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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 ($P \leq 0.001$) and total conjugated linoleic acids ($P \leq 0.001$). 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.

1. 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

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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.

2. Materials and methods

2.1. 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 16 to March 30, 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 2702 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.

2.2. Sampling procedures and laboratory analyses

2.2.1. 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.

2.2.2. Milk

Milk samples were collected after a 10-days period of adaptation to the diets (from January 16 to January 25). Individual daily milk yields were recorded once a week for 10 weeks. Two aliquots of each individual milk sample were collected during the morning milking every 3 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 7300 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, 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, Milano, Italy); C18:1 *c*6 (Supelco, Sigma–Aldrich Milano, 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 100 g^{-1} fat.