sainfoin was first dried few seconds by indirect contact with hot air (300–400 °C), leaving the drier at about 70 °C. The forage was then cooled, crushed, ground, and granulated by mechanical pressure through a sieve at 60–70 °C. The chemical composition of sainfoin dehydrated pellets included in the concentrate can be found in Supplementary Table 1. All the ingredients of the concentrates were mixed and pelleted (3.5 mm-diameter) to avoid selection and were formulated to be isoenergetic and isoproteic (Table 1). The increased level of sainfoin inclusion was counterbalanced by decreases in barley contents and increases in wheat and gluten feed to meet the condition of isoproteic and isoenergetic among the three concentrates.

Twenty–six male lambs were selected from the experimental flock of Rasa Aragonesa breed, reared with their dams and managed identically until weaning. Before weaning, lambs had free access to a commercial starter concentrate to promote concentrate consumption and facilitate weaning. After, the lambs were randomly distributed into three groups balanced for age (30.0 ± 1.99 d) and body weight (14.0 ± 0.49 kg BW). The lambs were individually housed indoors with free access to concentrate, water, and mineral blocks. Each group received for 40 days one of the three concentrates (0SF, 20SF, and 40SF). Concentrates were offered at +15% of the previous day's refusal to allow ad libitum feeding. The concentrate offered and refused was recorded daily per lamb to calculate the individual DMI. Samples were taken daily from each concentrate to obtain a weekly composite sample for chemical composition. Lambs were weighed once a week at 8:00 am using an electronic scale (0.1 kg precision) and the average daily gain (ADG) was calculated. Blood samples from jugular vein were obtained fortnightly (weeks 0, 2, 4, and 6) into heparin tubes (Vaccuette, Madrid, Spain), immediately centrifuged (3000 g for 15 min at 4 °C) and stored at −20 °C until metabolite analyses.

2.2. Slaughter procedures

After 40 days, lambs were slaughtered without prior fasting in the experimental abattoir of the CITA Research Centre, in accordance with Council Regulation (EC) Nº 1099/2009. To avoid further stress, the animals were kept in the experimental cages until slaughter and taken to the slaughterhouse in groups of 4–5 animals. The ruminal contents were extracted and filtered through a double cheesecloth before being stored in sterile jars. Immediately, the pH of the ruminal liquid was measured using a micropH 2002 pH meter (Crison Instruments S.A., Barcelona, Spain). Then, 2.5 mL of the ruminal liquid was mixed with 2.5 mL HCl 0.1 N to analyze the ammonia (NH_3-N) and 0.5 mL of the liquid was added to 0.5 mL of deproteinizing solution and 1 mL of distilled water to analyze volatile fatty acids (VFA). Both dilutions were stored at −20 °C until NH_3-N and VFA determinations. The rumens were then thoroughly cleaned and the color was measured on the inner side (in contact with the ruminal papillae) of the ventral sac using a Minolta CM−2006d spectrophotometer (Konica Minolta Holdings, Inc., Osaka, Japan).

Carcasses were weighed without head and offal to obtain the hot carcass weight (HCW) and, after chilling at 4 °C for 24 h, the cold carcass weight (CCW) was recorded. These data were used to calculate the dressing percentage (HCW × 100/slaughter weight) and the carcass shrinkage [(HCW−CCW) × 100/HCW]. The fatness degree of the carcasses was then scored following the Community Scale for the Classification of Carcasses of Ovine Animals (EC, 1249/2008): from 1 (low) to 5 (very high). Carcass color was measured at the subcutaneous caudal fat at the tail root and at the *rectus abdominis* muscle using a Minolta CM−2006d spectrophotometer (Konica Minolta Holdings, Inc., Osaka, Japan). The absolute value of the sum of the translated spectrum (SUM), used as an estimator of carotenoid content, was calculated following an equation based on reflectance values, as explained in Prache and Theriez (1999). Finally, the perirenal fat deposits were extracted and weighed with an electronic scale (0.1 g precision).

2.3. In vitro fermentation assay

To evaluate the ruminal degradability and fermentation, four Rasa Aragonesa wethers (65 ± 2,1 kg BW), used as donors of rumen inoculum, were individually fed twice a day with 700 g of alfalfa hay and 300 g of barley, resulting in an average concentrate to forage proportion in the diet of 70:30. For three consecutive weeks, rumen fluid was collected from wethers before morning feeding into a pre-warmed (39 °C) insulated thermos and transported to the laboratory, which was located next to the animal facilities. Rumen digesta was individually strained through four layers of cheesecloth and homogenized. Rumen fluid was mixed and a buffer solution was added based on the protocol of Menke and Steingass (1988) in a proportion of 1:2 (v:v) as detailly reported in Rufino-Moya et al. (2019). Samples of the three concentrates (500 mg) were incubated in triplicate in 60 mL of rumen fluid:buffer solution in each of the three runs conducted. Gas production was determined with the Ankom system (Ankom Technology, Macedon, NY, USA) and, at 48 h of incubation, the bottles were placed for 5–10 min in ice to stop fermentation and then tempered at room temperature (10–15 min). A sample of gas was collected from each bottle at atmospheric pressure with a syringe attached to a manometer and introduced to a

Vacutainer® tube and conserved at 4 ºC until CH_4 determination. The pH of the fermentation liquid was measured with a micropH 2002 pH meter (Crison Instruments S.A, Barcelona, Spain). The entire bottle content was filtered through a preweighed bag (50 μm; Ankom Technology, Macedon, NY, USA) to estimate the *in vitro* dry matter degradability (IVDMD).

2.4. Chemical analyses

2.4.1. Feedstuffs

All analyses of the chemical composition of the concentrates were performed in duplicate. Dry matter (DM) and ash contents were analyzed in oven–dried samples, and crude protein content was determined by the Dumas Procedure using a nitrogen analyzer (Model NA 2100, CE Instruments, Thermoquest SA, Barcelona, Spain) according to the AOAC methods (AOAC, 2000). The total starch of the concentrates was measured using the commercial kit K–TSTA–100 A (Neogen Corporation, Lansing, MI, USA) following the amyloglucosidase/α–amylase method (AOAC, 2000). Neutral detergent fiber (NDFom), acid detergent fiber (ADFom), and lignin contents of concentrates were analyzed following the sequential procedure of Mertens (2002) using the Ankom 200/220 fiber analyzer (Ankom Technology Corporation, Fairport, NY, USA). The NDFom was assayed with a heat stable amylase, while lignin was analyzed in ADFom residues by solubilization of cellulose with sulfuric acid (lignin (sa)). All values were corrected for ash–free content. Ether extract was determined using an Ankom XT10 extractor (Ankom Technology Corporation, Fairport, NY, USA) following the Ankom procedure (AOCS, 2005). The gross energy content was calculated through the combustion–specific heat obtained with a calorimetric bomb (Model Parr 1341 Plain Jacket Bomb Calorimeter, Parr Instrument Company, IL, USA). The total carotenoid content of the concentrates was analyzed as described in Blanco et al. (2019). The detailed procedure for the analysis of total polyphenols and extractable, protein–bound, and fiber–bound proanthocyanidins (PAC) of the concentrates can be found in Baila et al. (2022).

2.4.2. Plasma

Plasma concentrations of glucose and urea were analyzed by a kinetic method using an automatic analyzer (GernonStar, RAL/TRANSASIA, Dabhel, India), whereas non–esterified fatty acids (NEFA) concentrations were determined by an enzymatic method using a commercial kit (Randox Laboratories Ltd., Crumlin Co., Antrim, UK). The concentration of polyphenols was obtained using a 1:25 (plasma: milli–Q water) dilution and following the method of Leal et al. (2019). Plasma 2,2–azinobis–(3–ethylbensothiazoline)–6–sulfonic acid (ABTS) was studied as indicator of antioxidant activity, while lipid oxidation was determined through the determination of malondialdehyde (MDA). The method followed to analyze ABTS was based on Jiménez-Escrig et al. (2003) and the total MDA was determined as described in Bertolín et al. (2019).

2.4.3. Parameters and end products of fermentation

To study the *in vitro* curve of fermentation, gas production of 500 mg of concentrate samples was recorded hourly for 48 h using the Ankom system. The gas produced in batch cultures was adjusted to the model described by France et al. (1993): $P = A\ (1 - e^{-ct})$, where P is the cumulative gas production (mL) at time (h), A is the potential of gas production (mL), and c is the rate of gas production (h^{-1}).

The CH_4 was determined through an Agilent 7890B gas chromatograph (Agilent Technology, California-USA) with PAL3 autosampler, flame ionization detector (FID), and equipped with HPPlot Q column (15 m × 320 μm × 20 μm, Agilent Technology, California-USA), using the helium as carrier gas (5.6 mL × min^{-1}). The temperature was set at 40ºC for the injector and oven and 350 ºC for the detector. The injection volume was 300 μL. Methane identification was based on the retention time relative to the standard and methane production was calculated by the model proposed by Cattani et al. (2016) for the Ankom Gas Production System:

$$CH_4 = -0.0064\ x\ [CH_{4\ in\ \ the\ \ head\ \ space}\ \ x\ \ (head\ \ space\ \ volume + Gas\ \ \ Production)]^2$$
$$+ 0.9835\ x\ [CH_{4\ in\ \ the\ \ head\ \ space}\ \ x\ \ (head\ \ space\ \ volume + Gas\ \ \ Production)]$$

The content of NH_3-N in the ruminal fluid was assessed using the Berthelot reaction (Chaney and Marbach, 1962) and its determination was performed with a colorimetric method at 625 nm in an Epoch Microplate Spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA). The concentrations of VFA were determined using a Bruker Scion 460 gas chromatograph (Bruker, Billerica, MA, USA) equipped with a CP–8400 autosampler, flame ionization detection, and a BR–SWax capillary column (30 m × 0.25 mm ID × 0.25 μm film thickness, Bruker, Billerica, MA, USA).

2.5. Statistical analyses

Data were analyzed using SAS statistical software (v.9.3, SAS Inst. Inc., Cary, NC, USA).

The lamb was considered as the experimental unit. The DMI, BW, ADG, carcass traits, and rumen fermentation parameters were analyzed by a general linear model (GLM procedure) variance analysis with the diet (0SF, 20SF, and 40SF) as fixed effect. Plasma parameters were analyzed with mixed models (MIXED procedure) for repeated measures with the diet (0SF, 20SF, and 40SF), week (0, 2, 4, and 6), and their interaction as fixed effects and the lamb as random effect. The data from the first day of the experimental period were abnormal due to the adaptation of the animals, so they were discarded.

The statistical analyses made for the parameters and end products of fermentation are extensively described in Lobón et al. (2022). All data obtained from the *in vitro* assay were analyzed using mixed models (MIXED procedure) considering the diet (0SF, 20SF, and 40SF) as fixed effect and the run as random effect. The parameters of the gas production curve (*A* and *c*) were estimated with non-linear regression models using the NLIN programme.

Degrees of freedom were adjusted using the Kenward–Rodger correction. Data were reported as least squares means and their associated standard errors of the mean (SEM). Tukey's correction was used for pairwise comparisons. Effects were considered significant at $P < 0.05$ and trends were discussed when $0.05 \leq P < 0.10$.

3. Results

The results concerning the acid profile of diets and plasma, rumen and meat of lambs have been previously published in Baila et al. (2023).

3.1. Lamb performance and plasma metabolites

The effect of the diet on performance is presented in Table 2. Lambs were slaughtered at 70.6 (±1.95) days of age and at 25.0 (±0.71) kg BW as average. The DMI of the lambs was affected by the diet ($P < 0.001$) with higher intake in lambs fed 40SF than their counterparts. However, there was no effect of the diet on ADG ($P > 0.05$).

Plasma concentrations of metabolites, polyphenols, antioxidant activity, and lipid oxidation are shown in Fig. 1. There was an interaction between the diet and the week on glucose ($P < 0.001$), NEFA ($P < 0.001$), and urea ($P < 0.05$) concentrations. Plasma glucose concentrations kept steady until week 4, but from this moment to the slaughter (week 6) it decreased in both diets with sainfoin, with lower glucose in 20SF compared to values of 0SF lambs ($P < 0.05$). Plasma concentrations of NEFA at the beginning of the experiment were lower in 0SF lambs than their counterparts ($P < 0.05$) but were similar among diets thereafter. The NEFA concentrations remained constant in 0SF lambs during the period studied ($P > 0.05$), whereas in 20SF and 40SF decreased from week 0–2 ($P < 0.05$), remaining steady the rest of period. Regarding plasma urea concentration, all diets showed a decrease from week 0 to week 2 ($P > 0.01$), and thereafter 20SF and 40SF lambs remained steady, while 0SF lambs increased until the end of the study. Despite these differences, no significant effect was observed due to the diet within each week ($P > 0.05$).

Regarding polyphenols content, antioxidant capacity (ABTS), and lipid oxidation, no interaction was observed between diet and week ($P > 0.05$). The plasma polyphenols content and the antioxidant activity were affected only by the week ($P < 0.001$), increasing as the period studied advanced. Lipid oxidation was affected by the diet ($P < 0.05$) and the week ($P < 0.001$). The average lipid oxidation was greater in 0SF than in 40SF lambs ($P < 0.05$) while 20SF lambs presented intermedium values (5.86 ± 0.096, 5.54 ± 0.102, and 5.46 ± 0.096, for 0SF, 20SF, and 40SF, respectively). Similarly to polyphenols content and antioxidant capacity, plasma lipid oxidation increases over time ($P < 0.001$).

3.2. Carcass traits

The diet did not affect any carcass traits ($P > 0.05$; Table 3) and either the main parameters determining carcass color measured in rectus abdominis muscle and subcutaneous caudal fat and the heme pigments studied in rectus abdominis muscle ($P > 0.05$; Table 3). However, a trend towards a greater deposition of perirenal fat was recorded in 40SF than in 0SF lambs ($P < 0.10$).

3.3. Rumen and fermentation parameters

The color of the ruminal epithelium and the fermentation parameters at slaughter are shown in Table 4. The diet only affected the redness of the ruminal epithelium ($P < 0.01$), ruminal pH ($P < 0.05$), proportion of acetic acid ($P < 0.01$), and acetic:propionic ratio ($P < 0.01$). The 40SF lambs presented higher redness of ruminal epithelium than their counterparts ($P < 0.05$) and greater pH values than 20SF lambs ($P < 0.05$). The proportion of acetic acid and acetic:propionic ratio were lower in 0SF than in both diets with sainfoin ($P < 0.05$), regardless of inclusion level. No effect of the diet was observed on NH_3–N, total VFA content, or individual proportions of VFA ($P > 0.05$).

The results concerning the *in vitro* fermentation assay are presented in Table 5, and the fermentation curve during the incubation is represented in *Fig. 2* . The diet did not have effect on the final pH, gas production curve, NH_3-N, and total VFA production ($P > 0.05$). The CH_4 production showed a tendency to be lower in the diet 40SF and greater in 20SF diet ($P < 0.10$). The IVDMD decreased with the inclusion of sainfoin, being lower in 40SF than 0SF ($P < 0.05$) and intermediate in 20SF diet. Regarding the individual VFA

Table 2
Effect of the diet[a] on the performance of the finishing lambs.

	0SF	20SF	40SF	s.e.m.[b]	*P*-value
n	9	8	9		
Dry matter intake (g/d)	741[b]	745[b]	895[a]	17.8	<0.001
Average daily gain (g/d)	281	281	333	11.3	0.09
Slaughter age (d)	70.0	70.8	71.0	1.95	0.54
Slaughter weight (kg)	24.9	23.9	26.2	0.71	0.10

Means with a or b letter differ at P < 0.05.
[a] 0SF- 0% sainfoin; 20SF- 20% sainfoin; 40SF- 40% sainfoin in the diet.
[b] Standard error of the mean

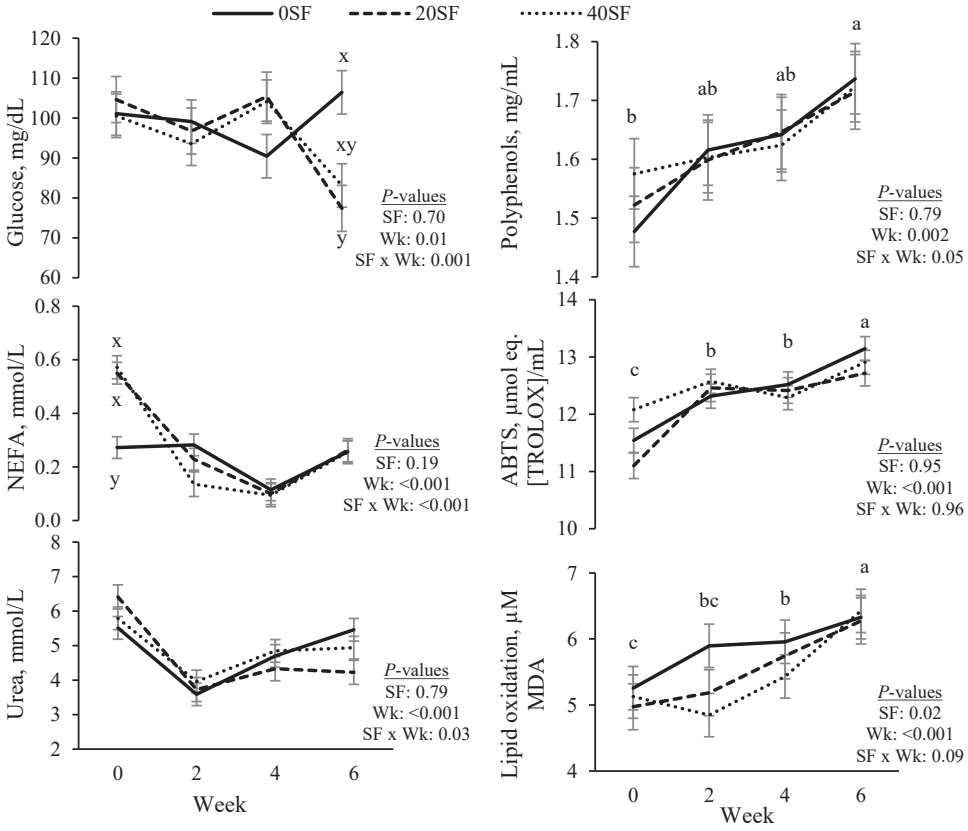

Fig. 1. Effect of the diet[1] (SF) and the week (Wk) on the concentrations on glucose, urea, and non-esterified fatty acid (NEFA), polyphenols, antioxidant activity [ABST: 2,2–azinobis–(3–ethylbensothiazoline)–6–sulfonic acid], and lipid oxidation, measured as malondialdehyde (MDA) in the plasma of the lambs. Means with a, b, or c letter differ at $P < 0.05$ among weeks. Means with x or y letter differ at $P < 0.05$ among diets within a week. [1]0SF- 0% sainfoin; 20SF- 20% sainfoin; 40SF- 40% sainfoin in the concentrate.

proportions, it was observed that 40SF presented greater propionic acid and lower butyric acid proportions than 0SF ($P < 0.05$), having the 20SF diet intermediate proportions.

4. Discussion

4.1. Lamb performance and plasma metabolites

The ADG of lambs was greater than 280 g in all diets, which is in concordance with the expected growth for male lambs of this breed (Ripoll et al., 2012; Lobón et al., 2020). Therefore, it can be stated that the lambs performed satisfactorily regardless of the diet.

The greater DMI recorded in the 40SF lambs was only reflected in a trend towards a greater ADG of these lambs. One possible explanation lies in the higher presence of PAC in 40SF diet, which increased with the inclusion of sainfoin in the concentrates, although the PAC content of sainfoin pellets was much lower than that of fresh sainfoin (Baila et al., 2022). In this line, some authors (Dey et al., 2008, Bonanno et al., 2011) have recorded higher intake and an improvement in the lambs' growth when lambs were fed diets including moderate to low concentrations of PAC (15 g/kg DM in and 20 g/kg DM, respectively), similar to those obtained in the present study. Therefore, we suggest that the absence of greater differences in the ADG values of 40SF compared to its counterparts must be due to the lower IVDMD observed in this diet in the *in vitro* assay caused by a higher fiber content in the diet, which can be explained by the lower degradability of the ration when the fibre content increases (Fimbres et al., 2002).

Regarding plasma metabolites, differences among treatments were found in plasma NEFA concentrations at the beginning of the study. This result could not be related to the diet, as the bleeding in week 0 took place just before the experiment started, and thus the lambs had not yet been fed the different concentrates. Those lipids act as an alternative pathway to glucose to provide energy when blood glucose decreases, but also can be raised under adrenaline releasing in response to stress (Stewart et al., 2007). Therefore, a possible explanation could be the bleeding time, as the lambs belonging to 0SF diet were the first bled at week 0 and the elapsed time may be too short to cause an increase of NEFA as an answer to stress. In contrast 20SF and 40SF lambs were bled after 0SF animals, spending longer time under stress condition, which could cause the differences in NEFA concentration between 0SF and the rest of diets. The type of diet did not have effect on glucose concentration during the study except at slaughtering, with differences between

Table 3
Effect of the diet[a] on the carcass traits and color of the finishing lambs.

	0SF	20SF	40SF	s.e.m.[b]	*P*-value
Carcass traits					
HCW[c] (kg)	12.3	12.0	13.3	0.46	0.16
CCW[d] (kg)	12.0	11.6	12.9	0.44	0.13
Dressing percentage[e] (%)	49.4	50.1	50.5	0.75	0.61
Carcass shrinkage[f] (%)	2.64	3.19	2.60	0.257	0.23
Fatness score[g]	2.11	2.29	2.22	0.092	0.39
Perirenal fat weight (g)	91	115	139	15.0	0.09
Rectus abdominis muscle					
Lightness	50.4	50.4	50.0	0.68	0.87
Redness	9.81	9.54	9.85	0.478	0.88
Yellowness	11.3	11.7	11.2	0.40	0.62
Hue angle	49.0	50.8	48.6	1.84	0.69
Chroma	15.0	15.2	15.1	0.40	0.91
Metmyoglobin	15.4	17.1	16.3	0.62	0.19
Oxymyoglobin	12.4	11.8	9.6	3.02	0.79
Deoxymyoglobin	72.2	71.1	75.1	2.91	0.60
Subcutaneous caudal fat					
Lightness	69.1	69.4	68.1	0.77	0.47
Redness	3.15	3.27	3.15	0.285	0.95
Yellowness	10.4	10.6	11.6	0.54	0.24
Hue angle	73.1	75.0	74.8	1.42	0.56
Chroma	10.9	11.1	12.1	0.57	0.29
SUM[h]	81.9	97.4	109.7	9.32	0.12

[a] 0SF- 0% sainfoin; 20SF- 20% sainfoin; 40SF- 40% sainfoin in the diet.
[b] Standard error of the mean
[c] HCW: Hot carcass weight
[d] CCW: Cold carcass weight
[e] HCW × 100/Slaughter weight
[f] (HCW – CCW) × 100/HCW
[g] Scale 1 (low) to 5 (high)
[h] Estimator of carotenoids as calculated in Prache and Theriez (1999).

Table 4
Effect of the diet[a] on the color of the ruminal epithelium, pH, ammonia (NH_3–N), and volatile fatty acids (VFA) of the rumen of the finishing lambs.

	0SF	20SF	40SF	s.e.m.[b]	*P*-value
Ruminal epithelium color					
Lightness	48	46	46	1.7	0.44
Redness	2.5[b]	2.6[b]	3.4[a]	0.19	0.005
Yellowness	7.0	6.7	7.5	0.72	0.71
Hue angle	69	67	65	2.4	0.52
Chroma	7.4	7.2	8.3	0.69	0.51
Ruminal fermentation parameters					
pH	5.9[ab]	5.7[b]	6.3[a]	0.16	0.03
NH_3–N (mg/L)	46	69	66	16.1	0.53
Total VFA (mmol/L)	95	96	96	13.3	0.99
Acetic acid (mmol/mol)	492[b]	606[a]	585[a]	2.2	0.003
Propionic acid (mmol/mol)	305	250	265	1.8	0.23
Butyric acid (mmol/mol)	154	102	109	2.1	0.49
Valeric acid (mmol/mol)	7.6	27	27	0.44	0.06
Iso-butyric acid (mmol/mol)	4.8	6.1	6.2	0.09	0.47
Iso-valeric acid (mmol/mol)	0.6	8.8	6.9	0.13	0.35
C_2:C_3 ratio (mmol/mol)	1.7[b]	2.6[a]	2.2[a]	0.18	0.002

Means with a or b letter differ at $P < 0.05$.
[a] 0SF- 0% sainfoin; 20SF- 20% sainfoin; 40SF- 40% sainfoin in the diet.
[b] Standard error of the mean

the 0SF and 20SF diets, and intermedium values in 40SF. At this sampling time, both diets with sainfoin showed low glucose concentration, outside of the normal range considered for Rasa Aragonesa lambs (87–122 mg/dl), according to Ramos et al. (1994). The 0SF diet was richer in starch whereas 20SF and 40SF had greater NDFom content. While the starch is efficiently and rapidly transformed into glucose, fiber needs to be transformed to VFA (Kaneko et al., 2008), which need to be converted to glucose, leading sometimes to a decrease in blood glucose levels (Farrer et al., 1995). Besides, it is known that glucose is an indicator of the liver's response to adrenaline during stress (Martin et al., 2011). Therefore, differences in glucose levels at slaughter could be the result of different stress responses among diets due to variations in starch and fiber contents among concentrates. Blood urea concentration was

Table 5
Effect of the diet[a] on gas production, *in vitro* dry matter digestibility (IVDMD), ammonia (NH3-N), methane (CH_4), and volatile fatty acids (VFA) after 48 h of incubation.

	0SF	20SF	40SF	s.e.m.[b]	*P*-value
Final pH	6.2	6.2	6.2	0.01	0.28
Gas production (mL/g DM)	312	336	311	11.2	0.30
Potential gas production (*A*) (mL)	141.3	148.9	137.3	5.85	0.44
Rate of gas production (*c*) (h^{-a})	0.10	0.10	0.10	0.007	0.73
Total CH_4 production (mL/g DM)	92.0	95.8	87.7	1.66	0.06
IVDMD (g/kg)	918[a]	882[ab]	857[b]	0.86	0.020
NH_3-N (mg/L)	518	524	555	26.8	0.61
Total VFA (mmol/L)	105	109	102	2.89	0.32
Acetic acid (mmol/mol)	603	604	607	0.2	0.49
Propionic acid (mmol/mol)	130[b]	131[ab]	136[a]	0.1	0.049
Butyric acid (mmol/mol)	187[a]	183[ab]	174[b]	0.2	0.04
Valeric acid (mmol/mol)	23	23	22	0.01	0.19
Iso-butyric acid (mmol/mol)	19	19	20	0.03	0.17
Iso-valeric acid (mmol/mol)	39	40	41	0.1	0.19
C_2:C_3 ratio (mmol/mol)	4.7	4.6	4.5	0.10	0.50

Means with a or b letter differ at $P < 0.05$.
[a] 0SF- 0% sainfoin; 20SF- 20% sainfoin; 40SF- 40% sainfoin in the concentrate.
[b] Standard error of the mean.

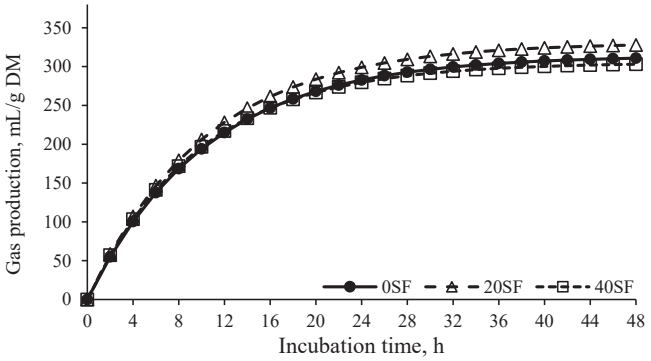

Fig. 2. Effect of the diet[1] on the fermentation curve during 48 h of *in vitro* incubation.[1]0SF- 0% sainfoin; 20SF- 20% sainfoin; 40SF- 40% sainfoin in the concentrate.

unaffected by the type of diet, which is related to compliance with the condition of isoproteic diets. A reduction in plasma urea has been reported in fresh sainfoin-fed ewes compared to those receiving sainfoin + PEG (a blocker of PAC), suggesting a reduced protein degradation due to the effect of PAC (Baila et al., 2022). The lack of effect in the current study, indicates that the PAC intake from sainfoin included in the concentrate was not enough to produce an effect on the protein metabolism.

In the same line, the plasma polyphenol concentration and antioxidant activity parameters were similar among diets, which is unexpected as sainfoin is known for its content of antioxidant compounds, including polyphenols, which should improve the antioxidant activity in lambs (Leal et al., 2019). This find could be related to the low content of antioxidant compounds in the pelleted sainfoin in this study, caused by its deterioration at high temperatures (Maillard and Berset, 1995) during the dehydration and pelleting processes.

4.2. Carcass traits

Forage diets are related to greater digestive development than rich–concentrate diets (Borton et al., 2005; Joy et al., 2008), therefore a decrease in dressing percentage would have been expected due to the sainfoin inclusion in the diet. However, as sainfoin was included in the concentrate, the forage particle size might be too small to be considered "physically effective fiber" (Banakar et al., 2018) and no effect was observed.

Carcass characteristics were similar among the diets, with no differences in carcass fatness degree, which is one of the major concerns of consumers (Bernués et al., 2012). Carcass color, measured in the *rectus abdominis* muscle and in the subcutaneous caudal fat, was also unaffected by the diet. Grazing systems lead to increase redness and chroma in *rectus abdominis* color (Carrasco et al., 2009; Ripoll et al., 2012) and yellowness values in subcutaneous caudal fat (Joy et al., 2008; Ripoll et al., 2008) compared to lambs concentrate–fed. These color changes are related to the deposition of carotenoids present in fresh forages but, when forages are preserved, the carotenoid content decreases considerably (Rufino-Moya et al., 2022). In the present study, sainfoin was dehydrated

and, despite the differences in carotenoid concentration among the diets, their presence was insufficient to induce significant changes on fat color. Besides, the color can also be affected by the presence of PAC in the diet, due to a delay in metmyoglobin formation leading to a lighter meat with an increase in color stability (Priolo et al., 2005; Luciano et al., 2011; Lobón et al., 2017). Nevertheless, herein, no effect on heme pigments formation in *rectus abdominis* was observed among diets. It is important to highlight that meat and fat color are one of the major characteristics that determine the purchase and, so, the lack of differences in these parameters in the present study confirms that the effect of the inclusion of sainfoin in the concentrate made it possible to produce homogeneous carcasses, as demanded by consumers.

4.3. Rumen and fermentation parameters

Previous research with Rasa Aragonesa lambs indicated that those grazing alfalfa had light brown rumen epithelium while concentrate–fed lambs had dark and grey epithelium (Álvarez-Rodríguez et al., 2012). Although the decrease in pH has been associated with darker rumen color (Álvarez-Rodríguez et al., 2012; Blanco et al., 2015), in the present study only a higher redness value in the ruminal epithelium was observed in 40SF lambs compared to 20SF and 0SF, suggesting that differences in the proportion of fiber or secondary compounds among concentrates may have been sufficient to cause this effect, but not on the rest of color parameters. In that sense, Blanco et al. (2015) observed higher redness value in ruminal epithelium of lambs fed alfalfa hay compared to lambs concentrate–fed with barley straw up to 25%. This suggests that the effect on rumen redness may be due to the deposition of some organic pigments (such as carotenoids) in the rumen wall, which were more abundant in the 40SF concentrate in the current experiment.

The values observed in ruminal pH agree with those recorded by Álvarez-Rodríguez et al. (2010), ranging from 5.5 in lambs fed concentrate (close to those obtained in 0SF and 20SF lambs) to 6.5 in lambs grazing alfalfa plus concentrate (similar to the pH of 40SF lambs). Higher values of pH improve the growth conditions for cellulolytic bacteria that need a ruminal pH range of 6.2–7.2 (Van Soest, 1994). The increase in ruminal pH, in turn, is related to the fiber content which is in line with the pH value of 40SF lambs, however, this result was not reflected in 20SF lambs, as they had lower fiber contents in the diet.

In ruminants, VFA are the main source of energy and NH_3-N reflects protein intake (Hatfield et al., 1998). In the present study no effect was observed in the total VFA and NH_3–N contents, reflecting similar energy and protein utilization, which is consistent with the results observed for plasma metabolites. The diet affected the proportion of acetic acid, which was increased with the inclusion of sainfoin in the concentrate, consequently increasing the acetic:propionic ratio. However, it must be taken into account that the inclusion of sainfoin in the concentrates led to some changes among the chemical composition of the diets, decreasing the starch content and increasing fiber fractions. Therefore, the differences found in acetic acid proportion could be explained by the greater NDFom and lower starch content in 40SF and 20SF diets, which favor the development of cellulolytic bacteria, responsible for the acetic acid production. The lack of effect of the diet on the ruminal NH_3–N content reflected the similar crude protein content of all diets, rather to the presence of sainfoin. Moreover, the presence of PAC can reduce ruminal NH_3–N concentrations by decreasing protein degradability (Frutos et al., 2004). In this line, a reduction in ruminal NH_3–N was observed in animals fed diets containing 20% pelleted sainfoin with 223 g of PAC/kg DM (Grosse Brinkhaus et al., 2016), contents 10 and 7 times greater than those recorded in the 20SF and 40SF diets of the present study, respectively. Thus, the lack of effect of PAC in the present study might be related to the low content of PAC in sainfoin concentrates which was not sufficient to modify NH_3–N concentrations, as confirmed by the similar plasma urea concentrations observed among diets.

4.4. In vitro fermentation trial

A high proportion of fiber in the diet increases gas production (Russell, 1998), while PAC is associated with a reduction in gas production (Waghorn, 2008). In the present study, the combination of both factors could counteract gas production, which would explain the lack of effect among diets. In this regard, previous studies have demonstrated the efficacy of sainfoin PAC in reducing *in vitro* gas production (Toral et al., 2016), which is desirable from an environmental standpoint and a growing concern within the industry. The trend towards lower CH_4 production from the diet with 40% sainfoin may be due to the fact that moderate condensed tannin content may have beneficial effects reducing rumen CH_4 emission production (Bodas et al., 2012).

As previously discussed, the 40SF diet led to a lower IVDMD. However, the reduction in IVDMD was not reflected in a decrease in VFA production, suggesting similar efficiency of the process on producing energy substrates, as VFA are the main energy source of ruminants (Hatfield et al., 1998). In the present study no effect was observed on NH_3–N contents, in agreement with the results observed in ruminal fluid of lambs, reflecting similar energy and protein utilization, which is in line with the results observed for plasma metabolites.

The finding in the *in vitro* assay of higher propionic acid proportions and the absence of an increase in the acetic acid in the 40SF diet was unexpected, since this diet presented lower starch content and higher fiber content than the 0SF, so it would expected to obtain the opposite result (Russell, 1998). Nevertheless, the lack of an increase in the proportion of acetic acid in the 40SF diet is consistent with the trend toward lower CH_4 production (Beauchemin et al., 2009). Besides, the proportions of VFA recorded in the ruminal fluid of lambs and *in vitro* assay did not followed the same pattern. However, this discrepancy was only observed in the proportions and not in the total and individual production of VFA, thus the effect on acetic an propionic between *in vitro* and ruminal fluid was diluted when the amount was studied.

5. Conclusions

Performance of finishing lambs fed 20% or 40% sainfoin included in pelleted concentrates was comparable to that of commercial concentrates without affecting carcass characteristics. Therefore, it can be concluded that the inclusion of up to 40% sainfoin in the concentrate of light lambs can be used without affecting their performance, ruminal fermentation, and carcass characteristics. However, to better understand the implications of the present study, it would be advisable to carry out a test under commercial conditions and to evaluate the effects of the diets on meat quality.

CRediT authorship contribution statement

Clàudia Baila Bigné: Writing – original draft, Formal analysis, Data curation. **Mireia Blanco:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Sandra Lobón:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Conceptualization. **Guillermo Ripoll:** Writing – review & editing, Data curation. **Isabel Casasús:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Margalida Joy:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of Competing Interest

None.

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Author declaration

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property. We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). She is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from mjoyt@ unizar.es

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.anifeedsci.2024.115975.

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