Use of *Pisum sativum* (L.) as alternative protein resource in diets for dairy sheep: effects on milk yield, gross composition and fatty acid profile

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ABSTRACT

Aim of the study was to evaluate the use of home-grown pea seeds as protein source in diets for lactating sheep. Two isonitrogenous and isoenergetic diets were fed to 12 mid-lactating Delle Langhe ewes for 73 days. The animals were fed with 1.5 kg alfalfa hay and either 0.7 kg commercial concentrate (control group, C) or 0.6 kg home-grown pea-barley mix (experimental group, PB). The main protein sources in the supplements were sunflower meal and soybean seeds for C group, and pea seeds for PB group. Milk yield was recorded and milk samples were analysed for fat, protein, lactose, casein, solids non-fat, somatic cell count, total bacterial count and fatty acids. Results showed that milk yield and gross composition were not significantly affected by the supplementation types. Differences were instead observed in milk fatty acid profile essentially as a consequence of variations in dietary fatty acids supplies. Milk from the PB group had higher concentrations of short-chain ($P \leq 0.05$) and saturated fatty acids ($P \leq 0.01$) and lower concentrations of long-chain ($P \leq 0.05$), monounsaturated ($P \leq 0.01$), *trans* fatty

acids (P≤0.001) and total conjugated linoleic acids (P≤0.001). The use of home-grown *Pisum sativum* in diets for dairy ewes could enhance farm sustainability without affecting milk production, but possible modifications in milk fatty acid composition have to be taken into consideration.

Keywords: dairy ewes, pea seeds, milk yield, milk quality, fatty acids

INTRODUCTION

At the European level, needs for vegetable protein sources suitable for ruminant nutrition is impellent. Recently, the substitution of imported protein-rich feedstuffs (e.g., soybean and its derivatives) with regionally cultivated and locally processed alternative vegetable protein sources (AVPS) has been largely advocated (Jensen, 2002).

Grain legumes may represent a valid solution to meet increasing plant protein requirements in animal husbandry, with the additional benefit of ensuring positive ecological and environmental roles. In fact, home-grown legume crops can prevent the degradation of soil fertility (Hauggaard-Nielsen, 2002; Watson et al., 2008), break pest and disease cycles (Caballero, 1999), and reduce negative environmental impacts such as greenhouse gas emissions, eco- and human-toxicity, acidification, etc. (Hörtenhuber and Zollitsch, 2010; Nemecek et al., 2008). This in turns improves the livestock farming sustainability as well.

Home-grown legume crops seem advantageous especially in organic farming. They can be used as a replacement of expensive commercially available organic protein-rich sources, to avoid risks of soybean contamination with genetically modified organisms (Hewlett and Azeez, 2008), and for the compulsory needs of ensuring the complete traceability of animal-derived food products (Froidmont and Bartiaux-Thill, 2004).

Nevertheless, legume crops are currently under-used, being only a marginal component of crop production within the European Union.

Globally, pea (*Pisum sativum* L.) is the second most important feed legume grain after soybean (Mikić et al., 2009). Its low amount of anti-nutritional factors confers good palatability (Liponi et al., 2006). Moreover, pea has good crude protein and starch contents, that make it a high quality and cost-efficient source of both protein and energy (Jezierny et al., 2010).

Information on the effective suitability of pea for small ruminant nutrition is very limited. The few existing studies reported that the use of pea does not affect health status, diet palatability or milk production performance (Bonomi et al., 2003; Liponi et al., 2007). Liponi et al. (2007) showed a significant decrease in the milk protein percentage while substituting dietary soybean meal with pea. However, the same was not observed by Bonomi et al. (2003). To the best of our knowledge, no information is currently available on the effect of dietary pea on milk fatty acid (FA) profile with the existing literature mainly devoted at verifying the effects of partially or totally replacing soybean meal and maize meal with pea. Since commercial concentrates formulated for dairy sheep are widely used, the possibility of replacing them with home-grown feed resources must be further investigated.

The use of pea in combination with cereals (which are still extensively cultivated at farm level) could represent a valid alternative to the use of commercial concentrates in diets for small ruminants, enhancing self-supply energy and protein requirements for livestock activities, thus reducing feeding costs at farm level. Among cereals, barley seems to be particularly appropriate to be used in combination with pea because its rapid rate of starch degradation could be well balanced by the slow degradation rate of starch in pea (Corbett, 1997).

The aim of this study was to evaluate performance in production, gross composition,

and fatty acid profile of milk from ewes fed two different diets with home-grown pea seeds or commercial sunflower meal and soybean seeds as the main protein sources.

MATERIALS AND METHODS

Animals, experimental design and feeding treatments

The experiment was carried out in a farm breeding Delle Langhe ewes located in North-Western Italy (latitude: 44°28'35'' N; longitude: 08°03'62'' E; altitude: 640 m a.s.l.) from January 16th to March 30th, 2009. Delle Langhe is a local dairy sheep breed whose number of purebreds registered in the Herd Book has been recently estimated to be approximately equal to 2,702 only (FAO, 2009). According to the European legislation for the support of rural development (Commission Regulation No. 1974/06, 2006), this breed has to be considered in danger of being lost to farming, since the number of females available for purebred reproduction is lower than the established limit threshold of 10,000 heads. Twelve multiparous Delle Langhe ewes in mid-lactation (108±18 days in milk) were selected from 50 lactating ewes and allocated to two balanced groups according to their stage of lactation, lactation number, milk yield and milk composition (fat, protein, lactose and casein contents). The groups were then randomly assigned to control or experimental diets. The ewes were housed indoors on straw litter in individual pens. Water was available at all time.

During the experimental period, the control group (C) was fed 1.5 kg head^{-1} day^{-1} alfalfa hay and 0.7 kg head^{-1} day^{-1} commercial concentrate containing sunflower meal and soybean seeds as main protein source. The experimental group (PB) was offered the same amount of alfalfa hay and 0.6 kg head^{-1} day^{-1} of home-grown 1:1 pea-barley mix. Diets were formulated in order to be isonitrogenous and isoenergetic. Animals were manually milked twice a day (at 8.00 and 18.00 h) and feed was provided after milking. Feed refusals were controlled once a week throughout the trial.

Sampling procedures and laboratory analyses

Feed - Representative feedstuffs samples were ground (cutting mill Pulverisette 15 - Fritsch GmbH, Idar-Oberstein, Germany) to pass a 1-mm screen. The samples were analysed for dry matter (DM), crude protein (CP), ether extract (EE), ash, neutral detergent fibre (NDF) and acid detergent fibre (ADF) according to AOAC procedures (2000). Starch was analysed by using a POLAX-2L polarimeter (ATAGO CO., LTD. Japan) according to "Gazzetta Ufficiale della Repubblica Italiana" (2000). For FAs analysis, total lipids were extracted according to Folch et al. (1957). Fatty acid methyl esters (FAMEs) were prepared by using a solution of KOH in methanol (IOfS, 2002), then separated and quantified by gas chromatography (Shimadzu GC17A, Shimadzu Corporation Analytical Instruments Division, Kyoto, Japan) equipped with a CP-Sil 88 capillary column (100 m × 0.25 mm ID, 0.20 μm film thickness; Varian Inc., Lake Forest, CA). The column temperature was held at 45°C for 5 min, then raised 20°C min^{-1} up to 195°C and maintained for 65 min. The temperature of the injector and the flame-ionization detector was maintained at 250°C and 280°C, respectively. The injection volume was 0.1 μL. Nitrogen constant linear flow rate was set at 40 mL min^{-1}. Peaks were identified by comparison of retention times with FAME standards (Restek Corporation, Bellefonte, PA, USA). Results are expressed as a percentage of each individual FA per total FAs detected. All analyses were done in duplicate.

Milk – Milk samples were collected after a 10-days period of adaptation to the diets (from January 16th to January 25th). Individual daily milk yields were recorded once a week for ten weeks. Two aliquots of each individual milk sample were collected during the morning milking every three weeks and were immediately stored at 4°C in a portable refrigerator. The former aliquot was analysed for fat, protein, lactose, casein, solids non-fat (SNF), somatic cell count (SCC) (MilkoScan FT 6000 and Fossomatic

5000 connected in series, Foss Electric, Hillerød, Denmark), and total bacterial count (TBC) (BactoScan FC 50, Foss Electric, Hillerød, Denmark). The latter one was frozen at –20°C and successively analysed for FA composition. Milk fat extraction was obtained by centrifugation at 7,300 rpm for 30 min at –4°C. The resulting molten butter has been then filtered through a hydrophobic filter (Whatman 1, Whatman International Ltd, Maidstone England), the pure milk fat was dissolved in heptane and FAMEs were obtained by *trans*-esterification of glycerides by using a solution of KOH in methanol (IOfS, 2002). FAs were determined, as previously reported by Collomb and Bühler (2000), by using the same analytical instruments and procedures described for the analysis of feed samples. Peaks were identified by injecting pure FAME standards and by comparison with the chromatogram published by Collomb and Bühler (2000). The following FAME standards were used: C4, C5, C6, C7, C8, C9, C10, C12, C13, C14, C15, C16, C17, C18, C19, C20, C22, C14:1 *t*9, C14:1 *c*9, C16:1 *t*9, C16:1 *c*9, C18:1 *t*9, C18:1 *c*9, C18:1 *c*11, C18:1 *c*12, C20:1 *c*11, C18:3 *c*9*c*12*c*15, C20:3 *c*8*c*11*c*14, C20:5 *c*5*c*8*c*11*c*14*c*17, C22:5 *c*7*c*10*c*13*c*16*c*19 (Fluka, Sigma-Aldrich Milano, 20151 Milano, Italy); C13 *iso*, C14 *iso*, C15 *iso*, C16 *iso*, C17 *iso*, C18:1 *t*6, C18:1 *t*11, C18:1 *c*7, C18:2 *t*9*t*12, C18:2 *c*9*t*12, C18:2 *t*9*c*12, C18:2 *c*9*c*12, C18:3 *c*6*c*9*c*12, C20:3 *c*11*c*14*c*17, C20:4 *c*5*c*8*c*11*c*14, C22:6 *c*4*c*7*c*10*c*13*c*16*c*19 (Sigma, Sigma-Aldrich Milano, 20151 Milano, Italy); C18:1 *c*6 (Supelco, Sigma-Aldrich Milano, 20151 Milano, Italy); C18:2 *c*9*t*11, C18:2 *t*10*c*12, C18:2 *c*9*c*11, C18:2 *t*9*t*11, C20:2 *c*11*c*14 (Matreya Inc., Pleasant Gap, PA, USA). Quantification was assessed by using nonanoic acid as internal standard. The results are expressed as absolute values as g $100g^{-1}$ fat.

Estimation of individual fatty acids intake

The percentages of total FAs on the total EE content for the home-grown feedstuffs (alfalfa hay, pea seeds and barley) have been obtained from the INRA tables of

composition and nutritional value of feed materials (INRA-AFZ, 2002). The same information was instead provided by the feed industry for the commercial concentrate. The daily intake (g head^{-1} day^{-1}) of each dietary FA in the two experimental groups was then estimated on the basis of the above mentioned percentages and of the results obtained with the laboratory analysis of feedstuffs.

Statistical analysis

The Kolmogorov-Smirnov test was used to check dependent variables for normality. Variables which were not normally distributed (SCC and TBC) were log-transformed prior to further statistical analysis, but results are presented as non-transformed data.

The changes in milk yield, main constituents and FAs were analysed as a repeated measures design using the MIXED procedure of SAS (2006) according to the following model:

$$Y_{ijk} = \mu + D_i + E_{(i)j} + SD_k + (D \times SD)_{ik} + \varepsilon_{ijk}$$

where Y_{ijk} = mean of response variable, μ = population mean, D_i = effect of treatment (diet), $E_{(i)j}$ = random effect of ewe within the treatments, SD_k = effect of sampling date, $(D \times SD)_{ik}$ = effect of interaction between treatment and sampling date, and ε_{ijk} = experimental error. Schwarz Bayesian criterion was used to select the best covariance structure from among compound symmetry, first-order autoregressive, and unstructured (Littell et al., 1998). Significance was declared at $P \leq 0.05$. Results of statistical analysis are reported as estimate least-squares means.

RESULTS AND DISCUSSION

Characteristics of the feedstuffs

The chemical composition and FA profile of alfalfa hay, commercial concentrate, pea seeds and barley, as well as those of the C and PB diets are presented in Table 1. The chemical composition of pea seeds, barley, and alfalfa hay was in accordance with values previously reported for these feedstuffs in the literature (Jezierny et al., 2010;

INRA-AFZ, 2002). The nutritional characteristics of the two diets were similar if considering major components (DM, CP, fibre and net energy for lactation). As expected, within the lipid fraction the predominant FAs in alfalfa hay were α-linolenic (C18:3 *c*9*c*12*c*15, ALA), linoleic (C18:2 *c*9*c*12, LA) and palmitic (C16:0, PA) acids. As usually occurs in forages (Clapham et al., 2005), the alfalfa hay resulted considerably high in ALA, accounting alone for approximately 40% of the total FAs. ALA levels in the commercial concentrate and in barley represented 3% and 6% of total FAs, respectively, while in pea seeds they were set at approximately 10% of total FAs. Results of the laboratory analysis showed that all feedstuffs other than alfalfa hay were rich-LA sources (45-54% of total FAs). LA was followed in order of abundance by oleic acid (C18:1 *c*9, OA) in the commercial concentrate and pea, and by PA in barley (about 20% of total FAs).

Individual fatty acids intake

Daily intake of dietary individual FAs from the main feedstuffs and from C and PB diets are presented in Table 2. The estimation showed a higher ingestion of total FAs in the C group. Due to the notable contribution of alfalfa hay in total ALA ingestion, the two groups of animals ingested approximately the same amount of this polyunsaturated fatty acid (5.5 g $head^{-1}$ day^{-1}). Compared to the ewes of the PB group, the ewes of the C group ingested higher levels of LA (9.8 vs 5.7 g $head^{-1}$ day^{-1}), more than doubled levels of OA (4.5 vs 2.1 g $head^{-1}$ day^{-1}) and myristic acid (C14:0; 1.1 vs 0.4 g $head^{-1}$ day^{-1}), slightly higher levels of PA (4.2 vs 3.2 g $head^{-1}$ day^{-1}) and approximately the same amount of stearic acid (C18:0; ~0.8-0.9 g $head^{-1}$ day^{-1}).

Milk yield and gross composition

Feeds were totally consumed by the ewes and only negligible refusals were observed during the trial. Consequently, the total DM intake was not influenced by the diet (1.9 and 1.8 kg DM $head^{-1}$ day^{-1} for the C and PB diets, respectively). The two diets did not

affect milk yield and composition (Table 3). Crude protein in pea, as well as in other legume grains, is characterized by low levels of rumen escape due to its high degradability (Lanza et al., 2003). In high-producing dairy ruminants, this low amount of bypass protein could represent an impediment for meeting protein needs. On the contrary, in ruminants at low to moderate levels of performance this characteristic should not constitute a problem as the majority of protein requirements can be met by microbial sources (Corbett, 1997). The amount of protein fed in the PB diet was sufficient for matching the requirements for maintenance and lactation despite its higher rumen degradability if compared to that in the C diet. Our results indicate that pea can be considered a valid alternative to other protein sources in rations for low to medium producing dairy sheep.

Moreover, it is worth mentioning that starch in pea has a relatively slow degradation rate. Such characteristic could be useful to overcome the lack of rapidly available dietary energy deriving from low quality feedstuffs that occurs in local breeds conducted in disadvantaged marginal areas (Yáñez-Ruiz et al., 2009).

No significant differences were found in the TBC and SCC between groups. The TBC values were always below the threshold limits established by the current European legislation (Council Directive 92/46/EEC, 1992) during the whole experimental period, indicating that no management difficulties or unsanitary conditions occurred.

The statistical analysis showed that the sampling date significantly affected milk yield ($P \leq 0.01$), fat ($P \leq 0.05$), and TBC ($P \leq 0.001$). A tendency was also observed for SCC ($P < 0.10$). As expected, milk yield decreased, while fat and SCC increased throughout the lactation. No significant interactions between diet and sampling date were detected among the considered parameters.

Milk fatty acid composition

Groups of fatty acids - The mean concentrations of FAs groups in milk fat from the C

and PB groups are shown in Table 4. The obtained data are similar to those reported by Abilleira et al. (2009) for Latxa sheep milk in Spain, but quite different from those reported by Collomb et al. (2006a) for Friesian and Lacaune sheep in Switzerland. This is probably a consequence of the different management conditions (grazing at mountain pastures) applied in the latter trial.

Milk from the PB group had significantly higher amounts of short chain fatty acids (SCFA; +18.4%; $P \leq 0.05$) and saturated fatty acids (SFA; +9.4%; $P \leq 0.01$) than milk obtained from the C group. Moreover, it showed a tendency for higher values of medium chain fatty acids (MCFA; +8.7%; $P < 0.10$). Among MCFA, lauric (C12:0), myristic (C14:0), and palmitic (C16:0) acids are generally considered detrimental for human health due to their cholesterol-increasing potential (Ohlsson, 2010). The hypercholesterolemic saturated fatty acids level (HSFA, calculated as C12:0+4*C14:0+C16:0) tended to be higher in the PB group (+13.0%; $P < 0.10$). Milk obtained from the ewes of the C group had, instead, higher concentrations of long chain fatty acids (LCFA; +19.3%; $P \leq 0.05$), monounsaturated fatty acids (MUFA; +26.4%; $P \leq 0.01$), *trans* FA (TFA, both mono- and diunsaturated; +63.6% and +40.2%; $P \leq 0.001$) and conjugated linoleic acids (CLA; +50.0%; $P \leq 0.001$). Moreover, it showed a tendency for higher values of n6 FAs (+13.7%; $P < 0.10$). The type of supplementation did not significantly affect branched chain fatty acids (BCFA) nor polyunsaturated fatty acids (PUFA) or n3 FAs.

The ewes belonging to the C group ingested higher levels of PUFA (Table 2) and showed higher LCFA concentrations in milk (Table 4). Both PUFA and LCFA are known to be effective inhibitors of *de novo* synthesis of FAs in the mammary gland, by exerting a direct or indirect effect on the lipogenic enzymes acetyl-CoA carboxilase and fatty acid synthase (Palmquist et al., 1993; Chilliard et al., 2000). Such considerations are probably the explanation for the lower SCFA and MCFA concentrations found in

the C group. As the majority of *de novo* synthesized FAs are saturated (C4:0 to C16:0), the significantly lower SFA amount found in the C group was also expected. The higher intakes of unsaturated FAs (especially OA and LA) estimated for the C group is also the reason for the higher concentrations of TFA, n6 FAs and CLA.

The sampling date did not significantly affect milk FA profile. This result is consistent with the findings by De La Fuente et al. (2009) who observed that the main changes in sheep milk FAs occur in early lactation, while a relatively stable FA pattern is generally observed in mid and late lactation.

Individual fatty acids – The mean concentrations of individual FAs in milk fat from the C and PB groups are shown in Table 5. Compared to the C group, milk fat from the PB group had significantly higher concentrations of caproic (C6:0), enhantic (C7:0), and *trans*-eicosenoic (C20:1*t*) acids. Moreover, it showed a tendency ($P<0.10$) for higher concentrations of caprylic (C8:0), capric (C10:0), lauric (C12:0) and myristic (C14:0) acids. Conversely, almost all detected C18:1 *trans* isomers, oleic acid, most C18:2 *trans* FAs, including CLA isomers [*c*9*t*11+*t*7*c*9+*t*8*c*10] and *t*10*c*12, showed higher amounts in the C group. No significant differences between groups were observed for the concentrations of butyric (C4:0) and stearic (C18:0) acids, ALA and other n3 FAs, linear odd-chain, monomethyl *iso*- and *anteiso*-BCFA, and CLA isomers [*c*9*c*11+*t*11*c*13] and *t*9*t*11.

The observed differences can be explained by considering the ways individual FAs are formed within the rumen or in the mammary gland.

The lower concentrations of *de novo* synthesized saturated SCFA found in the C group could be related, as previously discussed, to the higher PUFA supply from the C diet.

Concerning octadecenoic TFA, the patterns of their formation during ruminal biohydrogenation greatly depend on dietary profile of unsaturated fatty acids (UFA) (Loor et al., 2002). In the current trial, the peak referred to the most abundant isomer

trans-vaccenic acid (C18:1 *t*11, TVA) coeluted with that of other isomers (C18:1 *t*6-10). TVA is synthesized in the rumen during the biohydrogenation of dietary PUFA by the activity of isomerases and reductases of microbial origin (Jenkins et al., 2008). It can also originate, like all other *trans* octadecenoic FAs, through isomerization of OA and other *cis* or *trans* C18:1 FAs (Cuvelier et al., 2005). Since ALA intake was estimated to be approximately the same between groups, the higher concentrations of C18:1 *trans* FAs in the C group have to be related to the higher OA and LA intakes with the C diet. Such results are consistent with the previous findings by Loor et al. (2002) and Collomb et al. (2004a) who reported a positive correlation between the concentrations of many C18:1 *trans* isomers in rumen fluid or milk fat and the dietary OA and LA supplies.

Detected monounsaturated *trans* FAs other than octadecenoic ones (C14:1 *t*, C16:1 *t*, C17:1 *t*, and C20:1 *t*) were found only in low concentrations in sheep milk fat, confirming the results previously reported by other authors (Collomb et al., 2006a; Abilleira et al., 2009). Dienoic TFA other than CLA are known (some of them still only supposed) to be formed during the biohydrogenation of dietary LA and ALA (Chilliard et al., 2007). In this trial, some of them (essentially the ones deriving from LA: C18:2 *t*,*t*NMID+*t*9*t*12, C18:2 *c*9*t*12 and C18:2 *t*8*c*13+*c*,*c*MID) were significantly higher in milk fat from the C group, while others (C18:2 *c*9*t*13+*t*8*c*12, C18:2 *t*11*c*15 and C18:2 *t*9*c*12) did not statistically differ between groups.

Regarding CLA, under the chromatographic conditions applied in the current trial, the peak referring to the predominant isomer (C18:2 *c*9*t*11, rumenic acid, RA) coeluted with those of two other isomers: C18:2 *t*7*c*9 which has been reported as the second/third most abundant isomer depending on dietary conditions (Kraft et al., 2003; Luna et al., 2005), and C18:2 *t*8*c*10 which is instead only present in low amounts in milk and dairy products (Ostrovský et al., 2009; Renna et al., 2010). The sum of these three isomers was significantly higher ($P \leq 0.01$) in milk fat from the ewes fed the C diet. CLA isomers

containing a *cis*-9 double bond (C18:2 *c*9*t*11 and C18:2 *t*7*c*9) originate predominantly in the mammary gland thanks to the activity of Δ9-desaturase. This enzyme is able to add a *cis*-9 double bond to the octadecenoic acids C18:1 *t*11 and C18:1 *t*7 which escape complete rumen biohydrogenation of dietary UFA. While CLA *t*7*c*9 is almost exclusively synthesized endogenously, a small part of RA present in milk fat derives from escaping complete biohydrogenation, too. RA is, in fact, the first intermediate formed by means of an isomerization during the biohydrogenation of LA. Differently from C18:2 *c*9*t*11 and C18:2 *t*7*c*9, C18:2 *t*8*c*10 has been hypothesized to originate entirely within the rumen during the microbial biohydrogenation of dietary PUFA (Piperova et al., 2002). Previous findings indicated a significant positive correlation existing between the LA content in the diet and the amount of C18:2 *t*8*c*10 in milk fat (Collomb et al., 2004b). The whole above-mentioned concerns allow to explain the significantly higher level of the sum [C18:2 *c*9*t*11+C18:2 *t*7*c*9+C18:2 *t*8*c*10] found in milk fat from the C group. In fact, as previously mentioned, the group C18:1 *t*6-11, among which the precursors for CLA *c*9*t*11 and *t*7*c*9 synthesis belong, was also significantly higher in milk fat from the ewes fed the C diet ($P \leq 0.001$). TVA is an intermediate of the biohydrogenation processes and is formed from both dietary LA and ALA. C18:1 *t*7 is also formed within the rumen, deriving from isomerization of OA (Mosley et al., 2002; van de Vossenberg and Joblin, 2003) and other octadecenoic acids formed during the biohydrogenation of dietary UFA (Proell et al., 2002; van de Vossenberg and Joblin, 2003). The higher supply of both LA and OA in the C diet relative to the PB one suggests a higher formation of RA, CLA *t*8*c*10, TVA and C18:1 *t*7 in the rumen as well as a higher absorption and availability of the latter two C18:1 *trans* isomers in the mammary gland as a substrate for Δ9-desaturase activity.

According to the high forage levels of both experimental diets (Fuentes et al., 2009), CLA *t*10*c*12 amounts were found to be very low or almost null in milk fat from the C

and PB ewes, respectively. Despite the very low concentrations, this isomer showed significantly higher amounts in the C group (P≤0.001). CLA *t*10*c*12 is only synthesized within the rumen, being an intermediate product of the biohydrogenation of dietary LA (Jenkins et al., 2008). The obtained results have to be associated to the higher LA ingestion by the ewes belonging to the C group and confirm previous findings by other authors (Gómez-Cortés et al., 2011; Hervás et al., 2008) who reported statistically significant increasing concentration of C18:2 *t*10*c*12 in ewe milk fat associated with incremental amounts of dietary LA.

The differences between C and PB groups regarding the concentrations of n6 FAs were essentially related to the octadecenoic and some octadecadienoic acids (C18:2 *tt*NMID+*t*9*t*12 and C18:2 *c*9*t*12), while all other detected n6 FAs did not vary significantly between groups. n6 FAs originate predominantly from dietary LA, but ALA and OA can also be indirect precursors of some n6 FAs via isomerization of ruminal biohydrogenation intermediates. Considering the higher dietary LA and OA supply from the C diet, the obtained results were not surprising.

Many researchers have focused on dietary FAs and their effects on human health. It is well known that it is necessary to differentiate among individual FAs while referring to their biological activities. Some of the FAs that significantly differed between the two experimental groups in this trial have been reported to affect human health some way. The medium chain lauric, myristic, and palmitic acids increase plasma LDL cholesterol levels, the latter being a well-known risk factor for cardiovascular diseases (CVD) (Ohlsson, 2010). Both mono- and diunsaturated TFA, especially the latter ones, have been also reported to increase risk factors for CVD (Baylin et al., 2003), with a negative health impact even stronger than that observed for SFA (Willett, 2006). However, recent findings highlighted that, differently from other C18:1 *trans* FAs, the predominant *trans* isomer in milk fat (TVA) could even exert protective effects against

such disease (Tyburczy et al., 2009). At the same time, positive health impacts (protection against carcinogenesis, obesity, diabetes, arteriosclerosis and other inflammatory diseases) have been attributed to CLA (Collomb et al., 2006b). On the basis the results obtained in this experiment, the pea-barley mix supplementation led to a slight worsening of the milk lipid fraction, particularly since it increased some hypercholesterolemic saturated FAs and decreased both TVA and total CLA contents.

CONCLUSION

The replacement of a commercial concentrate containing sunflower meal and soybean seeds as main protein source with an experimental concentrate consisting of a 1:1 home grown pea-barley mix did not affect milk yield nor milk gross composition or somatic cell count of mid-lactating dairy ewes. Differences in fat levels and fat compositions of concentrates, however, led to significant variations in milk FA profile. Milk from the ewes fed the PB diet showed higher levels of SCFA, total SFA and a tendency for higher levels of MCFA and HSFA. At the same time, their milk had significantly lower levels of LCFA, TFA, CLA and a tendency for lower level of n6 FA.

An increased self-supply of both energy and protein sources destined to ruminant nutrition is recommended in order to increase the sustainability of livestock activities.

Our results showed that it is possible to formulate rations for dairy ewes balanced for nutrients and energy by using supplements based on pea seeds in combination with barley without affecting milk production performance. However, possible slight worsening in the FA profile of milk due to different dietary FAs supplies should be taken into account.

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Table 1

Table 1. Chemical composition of main feedstuffs (alfalfa hay, commercial concentrate, pea seeds and barley) and of the control (C) and the experimental (PB) diets.[†]

	Alfalfa hay	Commercial concentrate[‡]	Pea seeds	Barley	Diet C[§]	Diet PB[§]
Main nutrients						
DM (%)	86.1	88.1	86.8	87.3	86.7	86.4
Ash (%DM)	9.1	8.8	3.4	2.3	9.0	7.3
CP (%DM)	14.7	20.7	24.3	13.3	16.6	16.0
NDF (%DM)	52.5	31.8	19.2	21.4	45.8	43.2
ADF (%DM)	39.3	12.8	9.8	5.0	30.7	30.1
EE (%DM)	2.0	2.4	1.1	1.5	2.1	1.8
Starch (%DM)	5.4	32.0	47.1	58.2	14.0	19.0
NSC[¶]	21.7	36.3	52.0	61.5	26.5	31.8
NE_L (MJ/kgDM)	4.8	7.2	8.2	8.5	5.6	5.8
Fatty acids (% of total fatty acids)						
C12	0.55	0.11	n.d.	n.d.	0.41	0.39
C14	1.84	6.04	4.65	2.13	3.18	2.28
C15	0.33	0.12	0.24	0.10	0.26	0.28
C16	18.09	13.46	11.42	19.67	16.62	17.36
C16:1 *c*9	0.96	0.32	0.51	0.19	0.76	0.79
C17	n.d.	0.12	0.17	0.09	0.04	0.04
C18	4.67	1.83	3.64	1.70	3.77	4.10
C18:1 *c*9	9.16	23.94	21.22	13.82	13.86	11.55
C18:1 *c*11	0.41	1.24	1.33	0.88	0.67	0.61
C18:2n6	23.50	48.86	45.01	54.54	31.57	31.01
C18:3n3	39.23	3.17	10.62	5.82	27.76	30.37
C20	0.68	0.28	0.51	0.17	0.55	0.58
C20:1 *c*9	n.d.	0.55	0.68	0.88	0.18	0.22
C22	0.59	n.d.	n.d.	n.d.	0.40	0.40
ΣSFA	26.75	21.94	20.63	23.86	25.23	25.43
ΣMUFA	10.53	26.05	23.73	15.77	15.47	13.17
ΣPUFA	62.72	52.03	55.63	60.37	59.33	61.38

[†] Diet C, control diet based on 1.5 kg alfalfa hay and 0.7 kg commercial concentrate; Diet PB, experimental diet based on 1.5 kg alfalfa hay + 0.3 kg pea seeds + 0.3 kg barley; DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; EE, ether extract; NSC, nonstructural carbohydrates; NE_L, net energy for lactation; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n.d., not detected.

[‡] Commercial concentrate based on: wheat bran (22%), maize (17%), wheat middlings (15%), sunflower meal (15%), barley (11%), extruded soybean (9%), alfalfa meal (6%), sugarcane molasses (2%), calcium carbonate (2%), sodium chloride (0.5%) and vitamin-oligoelements mix (0.5%).

[§] The reported values are calculations made on the basis of the analytical determinations of the feedstuffs.

[¶] Calculated as 100 – (NDF + CP + EE + ash).

Table 2

Table 2. Daily intake (g head^{-1} day^{-1}) of dietary individual fatty acids from the main feedstuffs (hay, commercial concentrate, pea seeds and barley) and from the control (C) and the experimental (PB) diets.[†]

	Alfalfa hay	Commercial concentrate[‡]	Pea seeds	Barley	Diet C	Diet PB
C12	0.071	0.015	-	-	0.086	0.071
C14	0.238	0.831	0.107	0.063	1.069	0.407
C15	0.043	0.017	0.005	0.003	0.059	0.051
C16	2.336	1.853	0.262	0.580	4.189	3.178
C16:1 *c*9	0.124	0.044	0.012	0.006	0.168	0.141
C17	-	0.017	0.004	0.003	0.017	0.007
C18	0.603	0.252	0.083	0.050	0.855	0.737
C18:1 *c*9	1.183	3.295	0.486	0.407	4.478	2.076
C18:1 *c*11	0.053	0.171	0.030	0.026	0.224	0.109
C18:2 *c*9*c*12	3.035	6.725	1.031	1.607	9.760	5.673
C18:3 *c*9*c*12*c*15	5.067	0.436	0.243	0.171	5.503	5.481
C20	0.088	0.039	0.012	0.005	0.126	0.105
C20:1 *c*9	-	0.076	0.016	0.026	0.076	0.042
C22	0.076	-	-	-	0.076	0.076
ΣSFA	3.455	3.020	0.473	0.703	6.475	4.631
ΣMUFA	1.360	3.586	0.544	0.465	4.946	2.368
ΣPUFA	8.100	7.162	1.275	1.779	15.262	11.154

[†] Diet C, control diet based on 1.5 kg alfalfa hay and 0.7 kg commercial concentrate; Diet PB, experimental diet based on 1.5 kg alfalfa hay + 0.3 kg pea seeds + 0.3 kg barley; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

[‡] Commercial concentrate based on: wheat bran (22%), maize (17%), wheat middlings (15%), sunflower meal (15%), barley (11%), extruded soybean (9%), alfalfa meal (6%), sugarcane molasses (2%), calcium carbonate (2%), sodium chloride (0.5%) and vitamin-oligoelements mix (0.5%).

Table 3

Table 3. Mean values of yield, main constituents and microbial counts of milk from ewes fed the control (C) and the experimental (PB) diets.[†]

	Diet C	Diet PB	S.E.M.	Significance[‡]	
				D	SD
Milk yield (kg head^{-1} day^{-1})	1.02	1.06	0.046	ns	**
Fat (%)	6.52	6.65	0.371	ns	*
Protein (%)	5.80	6.04	0.219	ns	ns
Lactose (%)	4.51	4.48	0.082	ns	ns
Casein (%)	4.61	4.78	0.198	ns	ns
SNF (%)	11.03	11.24	0.179	ns	ns
SCC (n*10^3 mL^{-1})	136.38	138.13	66.874	ns	0.07
TBC (CFU*10^3 mL^{-1})	42.25	63.74	15.201	ns	***

[†] Diet C, control diet based on 1.5 kg alfalfa hay and 0.7 kg commercial concentrate; Diet PB, experimental diet based on 1.5 kg alfalfa hay + 0.3 kg pea seeds + 0.3 kg barley; S.E.M., standard error of mean; D, effect of diet; SD, effect of sampling date; SNF, solids non-fat; SCC, somatic cell count; TBC, total bacterial count.

[‡] Probability: *: P≤0.05; **: P≤0.01; ***: P≤0.001; the P-value is shown if, thus being not significant, it shows a tendency (P<0.10); ns, not significant (P≥0.10). The effect of interaction between diet and sampling date (D×SD) was not significant; therefore significance are only presented for diet (D) and sampling date (SD).

Table 4

Table 4. Mean contents (g $100g^{-1}$ fat) of groups of fatty acids in milk fat of ewes fed the control (C) and experimental (PB) diets.[†]

	Diet C	Diet PB	S.E.M.	Significance[‡]
				D
Σ short chain[a]	12.92	14.82	0.697	*
Σ medium chain[b]	42.05	44.57	1.247	0.07
Σ long chain[c]	31.99	27.59	1.638	*
Σ saturated[d]	60.47	64.75	1.320	**
Σ branched chain[e]	2.50	2.84	0.186	ns
Σ monounsaturated[f]	21.79	17.63	1.060	**
Σ C18:1[g]	19.60	15.86	1.063	**
Σ C18:1 *trans*[h]	2.15	1.35	0.102	***
Σ polyunsaturated[i]	4.68	4.34	0.293	ns
Σ C18:2[j]	3.47	2.98	0.221	*
Σ C18:2 *trans*[k]	1.38	0.99	0.084	***
Σ *trans* without CLA[l]	5.33	3.54	0.251	***
Σ n3 FA[m]	1.06	1.24	0.082	ns
Σ n6 FA[n]	3.31	2.91	0.210	0.08
Σ n6/n3	3.13	2.35	0.108	***
Σ CLA[o]	0.72	0.47	0.052	***
Σ unsaturated[p]	26.47	21.98	1.290	**
HSFA[§]	67.13	72.46	2.453	0.07

[†] Diet C, control diet based on 1.5 kg alfalfa hay and 0.7 kg commercial concentrate; Diet PB, experimental diet based on 1.5 kg alfalfa hay + 0.3 kg pea seeds + 0.3 kg barley; S.E.M., standard error of mean; D, effect of diet; CLA, conjugated linoleic acid; HSFA, hypercholesterolemic saturated fatty acids.

[‡] Probability: *: P≤0.05; **: P≤0.01; ***: P≤0.001; the P-value is shown if, thus being not significant, it shows a tendency (P<0.10); ns, not significant (P≥0.10). The effect of sampling date (SD) and interaction between diet and sampling date (D×SD) were not significant; therefore significance is only presented for diet (D).

[§] Calculated as C12+4*C14+C16.

[a] C4, C5, C6, C7, C8, C10, C10:1.

[b] C12, C13 *iso*, C13 *aiso*, C12:1 *c*+C13, C14 *iso*, C14, C15 *iso*, C14:1 *t*, C15 *aiso*, C14:1 *c*, C15, C16 *iso*, C16, C17 *iso*, C16:1 *t*, C17 *aiso*, C16:1 *c*.

[c] C17, C18 *iso*, C17:1 *t*, C18 *aiso*, C18, Σ C18:1, C19, Σ C18:2, C20, C20:1 *t*, C18:3 *c*6*c*9*c*12, C20:1 *c*5, C20:1 *c*9, C20:1 *c*11, C18:3 *c*9*c*12*c*15, C18:2 *c*9*t*11+*t*8*c*10+*t*7*c*9, C18:2 *t*11*c*13+*c*9*c*11, C18:2 *t*9*t*11, C20:2 *c*,*c* n6, C22, C20:3n6, C20:3n3, C20:4n6 (AA), C20:5n3 (EPA), C22:5n3 (DPA), C22:6n3 (DHA).

[d] C4, C5, C6, C7, C8, C10, C12, Σ branched chain, C14, C15, C16, C17, C18, C19, C20, C22.

[e] C13 *iso*+*aiso*, C14 *iso*, C15 *iso*+*aiso*, C16 *iso*, C17 *iso*+*aiso*, C18 *iso*+*aiso*.

[f] C10:1, C12:1 *c*+*C*13, C14:1 *ct*, C16:1 *ct*, C17:1 *t*, Σ C18:1, C20:1 *t*, C20:1 *c*5, C20:1 *c*9, C20:1 *c*11.

[g] C18:1 *t*4, *t*5, *t*6-11, *t*12-14+*c*6-8, *c*9, *c*11, *c*12, *c*14+*t*16.

[h] C18:1 *t*4, *t*5, *t*6-11, *t*12-14+*c*6-8.

[i] Σ C18:2, C18:3 *c*6*c*9*c*12, C18:3 *c*9*c*12*c*15, C20:2 *c*,*c* n6, C20:3n3, C20:3n6, C20:4n6 (AA), C20:5n3 (EPA), C22:5n3 (DPA), C22:6n3 (DHA).

[j] C18:2 *tt*NMID+*t*9*t*12, *c*9*t*13+*t*8*c*12, *c*9*t*12, *c*,*c*-MID+*t*8*c*13, *t*11*c*15, *t*9*c*12, *c*9*c*12, *c*9*c*15, *c*9*t*11+*t*8*c*10+*t*7*c*9, *t*10*c*12, *t*11*c*13+*c*9*c*11, *t*9*t*11.

[k] C18:2 *tt*NMID+*t*9*t*12, *c*9*t*13+*t*8*c*12, *c*9*t*12, *c*,*c*-MID+*t*8*c*13, *t*11*c*15, *t*9*c*12, C18:2 *c*9*t*11+*t*8*c*10+*t*7*c*9, C18:2 *t*10*c*12, C18:2 *t*11*c*13+*c*9*c*11, C18:2 *t*9*t*11.

[l] C14:1 *t*, C16:1 *t*, C17:1 *t*, Σ C18:1 *t* , Σ C18:2 *t* (without CLA *trans*), C20:1 *t*.

[m] C18:2 *t*11*c*15+C18:2 *c*9*c*15, C18:3 *c*9*c*12*c*15, C20:3n3, C20:5n3 (EPA), C22:5n3 (DPA), C22:6n3 (DHA).

[n] C18:1 *t*12, C18:1 *c*12, C18:2 *tt*NMID+*t*9*t*12, C18:2 *c*9*t*12, C18:2 *t*9*c*12, C18:2 *c*9*c*12, C18:3 *c*6*c*9*c*12, C20:2 *cc* n6, C20:3n6, C20:4n6 (AA).

[o] C18:2 *c*9*t*11+*t*8*c*10+*t*7*c*9, *t*10*c*12, *t*11*c*13+*c*9*c*11, *t*9*t*11.

[p] C10:1, C12:1 *c*+C13, C14:1 *ct*, C16:1 *ct*, C17:1 *t*, Σ C18:1, Σ C18:2, C20:1 *t*, C18:3 *c*6*c*9*c*12, C20:1 *c*5, C20:1 *c*9, C20:1 *c*11, C18:3 *c*9*c*12*c*15, C18:2 *c*9*t*11+*t*8*c*10+*t*7*c*9, C18:2 *t*11*c*13+*c*9*c*11, C18:2 *t*10*c*12,

C18:2 *t*9*t*11, C20:2 *c*,*c* n6, C20:3n6, C20:3n3, C20:4n6 (AA), C20:5n3 (EPA), C22:5n3 (DPA), C22:6n3 (DHA).

Table 5

Table 5. Mean contents (g $100g^{-1}$ fat) of individual fatty acids in milk fat of ewes fed the control (C) and experimental (PB) diets.[†]

	Diet C	Diet PB	S.E.M.	Significance[‡]
				D
C4	2.89	3.12	0.177	ns
C5	0.01	0.02	0.001	ns
C6	2.05	2.44	0.119	*
C7	0.02	0.03	0.003	*
C8	1.88	2.19	0.130	0.08
C10	5.74	6.69	0.448	0.09
C10:1	0.33	0.34	0.026	ns
C12	3.68	4.20	0.257	0.09
C13 *iso*	0.03	0.03	0.003	ns
C13 *aiso*	0.06	0.06	0.005	ns
C12:1 *c*+C13	0.16	0.17	0.011	ns
C14 *iso*	0.13	0.15	0.016	ns
C14	10.00	10.96	0.491	0.09
C15 *iso*	0.30	0.35	0.026	ns
C14:1 *t*	0.01	<0.01	0.001	*
C15 *aiso*	0.44	0.53	0.043	ns
C14:1 *c*	0.29	0.21	0.026	*
C15	1.03	1.22	0.095	ns
C16 *iso*	0.25	0.33	0.033	ns
C16	23.45	24.43	0.872	ns
C17 *iso*	0.38	0.41	0.038	ns
C16:1 *t*	0.08	0.05	0.008	**
C17 *aiso*	0.63	0.68	0.040	ns
C16:1 *c*	1.14	0.81	0.065	***
C17	0.61	0.72	0.057	ns
C18 *iso*	<0.01	<0.01	0.001	ns
C17:1 *t*	0.07	0.08	0.009	ns
C18 *aiso*	0.28	0.30	0.025	ns
C18	6.21	5.80	0.429	ns
C18:1 *t*4	<0.01	<0.01	0.001	ns
C18:1 *t*5	0.01	<0.01	0.001	**
C18:1 *t*6-11	1.72	1.06	0.079	***
C18:1 *t*12-14+*c*6-8	0.42	0.29	0.032	**
C18:1 *c*9	16.67	13.81	0.968	**
C18:1 *c*11	0.42	0.43	0.036	ns
C18:1 *c*12	0.18	0.13	0.053	***
C18:1 *c*14+*t*16	0.18	0.14	0.013	*
C19	0.11	0.12	0.012	ns
C18:2 *tt*NMID+9,12*t*	0.08	0.06	0.001	*
C18:2 *c*9*t*13+*t*8*c*12	0.03	0.03	0.003	ns
C18:2 *c*9*t*12	0.19	0.12	0.015	**
C18:2 *c*,*c*MID+*t*8*c*13	0.17	0.11	0.012	**
C18:2 *t*11*c*15	0.09	0.10	0.011	ns
C18:2 *t*9*c*12	0.10	0.10	0.095	ns
C18:2 *c*9*c*12 (LA)	2.09	1.98	0.152	ns
C18:2 *c*9*c*15	<0.01	<0.01	0.001	ns
C20	0.24	0.25	0.017	ns
C20:1 *t*	0.04	0.05	0.003	**
C18:3 *c*6*c*9*c*12 (GLA)	0.03	0.02	0.002	ns
C20:1 *c*5	<0.01	<0.01	0.001	ns
C20:1 *c*9	0.03	0.03	0.003	ns
C20:1 *c*11	0.06	0.04	0.004	**

Table 5. Continued.

C18:3 *c*9*c*12*c*15 (ALA)	0.70	0.84	0.069	ns
CLA *c*9*t*11+*t*8*c*10+*t*7*c*9	0.70	0.45	0.051	**
CLA *t*10*c*12	0.002	<0.001	0.0003	***
CLA *c*9*c*11+*t*11*c*13	0.02	0.02	0.002	ns
CLA *t*9*t*11	0.01	0.01	0.002	ns
C20:2 *c*,*c* n6	0.01	0.02	0.003	ns
C22	0.04	0.04	0.004	ns
C20:3n6	0.02	0.02	0.002	ns
C20:3n3	0.01	0.01	0.001	ns
C20:4n6 (AA)	0.18	0.17	0.011	ns
C20:5n3 (EPA)	0.06	0.07	0.006	ns
C22:5n3 (DPA)	0.13	0.15	0.015	ns
C22:6n3 (DHA)	0.07	0.08	0.018	ns

† Diet C, control diet based on 1.5 kg alfalfa hay and 0.7 kg commercial concentrate; Diet PB, experimental diet based on 1.5 kg alfalfa hay + 0.3 kg pea seeds + 0.3 kg barley; S.E.M., standard error of mean; D, effect of diet; CLA, conjugated linoleic acid; *t* = *trans*; *c* = *cis*; NMID, non methylene interrupted diene; MID, methylene interrupted diene; LA, linoleic acid; GLA, gamma-linoleic acid; ALA, alpha-linoleic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

‡ Probability: *: P≤0.05; **: P≤0.01; ***: P≤0.001; the P-value is shown if, thus being not significant, it shows a tendency (P<0.10); ns, not significant (P≥0.10). The effect of sampling date (SD) and interaction between diet and sampling date (D×SD) were not significant; therefore significance is only presented for diet (D).