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Legume Grains as an Alternative to Soybean Meal in the Diet of Intensively Reared Dairy Ewes

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Abstract: Grain legumes are feedstuffs with high nutritional value that could replace soybean in dairy sheep nutrition. This could be beneficial in terms of economic efficiency and environmental sustainability. However, research regarding their potential effects on animal performance is scarce. The objective was to assess milk production and body condition score (BCS) of intensively reared dairy ewes after replacing soybean meal with a mixture of lupin, pea, vetch, and faba bean in their ration. A total of 40 Chios dairy ewes were randomly selected. They were allocated in two (n = 20) groups C and T; both were fed same amounts of Lucerne hay and wheat straw as well as a concentrate feed that was formulated with either inclusion of soybean meal for Group C or a mixture of legumes for Group T. Both feeds had equal energy and protein contents. Data collection was performed every 15 days for a 60-day period (a total of five measurements). In each measurement, ewe BCS was assessed, milk yield was recorded electronically, and individual milk samples were collected to assess chemical composition (fat, protein, lactose, and solids-non-fat content); energy corrected milk yield and milk components yield were calculated. Moreover, feed refusals from each group were weighed to calculate feed intake. Average individual daily concentrate feed intake was lower in Group T compared to C. Nutritional management did not significantly affect ($p > 0.05$) milk production and BCS. An exception was the last measurement where ewes in Group T had significantly ($p < 0.05$) higher milk protein, lactose and SNF yield, and lactose content. Results suggest that the studied legumes could replace soybean without compromising productivity of intensively reared dairy ewes.

Keywords: dairy ewes; legumes; soybean; milk production; body condition score

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1. Introduction

Dairy sheep nutritional management during the milking period is characterised by increased energy and protein requirements. In practice, protein-rich feedstuffs such as soybean and soybean meal (co-product of soybean oil extraction) are dominant in ration formulation. However, the costs and environmental impact of soybean usage in livestock are issues of debate [1,2].

The European Union's (EU) self-sufficiency in soybean production is approximately 5% and ca. 13 million tonnes of soybean are annually imported from Brazil, Argentina, and the USA; 28% of these imports are used for ruminant nutrition [3,4]. Soybean cultivation and trade have been associated with increased deforestation, soil erosion and water pollution [5,6]. At the same time, soybean is mainly produced from genetically modified plants that opposes consumer awareness and renders its use limited in organic farming systems [7]. Non-genetically modified soybean is scarce, and its price is very high (80–100 euro per tonne) [4].

Taking into consideration these issues, the EU is promoting the use of alternative protein sources [4]. Grain legumes such as lupin (*Lupinus albus*), pea (*Pisum sativum*), faba bean (*Vicia faba*) and vetch (*Vicia sativa*) are feedstuffs of high nutritional value that could be used as alternatives to substitute or replace soybean/soybean meal in dairy sheep nutrition without adverse effects on nutrient digestibility [8–11]. Such an approach can improve the overall sustainability of the agricultural sector. Specifically, growing legumes in rotation with crop cereals has been shown to improve soil fertility and reduce the need for chemical fertilisers due to the high nitrogen amounts and organic matter they provide. Moreover, legumes can break pest cycles, reduce greenhouse gas emissions (GHG), and increase crop yields [7,10,12,13]. Additionally, grain legumes present a high market potential. They are cultivated in all temperate regions of Europe [13] and their production has increased over the last decade [10,14]. Moreover, lower production inputs are needed, and higher yields can be achieved compared to soybean (by 1 to 3 tonnes per hectare) [13]. As a result, the cost for a mixture with grain legumes is lower than a mixture with soybean meal, especially given the current increase in the price of the latter [15]. Overall, using grain legumes to replace soybean in animal nutrition could help to increase environmental sustainability and resource use efficiency [15].

However, grain legumes have a relatively high content in antinutritional factors (phenols, tannins, condensed tannins, and alkaloids) and their use has raised concern regarding adverse effects on animal productivity [10,13,16]. Hence, it is important to ensure the safety of using legumes as alternative to soybean in dairy sheep nutrition. A plethora of studies in dairy cows have shown that lupin, pea and/or faba bean could substitute soybean without any effects on milk production or body condition score (BCS) [17–22]. Nevertheless, a negative effect on milk yield was reported when vetch was included in the diet of dairy cows [23]. Studies in sheep have mostly focused on meat production suggesting no adverse effects on growth, carcass characteristics and meat quality by replacing or substituting soybean and its derivatives with grain legumes [24–30]. However, relevant literature in dairy ewes is scarce. Studies so far have investigated the effects of replacing soybean or soybean meal with lupin, pea or faba bean on ewe milk yield and composition indicating no unfavourable associations [31–36]. To the best of our knowledge, there are no available studies regarding potential effects on BCS. Moreover, the effect of replacing soybean meal with vetch (a feedstuff of low production cost and high nutritional value [23]), or with a mixture of the above grain legumes on dairy ewe performance has not been investigated.

Therefore, the objective of the present study was to assess milk production and BCS of intensively reared dairy ewes after replacing soybean meal with a mixture of lupin, pea, vetch, and faba bean in their ration.

2. Materials and Methods

2.1. Facilities, Animals, and Study Design

The study was conducted in the facilities of the Veterinary Faculty of Aristotle University of Thessaloniki farm in Kolchiko, Lagada. The duration was 75 days starting on the 15 January 2021 and ending on the 30 March 2021. The study was conducted in accordance with ethical guidelines and received approval by the Directorate of Veterinary Medicine of the Region of Central Macedonia (approval protocol number 10859(43)).

A total of 40 dairy ewes, which were purebred representatives of the Chios breed, were selected for the study. Minimum sample size was calculated using appropriate software (G*Power v.3.1.9.7) under the hypothesis of a 10% difference in milk yield and 20% variability within each experimental group; probability for Type I and II errors were set at 0.05 and 0.30, respectively (Figure S1). Selected animals were at the third month of their first to fifth lactation period and were allocated in two equal groups (n = 20; Group C: Control and Group T: Treatment). The two groups were balanced in terms of milk production and number of lactations (Table 1) and housed in two different pens with straw bedding.

Table 1. Mean milk production after weaning and number of lactation periods in the two studied groups (n = 20 per group).

Trait	Group	Mean	SD [1]
Milk yield after weaning (g/d)	Control	814.5	310.10
	Treatment	793.8	344.16
Number of lactations	Control	2.8	1.28
	Treatment	2.6	1.29

[1] SD = standard deviation.

Ewes were fed a pelleted concentrate feed together with lucerne hay and wheat straw (1.5, 1.5 and 0.3 kg/animal/day) and had ad libitum access to water. Group C received a conventional concentrate feed with soybean meal, whereas Group T received a concentrate feed of equal energy and protein supply where soybean meal was replaced with a mixture of lupin, pea, vetch, and faba bean (commercial varieties Multi Italia, Dodoni, Evinos, and Tanagra, respectively). Conventional and experimental diets were formulated to meet animal requirements for body weight, daily milk yield, fat yield, and protein yield of 60 kg, 2.5 L, 72 g/L, and 54 g/L, respectively, according to INRA recommendations. The physical composition and the energy and protein content of the two diets are provided in Tables 2 and 3, respectively. The formulated diets were offered twice daily.

Table 2. Physical composition of conventional (Group C) and experimental (Group T) concentrate feeds used in the study.

Feeds (g/kg)	Group C	Group T
Maize	585	409,540.95
Wheat bran	227.5	240,524.05
Soybean meal	162.5	-
Lupin	-	81.25
Vetch	-	81.25
Faba bean	-	81.25
Pea	-	81.25
Vitamins and minerals	25	25

Table 3. Energy and protein contents of the diets used in the study.

Parameter	Lucerne Hay	Wheat Straw	Pellet
DM [1] (g/kg)	850	880	870
UFL [2] (/kg DM)	0.57	0.37	1
Crude protein (g/kg DM)	148	35	158.6

[1] DM = dry matter [2] UFL = units for lactation (net energy for lactation (kcal/kg)/1760) [37].

2.2. Grain Legumes Analyses

Samples were obtained from the studied legumes for chemical composition analysis. Dry matter, starch, crude fat, crude protein, crude fiber, crude ash, units for lactation (UFL), true protein digested in the small intestine when fermentable N is limiting (PDIN), and when fermentable energy is limiting (PDIE) were determined with standard procedures according to the Association of Official Agricultural Chemists [37,38] by an accredited laboratory (UPSCIENCE Lab Solutions, Saint Nolff, France). Moreover, neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined with the ANKOM fiber 220 analyzer (ANKOM Technology Corporation, Fairport, NY, USA). NDF was estimated with the addition of sulphite, and α- amylase to the solution for its determination. ADF analysis was performed after the NDF analysis. ADF samples were incubated with 70% sulphuric acid for the determination of acid detergent lignin (ADL). All analyses were carried out on duplicate samples, and results were reported on a DM basis.

Antinutritional compounds, namely phenols, tannins, condensed tannins, and alkaloids were also determined. Concerning the determination of phenolic extracts, the ground material (0.4 g) was extracted with 70% aqueous acetone (5 mL), vortexed for 30 s, and then sonicated for 15 min at room temperature. The supernatant was collected after centrifugation at 2200× *g* for 10 min, and the extraction was repeated twice. The combined phenolic extracts were used for the determination of total phenolic content (TP), total hydrolyzed tannins content (TN), and total condensed tannins content (CT).

TP was determined by the Folin–Ciocalteu method [39] and the results were expressed as gallic acid equivalents per kilogram of dry weight (g GAE kg^{-1}). TN was determined according to Makkar et al. [40] as follows: Aliquots of phenolic extracts (400 μL) were mixed with 400 mg polyvinylpolypyrrolidone (PVPP), followed by centrifugation at 10,000× *g* at 4 °C for 20 min. Supernatants were transferred into test tubes for the determination of the non-tannin phenolics according to the Folin–Ciocalteu method. Non-tannin phenolics were subtracted from total phenolics for the estimation of TN which were expressed as g of gallic acid equivalents per kg of dry weight (g GAE kg^{-1}). CT was measured according to butanol–acid assay [41] and was expressed as g of procyanidin B2 equivalents per kg dry weight (g PCBE kg^{-1}).

Moreover, total alkaloids (TA) of Lupin were determined by spectrophotometer using the method outlined by Fadhil et al. [42], based on the reaction with bromocresol green, and the results were expressed as the atropine equivalents.

The chemical composition and antinutritional compounds of the four studied grain legumes are presented in Tables 4 and 5, respectively.

Table 4. Chemical composition of the four studied legumes.

Parameter	Lupin	Pea	Vetch	Faba Bean
DM [1] (g/kg)	920	900	900	900
Starch (g/kg DM)	-	410	379	428
Crude Fat (g/kg DM)	87	15	10	11
Crude Fiber (g/kg DM)	128	93	43	64
Crude Ash (g/kg DM)	35	41	36	36
Crude Protein (g/kg DM)	392	271	315	276
UFL [2] (/kg DM)	1.31	1.16	1.19	1.19
PDIN [3] (g/kg DM)	250	173	203	173
PDIE [4] (g/kg DM)	136	108	122	99
NDF [5] (g/kg DM)	199.90	149.35	149.84	140.74
ADF [6] (g/kg DM)	133.46	75.97	60.93	97.52
ADL [7] (g/kg DM)	6.18	5.57	5.06	2.45

[1] DM = dry matter; [2] UFL = units for lactation (net energy for lactation (kcal/kg)/1760) [37]; [3] PDIN = true protein digested in the small intestine when fermentable N is limiting [37]; [4] PDIE = true protein digested in the small intestine when fermentable energy is limiting [37]; [5] NDF = neutral detergent fiber; [6] ADF = acid detergent fiber; [7] ADL = acid detergent lignin.

Table 5. Antinutritional compounds of the four studied legumes.

Parameter	Lupin	Pea	Vetch	Faba Bean
TP [1] (g GAE [5] /kg)	3.77	4.33	3.81	5.21
TN [2] (g GAE [5] /kg)	2.98	2.84	2.91	3.28
CT [3] (g PCBE [6] /kg)	0.18	4.26	1.71	9.00
TA [4] (%)	1.70	-	-	-

[1] TP = total phenolic content; [2] TN = total hydrolyzed tannins content; [3] CT = total condensed tannins content; [4] TA = total alkaloid content; [5] GAE = gallic acid equivalents; [6] PCBE = procyanidin B2 equivalents.

2.3. Data Collection

Following an acclimatization period to the feeding treatments of 15 days, data collection was performed in five time points corresponding to study days 0, 15, 30, 45 and 60 (days of lactation 90, 105, 120, 135, and 150, respectively). Prior to milking, the BCS of each ewe was assessed by palpation of the dorsal lumbar region and recorded on the 5-point scale (1: emaciated to 5: obese, in 0.5-point increments) of Russel et al. [43]. The evaluation was always performed by the same qualified veterinarian. Then, individual ewe milk yield was recorded using electronic milk meters and a milk sample was collected in 50 mL tubes to assess milk composition; fat, protein, lactose, and solids-non-fat (SNF) content. Milk samples were transported to the laboratory at 4 °C and milk composition was determined with Near Infrared Spectroscopy using a DA 7250 NIR analyser (PerkinElmer, Waltham, MA, USA). Finally, pellet and lucerne hay refusals from each group were collected, weighed, and recorded in a designated sheet.

2.4. Data Handling

Individual daily milk yield was calculated according to the official AT method of the International Committee of Animal Recording [44]. Milk fat, protein, lactose and SNF yields were calculated based on milk yield and the respective content records. Energy corrected milk yield (ECMY) for 6% fat was also calculated according to the following formula described by Tsiplakou et al. [45]:

$$\text{ECMY} = [0.28 + (0.12 \times \text{Fat}\ (\%))] \times \text{Milk yield}\ (\text{kg}), \quad (1)$$

Based on the recorded pellet refusals, the respective group feed intake was calculated. Individual feed intake was considered as the group feed intake divided by the number of ewes in each group.

Quality control of milk composition records was implemented according to the average reported values for sheep milk and the respective capacity of the studied breed [46,47]. Based on the quality control, six, four, and nine records for milk protein, lactose and SNF contents, respectively were set as missing values. Moreover, nine records of milk composition were not estimable and were also set as missing. Additionally, seven records of daily milk yield that were below 0.2 kg were removed according to ICAR recommendations [46]. Finally, during the study, two animals in Group C died and hence, the relevant records were not included in the ensuing analyses. The final dataset used for the analysis is available in Dataset S1.

2.5. Statistical Analysis

Descriptive statistics of all studied traits per nutritional management were calculated using the R statistical package psych [48]. Daily milk yield, ECMY, milk fat, protein, lactose and SNF yields, and lactose content were logarithmically transformed to ensure normality of distribution. Preliminary analyses were performed to test for significant effects of milk yield after weaning and lactation period on the studied traits, since the design was no longer balanced following the death of two animals in Group C. Then, the effect of nutritional management on milk yield and quality and BCS were tested using mixed linear models, which included all significant effects from preliminary analyses. Specifically, the following model was used:

$$Y_{ijhmn} = \mu + L_i + b_1 \times M + G_i + S_h + G_i S_h + A_m + e_{ijhmn} \quad (2)$$

where: Y_{ijhmn} = dependent variable (nth trait measurement on animal m); μ = overall population mean; L_i = fixed effect of lactation period (five levels); b1 = regression coefficient on milk yield after weaning (M); G_j = fixed effect of treatment group (two levels); S_h = fixed effect of time/sampling number (five levels); $G_j S_h$ = fixed effect of the interaction between treatment group and time; A_m = random effect of the animal (m = 1–38 ewes); e_{ijhmn} = random residual effect

All analyses were performed using the R statistical package "lme4" [49]. The level of statistical significance was set at 0.05.

3. Results

3.1. Descriptive Statistics

Descriptive statistics of all studied traits per nutritional management are presented in Table 6. Average daily concentrate intake per group of nutritional management is presented in Figure 1. Throughout the study, ewes in Group T consumed smaller amounts compared to ewes in Group C.

Table 6. Descriptive statistics of studied traits per nutritional management (Control = conventional diet with soybean meal; Treatment = experimental diet with grain legumes).

Trait	Nutritional Management	N	Mean	SD [3]
Daily milk yield (kg)	Control	87	1.16	0.60
	Treatment	96	1.23	0.45
	Total	183	1.20	0.53
ECMY (kg)	Control	86	1.21	0.54
	Treatment	95	1.35	0.56
	Total	181	1.28	0.55
Fat content (%)	Control	86	6.47	1.16
	Treatment	95	6.49	1.56
	Total	181	6.59	1.38
Fat yield (g)	Control	86	73.29	32.05
	Treatment	95	83.34	37.08
	Total	181	78.57	35.05
Protein content (%)	Control	82	5.33	0.84
	Treatment	93	5.37	0.80
	Total	175	5.35	0.81
Protein yield (g)	Control	82	64.61	35.78
	Treatment	93	67.52	25.33
	Total	175	66.16	30.62
Lactose content (%)	Control	85	4.65	0.34
	Treatment	92	4.70	0.32
	Total	177	4.68	0.33
Lactose yield (g)	Control	85	55.25	29.82
	Treatment	92	59.69	21.78
	Total	177	57.56	25.97
SNF [1] content (%)	Control	83	11.73	0.73
	Treatment	93	11.71	0.80
	Total	176	11.72	0.77
SNF [1] yield (g)	Control	83	140.31	73.13
	Treatment	93	146.87	50.47
	Total	176	143.72	62.10
BCS [2] (1–5)	Control	90	2.89	0.23
	Treatment	100	2.88	0.21
	Total	190	2.89	0.22

[1] SNF = solids-non-fat [2] BCS = body condition score [3] SD = standard deviation.

3.2. Effect of Nutritional Management on Animal Performance

Estimates of the effect of nutritional management on ewe milk production traits and BCS are presented in Table 7. No statistically significant differences ($p > 0.05$) between the two studied groups were reported. Moreover, the effect of the interaction between treatment and time on the studied traits was not statistically significant ($p > 0.05$). The only exception was reported in the 5th measurement (day 60 of the study) where ewes in Group T had significantly ($p < 0.05$) higher milk protein, lactose and SNF yields, and lactose content compared to ewes in Group C (difference by 16.3 g, 15.1 g, 30.0 g, and 0.3%, respectively, Figure 2).

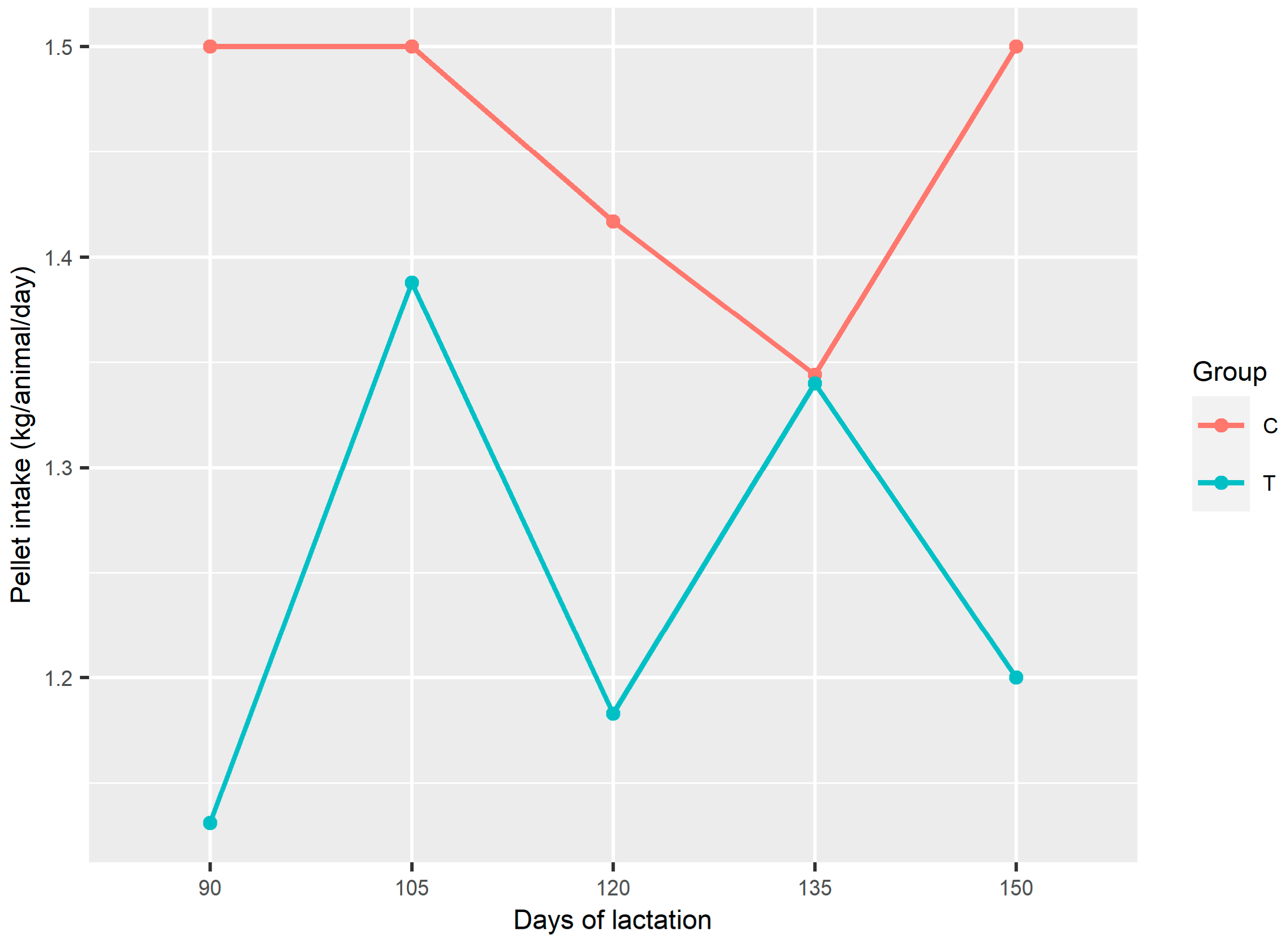

Figure 1. Average daily concentrate (pellet) intake per group of nutritional management (Group C= conventional diet with soybean meal; Group T= experimental diet with grain legumes) for a period of five measurements (days of lactation 90, 105, 120, 135, and 150).

Table 7. Effect of nutritional management (β-coefficient and standard error in parenthesis) on milk production traits and BCS.

Trait	Comparison	β (SE [3])	*p*-Value
Daily milk yield (kg, ln)	T-C	0.097 (0.107)	0.369
Energy corrected milk yield (kg, ln)	T-C	0.082 (0.113)	0.473
Fat content (%)	T-C	0.342 (0.289)	0.245
Fat yield (kg, ln)	T-C	0.082 (0.119)	0.497
Protein content (%)	T-C	0.070 (0.149)	0.640
Protein yield (g, ln)	T-C	0.101 (0.099)	0.315
Lactose content (%, ln)	T-C	0.003 (0.016)	0.852
Lactose yield (g, ln)	T-C	0.106 (0.128)	0.412
SNF [1] content (%)	T-C	−0.019 (0.161)	0.906
SNF [1] yield (g, ln)	T-C	0.079 (0.098)	0.423
BCS [2] (1–5)	T-C	−0.012 (0.056)	0.837

[1] SNF = solids-non-fat [2] BCS = body condition score [3] SE = standard error.

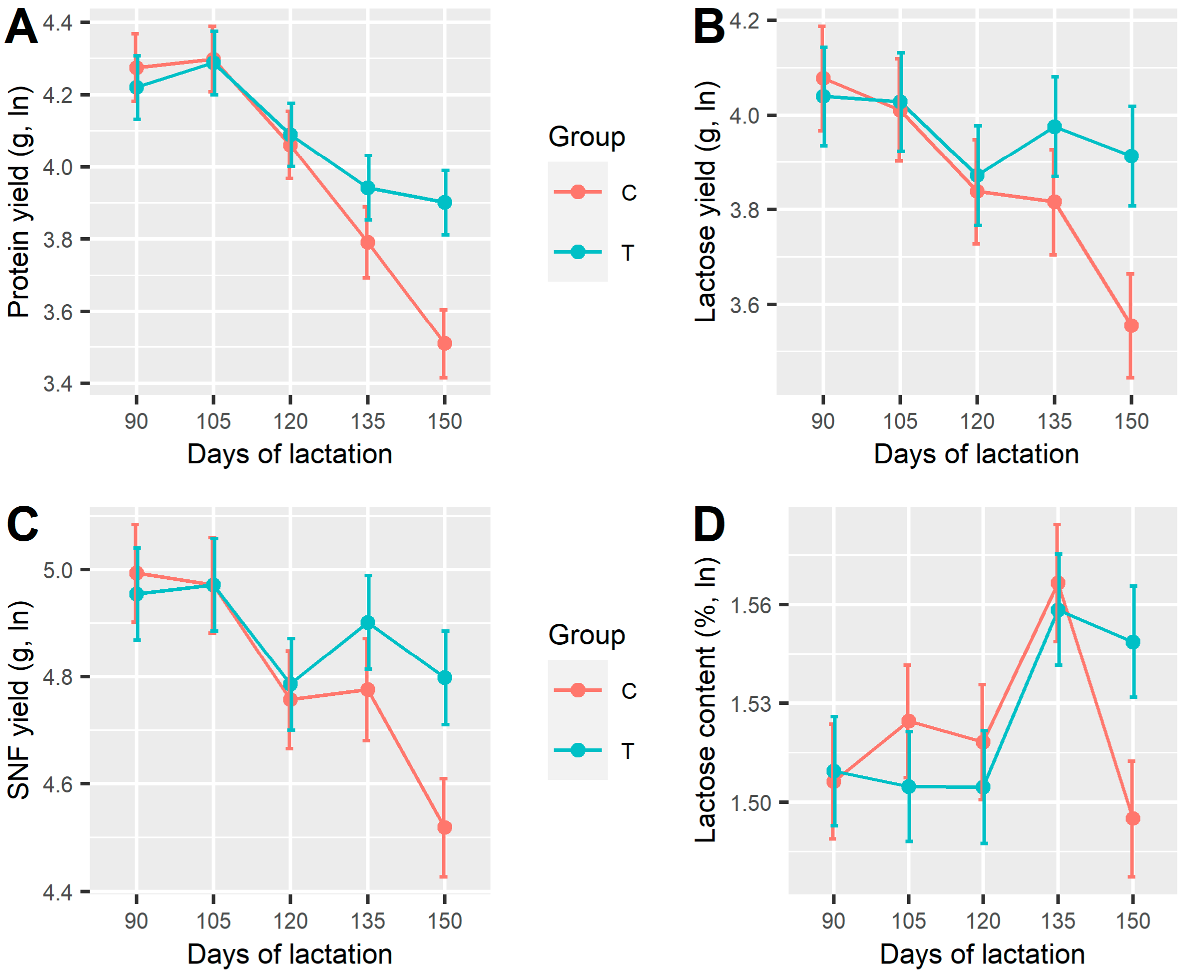

Figure 2. Plots of least square means of protein yield (**A**), lactose yield (**B**), SNF yield (**C**), and lactose content (**D**) per group of nutritional management (Group C= conventional diet with soybean meal; Group T= experimental diet with grain legumes) for a period of five measurements (days of lactation 90, 105, 120, 135, and 150); in all cases significant differences between groups were reported in 150 days of lactation ($p < 0.05$).

4. Discussion

As asserted in the introduction, this study assessed milk production and BCS of intensively reared Chios dairy ewes after replacing soybean meal with a mixture of grain legumes in their ration. To the best of our knowledge, it is the first study to investigate the use of a mixture of lupin, pea, vetch and faba bean. Overall, replacement of soybean meal with the studied legumes did not adversely affect milk yield and composition, and BCS of the studied Chios dairy ewes.

At the start of the study, the two groups of nutritional management were equal in sample size (n = 20 in each group) and balanced for ewe milk yield after weaning and number of lactation period. However, during the sampling period, two ewes from Group C died reducing the total sample size to 38 animals. As a result, the two groups were no longer balanced. To compensate for the latter, milk yield after weaning and lactation period were included as explanatory factors in statistical analyses.

In our study, ewes receiving each diet were group housed. Individual housing would cause unnecessary stress to the animals, which could have potentially affected their production. Our goal was to assess the impact of replacing soybean meal with grain legumes on ewe performance under typical rearing conditions in an intensive farming system. The two studied groups were housed in the same premises under identical conditions to ensure that any potential differences in animal performance are attributed to nutritional management, rather than environmental factors. Nevertheless, it is acknowledged that in this experiment pen was confounded with treatment, and this is a limitation of the study.

Although animals were not individually housed, for purposes of completeness and towards interpreting the results, the average individual concentrate feed intake was calculated as the respective group feed intake divided by the number of ewes in each group. The same approach was followed by Masucci et al. [31] that reported no significant differences in dry matter intake of Sarda ewes between conventional mixtures with soybean meal and mixtures with lupin. On the contrary, the study of Bonnano et al. [34] suggested a lower consumption of concentrate mixtures with chickpea or pea compared to those with soybean in Comissana ewes under individual housing conditions. The latter findings are in accordance with those of the present study where average concentrate feed intake of Chios ewes was lower in the case of the legume-based diet compared to the soybean-based one throughout the study period.

The observed lower feed intake in our study should be interpreted with caution given that animals were not individually housed. However, such differences could be potentially attributed to the antinutritional compounds of grain legumes, especially tannins. Although present in all legume species, tannins are the main antinutritional factor of faba bean and pea. Herein, tannins content in the latter legumes was within the range of existing literature [50]. However, under typical concentrations, tannins can cause both short and long-term reduction of appetite. Short-term reduction of feed intake has been associated with effects on the oral epithelium, whereas long-term effects have been related to the reduced ammonia and volatile fatty acids levels in the rumen fluid [51]. Therefore, to reduce potential effects of antinutritional factors and increase feed intake, processing of grain legumes such as soaking, fermentation or extrusion could be utilised [52,53]. In all cases, further research regarding feed intake of ewes with legume-based diets under individual housing conditions is warranted.

Research on the impact of replacing soybean meal with grain legumes on BCS of dairy ewes is scarce. The only available study is that of Kalogianni et al. [54], that reported a similar trend in BCS of dairy ewes receiving a concentrate diet with soybean meal and those fed a diet in which soybean had been substituted with a mixture of cottonseed cake, rapeseed meal, and faba bean. Given that in the latter study, the mixture used to substitute soybean was not entirely composed of legumes and only descriptive information for BCS was provided, results are not directly comparable with ours. Research in dairy cows has shown that no adverse effects on BCS could be expected by replacing or substituting soybean with faba beans or peas [20,21,55]. The above findings are in general agreement with those herein, for the mixture of the studied legumes.

Previous studies on the effect of replacing or substituting soybean and its derivatives with grain legumes on milk production of dairy ewes are limited and, in all cases, only one legume species was used. Masucci et al. [31], reported no significant effects on milk yield and composition of Sarda ewes when soybean meal was replaced with lupin seeds. Similarly, the milk production of Massese, Delle Langue, Comissana, Sicilo-Sarde and Merinolandschaf ewes was not significantly affected following the replacement of soybean or soybean meal with pea or faba bean [32–36]. Moreover, substitution of soybean meal with a mixture of faba bean, rapeseed meal, and cottonseed cake did not adversely affect milk production of Chios and Frizarta ewes [54]. Results from the above studies are in agreement with those herein, which further suggest the possibility of replacing soybean meal with a mixture of lupin, pea, faba bean and vetch without compromising milk yield and composition of Chios dairy ewes. Our results are further corroborated by studies in dairy cows that have also reported no adverse effects on milk production by partial or total replacement of soybean with faba bean, pea, a mixture of faba bean and pea, or lupin and pea [17–22]. In the study of Huang et al. [23], however, inclusion of vetch in the diet of dairy cows resulted in lower milk yield. According to our findings, a mixture of vetch, lupin, pea, and faba bean is not expected to adversely affect milk production.

Overall, results of the present study indicate the potential of replacing soybean meal with a mixture of the studied legumes in the diet of intensively reared dairy ewes without compromising productivity. Ewes receiving the diet with grain legumes were able to utilize energy and protein supply towards meeting the demands for maintenance and milk production in an equally efficient way with those receiving soybean. Grain legumes have a high starch content and previous research has suggested that such diets could potentially favour fat deposition over milk production [56]. The latter was not corroborated from results in our study. This, however, could be attributed to the lower feed intake observed in the case of the diet with grain legumes. Therefore, future research investigating the effects of legume-based diets on ewe productivity after a processing treatment to increase feed intake is warranted.

Moreover, our results suggest that towards the end of the lactation period, grain legumes may have a favourable effect on milk composition (protein, lactose, and SNF yields). Previous research has also reported favourable effects on milk protein yield of dairy goats receiving diets with grain legumes [57,58]. In our study, to achieve an equal energy and protein content in the two nutritional treatments, the experimental diet with legumes had a lower percentage of cereal grains compared to the conventional one. Starches from pea and faba bean present a lower degradability in the rumen than starches from cereals, which according to literature, in dairy cows, may favour milk production traits [10]. Further relevant research in dairy ewes could help to explain potential favourable effects of grain legumes on milk composition.

Replacement of soybean meal with the studied legumes is expected to be beneficial both environmentally and economically. According to studies in dairy cows, replacement of soybean with grain legumes can reduce adverse environmental impacts and GHG carbon footprint in milk [10,59]. Future research in dairy sheep undertaking life cycle assessments could help estimate the relevant impacts and help farmers towards meeting the desirable goals set by the European Green Deal [60]. Moreover, given that the price for soybean is continuously increasing, replacement with legume grains could help towards reducing production costs. However, a comprehensive economic analysis is needed to quantify the relevant impacts on financial performance. Finally, the feasibility of replacing soybean meal with grain legumes in the diet of dairy ewes is highly dependent on their availability. In this regard, EU support for the cultivation of legumes across member states is considered imperative towards enhancing the sustainability of the dairy sheep sector.

5. Conclusions

Results of the present study indicate that a mixture of lupin, pea, faba bean, and vetch could be used to replace soybean meal in the diet of intensively reared dairy ewes without compromising milk production and BCS. The latter is expected to reduce environmental impacts and production costs of intensive dairy sheep farms, thus helping to increase the overall sustainability of the sector.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su15021028/s1, Dataset S1: Total dataset of traits used for the analyses; Figure S1: Graphical representation of minimum sample size calculation.

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