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Legume grain-based supplements in dairy sheep diet: effects on milk yield, composition and fatty acid profile

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Abstract. With the aim to find protein sources that are free of genetically modified organisms, the effects of legume grain-based concentrates, used as alternatives of a mixed concentrate feed containing soybean, were evaluated on sheep milk production. Twelve lactating ewes were divided into four groups, fed hay and, according to a 4 × 4 Latin square design, supplied with 800 g/day of a commercial mixed concentrate feed (MCF) containing maize and soybean, or the same amount of isoprotein concentrates consisting of chickpea (CH), faba bean (FB), or pea (PE) mixed with barley. The ewes ingested more of the concentrates with legume grains than the MCF (702, 702, 678 vs 587 g/day DM for CH, FB, PE and MCF; $P \leq 0.001$). Compared with CH, FB and PE resulted in greater ($P \leq 0.05$) milk yield (710, 718 vs 654 g/day for FB, PE and CH, respectively), and led to a greater ($P \leq 0.05$) efficiency of dietary protein utilisation for milk casein synthesis (94, 97 vs 87 g casein/kg crude protein intake for FB, PE and CH, respectively), whereas MCF resulted in intermediate levels of milk yield (677 g/day) and milk casein/crude protein intake (88 g/kg). Chickpea increased the milk content of *trans*-vaccenic and rumenic acids in comparison with FB and PE and, similarly to MCF, increased the milk content of linoleic acid, as well as total unsaturated fatty acids (24.3, 23.9 vs 17.2, 16.8 g/100 g fatty acid methyl esters for MCF, CH, FB and PE; $P \leq 0.001$), thereby improving the potential health-promoting index. Legume grains can replace soybean in diets of dairy ewes, as they do not adversely affect milk yield and composition.

Additional keywords: chickpea, faba bean, milk fatty acids, organic milk, pea, sheep milk, soybean.

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Introduction

Soybean and maize are the most widely cultivated crops for animal feed. However, the European regulations on organic production (Council Regulation No. 834/2007; Commission Regulation No. 889/2008) prohibit the use of feeds in livestock farming that contain genetically modified organisms. Therefore, there has been a need for organic farmers to introduce alternative vegetable protein sources in the diet of animals, as in Europe it is difficult and costly to find soybean and maize that are not genetically modified (Nabradi and Popp 2011). Moreover, in Italy, soybean and maize are often imported, and long and inadequate transport and storage conditions can promote the development of moulds that produce mycotoxins, such as aflatoxins, which are potent carcinogens that pose health risks to both animals and humans (Bryden 2012).

In view of the need to replace soybean with alternative protein sources, particular interest has been placed on legume grains, linked to many factors: their high rate of diffusion, and consequently, their ready availability in several local contexts (Sinclair and Vadez 2012), as well as in the Mediterranean areas; their suitability to different agronomic conditions (López-Bellido *et al*. 2005) and proper organic methods of cultivation (Badgley *et al*. 2007); the agronomic benefits in terms of soil fertility and structure due to the high levels of N and organic matter that they provide when included in crop rotations (Sinclair and Vadez 2012); the good nutritional value of their crop residues that can be directly exploited by grazing animals (Sinclair and Vadez 2012); the fact that they are not genetically modified; their low risk of mycotoxin contamination since they are less subjected to long distance transport or storage condition that could favour their development (Bryden 2012); the mostly present anti-nutritional compounds, such as lectins and protease inhibitors (trypsin and chymotrypsin inhibitors) (Dixon and Hosking 1992; Friedman 1996), that seem to be inactivated by rumen fermentation, thus they do not impair nutrients utilisation for ruminants (Dixon and Hosking 1992; Holmes *et al*. 1993); and their high content in crude protein (CP) [>24% of total dry matter (DM)], starch, and, on occasions lipids (Dixon and Hosking 1992; Cutrignelli *et al*. 2011), as in chickpea (~5% DM) (Priolo *et al*. 2003). Thus, the use of legume grains would allow the safer production of milk and cheese for consumers, since the animals would ingest feeds that have a lower risk to be contaminated by dangerous mycotoxins. These benefits could lead to an increase in the production of legume crops on both

organic and conventional farming systems, and could also promote a greater appreciation of relative livestock products.

Studies on the use of legume grains as alternative protein sources for sheep have largely been related to meat production (Vasta *et al*. 2008), and have shown partial or total replacement of soybean with chickpea (Hadjipanayiotou 2002; Christodoulou *et al*. 2005), faba bean (Lanza *et al*. 1999; Antongiovanni *et al*. 2002; Lanza *et al*. 2011), and pea (Lanza *et al*. 2003, 2011; Loe *et al*. 2004). These protein sources have no adverse effects on lamb growth rate or on carcass and meat characteristics, and are able to increase the amount of omega-3 fatty acids (FA) in the intramuscular fat (Priolo *et al*. 2003; Lanza *et al*. 2011; Scerra *et al*. 2011). When compared simultaneously, chickpea, faba bean, and pea were able to completely replace soybean concentrate, resulting in comparable growth rates, carcass characteristics, and meat quality of lambs slaughtered at 130 days of age (Bonanno *et al*. 2012).

Few studies have focussed on the use of grains from different species of legumes in the feed of lactating ewes and goats, and their relative effects on the quality of dairy products (Vasta *et al*. 2008). In general, the use of various legume seeds to replace soybean protein of the concentrate does not affect milk production of small ruminants, as demonstrated with chickpea (Christodoulou *et al*. 2005), faba bean (Liponi *et al*. 2007; Ramos Morales *et al*. 2008), and pea (Bonomi *et al*. 2003; Liponi *et al*. 2007; Renna *et al*. 2012). In particular, there have been a very limited number of studies on the impact of legume seeds on the milk FA profile. In regard to sheep dairy products, Di Grigoli *et al*. (2009) found that a concentrate based on barley and tick bean that was supplied to grazing ewes resulted in higher milk yield than an isoenergetic and isoprotein concentrate based on maize and soybean, as well as in an enrichment of the lipid fractions of milk and cheese with α-linolenic acid (ALA) (C18:3 n-3 *c9*, *c12*, *c15*), and consequently, with total omega-3 FA. More recently, Renna *et al*. (2012) observed an increase of short-chain and saturated FA in milk from ewes receiving a concentrate based on pea and barley compared with a commercial concentrate containing sunflower meal and soybean seeds, with no significant differences in milk yield.

Among the few studies that have investigated the effects of different legume grains on sheep dairy production, no information has been published on the effect of chickpea on the milk FA profile, nor has there been a direct comparison of grains from more common legume species, especially those dominant in the Mediterranean environment. Therefore, the aim of this study was to evaluate the effects of feeding supplements based on different Mediterranean legume grains, such as chickpea, faba bean, or pea, on ewes' feed intake and milk yield and quality, including the FA profile. In addition, the potential advantage of using these alternative protein sources was verified by comparing the legume grain-based concentrates with a concentrate feed containing soybean.

Materials and methods

Animals and experimental design

This experiment was carried out over the course of 14 weeks (January–April 2010) on the Pietranera Experimental Farm (Fondazione Lima-Mancuso, Università degli Studi di Palermo), which is located in the province of Agrigento in Sicily, Italy (37°32′N, 13°31′E; 178 m above sea level).

Twelve lactating ewes of the Comisana breed in their third or fourth lactation were homogeneously allocated into four groups based on parity, liveweight (56 ± 6 kg), body condition score (BCS) (2.87 ± 0.18) assessed according to Russel *et al*. (1969), days in milk (92 ± 9), and milk yield (875 ± 60 g/day). During the experiment, the ewes were housed in individual wheat straw-bedded pens (4 m^2), placed inside a semi-open shelter.

After a 2-week adaptation period to the experimental conditions, each group of ewes was fed in sequence with one of four diets, according to a 4 × 4 Latin-square design with periods comprised of 21 days, 16 days for adaptation to the diets and 5 days for measuring and sampling. With regard to the experimental diets, in each period, the ewes of each group were fed *ad libitum* with hay of berseem clover and spontaneous grasses, mainly *Lolium* spp. and *Phalaris* spp., plus 800 g/day divided into two meals of one of the following isoprotein concentrates obtained by mixing different proportions of chickpea (*Cicer arietinum* L.), faba bean (*Vicia faba* var. *minor* L.), or pea (*Pisum sativum* L.) with barley in the form of coarsely ground meal: 500 g chickpea and 300 g barley; 450 g faba bean and 350 g barley; 550 g pea and 250 g barley. The three legume grain-based concentrates were compared with a commercial mixed concentrate feed (MCF) containing maize and soybean, with the same CP content.

Measurements, sampling, and analyses

At the beginning and end of each experimental period, the ewes were weighed and the BCS was checked (Russel *et al*. 1969).

Feed

During the last 5 days of each experimental period, the offered and refused hay and concentrate of each ewe were weighed daily and sampled once to estimate the amount and quality of feed intake. The samples of hay and concentrates were analysed for determination of DM, CP (N × 6.25), ether extract (EE), ash (AOAC 2000), and structural carbohydrates, such as neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) (Van Soest *et al*. 1991). Non-fibre carbohydrates (NFC) content was calculated as follows: NFC = 100 – (%NDF + %CP + %EE + %ash). Energy content, expressed in Mcal of net energy for lactation (NE_L), was estimated using equations from the National Research Council (2001) based on digestibility and ADF content.

The FA composition of lyophilised feed samples (50 mg) was determined using the one-step extraction and transesterification procedure according to Sukhija and Palmquist (1988), with C19:0 as the internal standard (Sigma-Aldrich, Milano, Italy). Identification of feed FA was performed using the same procedure described below for milk FA.

Milk

During the last 5 days of each experimental period, individual milk yield was recorded daily, in the morning (7:00 a.m.) and evening milking (4:00 p.m.), and sampled three times, on Days 17, 19, and 21. Daily individual milk samples were composed by unifying amounts of morning and evening milk in proportion to

respective yields. Individual milk samples were analysed for lactose, fat, protein, casein, and somatic cell count (SCC) using the infrared method (Combi-Foss 6000, Foss Electric, Hillerød, Denmark). Total bacterial count (TBC) was determined using the BactoScan instrument (Foss Electric), pH was determined using the HI 9025 pH-meter (Hanna Instruments, Ann Arbor, MI, USA), titratable acidity was evaluated using the Soxhlet-Henkel method (°SH/50 mL), and urea levels were analysed by the enzymatic method using differences in pH (CL-10 Plus, Eurochem, Roma, Italy). Fat and protein-corrected milk with 6.5% fat and 5.8% protein was calculated as follows: fat and protein-corrected milk g/day = milk kg × (0.25 + %fat + %protein) (Pulina and Nudda 2002). Individual milk samples were also evaluated for their clotting ability by measuring coagulation time (r, min), curd firming time (k_{20}, min), and curd firmness after 30 min (a_{30}, mm), according to Zannoni and Annibaldi (1981), in 10 mL of milk at 35°C with 0.2 mL diluted solution (1.6 : 100) of rennet (1 : 15 000; Chr. Hansen, Parma, Italy), using the Formagraph (Foss Electric).

Milk FA were determined from the daily individual milk samples collected at the end of each experimental period, on Day 21. FA in lyophilised milk samples (100 mg) were directly methylated with 1 mL hexan and 2 mL 0.5 M $NaOCH_3$ at 50°C for 15 min, followed by 1 mL 5% HCl in methanol at 50°C for 15 min (Loor *et al*. 2002). FA methyl esters (FAME) were recovered in hexane (1.5 mL). One microlitre of each sample was injected by an autosampler into an HP 6890 gas chromatography system equipped with a flame-ionisation detector (Agilent Technologies, Santa Clara, CA, USA). FAME from all samples were separated using a 100-m length, 0.25-mm i.d., and 0.25-μm-film-thickness capillary column (CP-Sil 88, Chrompack, Middelburg, The Netherlands). The injector temperature was kept at 255°C, and the detector temperature was kept at 250°C, with an H_2 flow of 40 mL/min, an air flow of 400 mL/min, and a constant He flow of 45 mL/min. The initial oven temperature was held at 70°C for 1 min, increased 5°C/min to 100°C, held for 2 min, increased 10°C/min to 175°C, held for 40 min, and finally increased 5°C/min to a final temperature of 225°C, and held for 45 min. Helium, with a head pressure of 23 psi and a flow rate of 0.7 mL/min (linear velocity of 14 cm/s), was used as the carrier gas. A FAME hexane mix solution (Nu-Check-Prep, Elysian, MN, USA) was used to identify each FA. Individuals C15:0 *iso*, C15:0 *anteiso*, C17:0 *iso* and C17:0 *anteiso* (Larodan Fine Chemicals AB, Malmö, Sweden) were used to identify these FA. A standard mixture of methyl esters of C18:2 *c9*, *t11* and C18:2 *c10*, *t12* (Sigma-Aldrich) and published isomeric profile (Kramer *et al*. 2004; Luna *et al*. 2005) were used to help in identifying the conjugated linoleic acid (CLA) isomers. The health-promoting index was calculated according to Chen *et al*. (2004): total unsaturated FA/[C12:0 + (4 × C14:0) + C16:0].

Statistical analyses

Statistical analysis was carried out using the MIXED procedure in SAS (2010) 9.2 software. In the mixed model used for the data of ewes' liveweight and BCS and milk FA composition, the experimental period (four levels) and type of concentrate (four levels) were fixed factors, and the ewe was considered a random factor and used as error term. For data of ewes' feed intake and milk production, the effects of experimental period, day within experimental period (five or three levels) and type of concentrate were tested by means of a repeated-measures mixed model, with the experimental day being used as the repeated-measures unit, and the ewe being the repeated subject treated as a random factor. Before analysis, SCC and TBC values were transformed into logarithmic form ($\log_{10}$). When a statistically significant effect ($P \leq 0.05$) of the type of concentrate was detected, means were compared using *P*-values adjusted according to Tukey–Kramer multiple comparisons test. A *P*-value equal or less than 0.05 has been used to indicate statistically significant differences among means in the tables.

Results and discussion

Feed composition and intake

The chemical and FA composition of the dietary components and experimental concentrates are shown in Table 1. The legume grains, especially the faba bean, had a high protein content, whereas the chickpea were less fibrous and higher in ether extract than the other protein sources. Since legume grain-based concentrates were formulated to obtain a similar protein content between them and MCF, equal to 23% DM, the amount of barley was different among the mixtures (37.1%, 44.4%, and 31.8% DM for chickpea, faba bean, and pea concentrate, respectively), and was highest in the faba bean concentrate, since the faba bean had the highest protein content.

The concentrate mixtures showed differences in fibre content, expressed as NDF, which was lowest in the chickpea mix. They also showed differences in lipid level, which was high in the chickpea mix and even higher in MCF.

With regard to FA composition (Table 1), linoleic acid (LA) (C18:2 n-6 *c9*, *c12*) was the predominant FA in each of the used legume grains, making up nearly 50% of total FA, followed by oleic acid (C18:1 *c9*) (OA), as also reported by other authors (Priolo *et al*. 2003; Lanza *et al*. 2011; Renna *et al*. 2012). However, as a result of the lipid content of chickpea, higher than that of faba bean and pea, the chickpea-based concentrate provided higher amounts of LA and OA, as well as total polyunsaturated FA (PUFA) and unsaturated FA than the other legume grain-based concentrates, approaching those of MCF, which showed the highest lipid percentage.

Table 2 provides the daily DM and nutrient intake for concentrate, hay, and total diet. The voluntary intake of concentrates, expressed on an as-fed basis, indicated that alternative legume grain mixtures were not always completely consumed by ewes, with the exception of the concentrate with faba bean. However, the levels of DM intake of the three legume grains mixtures were higher than that of the MCF and, as a consequence, their percentages in the diet were higher by ~5% than that of the MCF (24.8% DM), suggesting a good acceptance of concentrates with legume grains for the lactating ewes.

Due to the lower DM intake, the amount of ingested protein from MCF tended to be lower than that of the experimental concentrates. Moreover, more intake of lignin and less of NFC and NE_L were also recorded with MCF. The concentrate with

Table 1. Chemical (% DM) and fatty acid composition (g/kg DM) of dietary components and concentrates
OA, oleic acid; LA, linoleic acid; ALA, α-linolenic acid; PUFA, polyunsaturated fatty acids

	Dietary components					Concentrates			
	Chickpea	Faba bean	Pea	Barley	Hay	Chickpea	Faba bean	Pea	MCF[A]
DM	90.5	86.8	86.9	89.0	91.7	89.9	87.7	87.6	88.0
Crude protein	28.1	30.2	26.9	13.9	12.1	22.9	23.0	22.8	23.3
Ether extract	5.09	1.39	1.48	1.76	1.67	3.85	1.55	1.57	5.09
Ash	3.56	3.35	3.16	2.98	10.74	3.34	3.19	3.10	9.22
NDF	12.4	19.3	20.0	18.3	54.8	14.6	18.9	19.5	23.2
ADF	5.50	12.6	8.95	9.03	39.9	6.81	11.0	8.98	11.0
ADL	0.28	0.58	0.18	1.03	5.29	0.56	0.78	0.45	2.60
NFC[B]	50.8	45.7	48.5	63.0	20.7	55.3	53.4	53.0	39.2
NE_L (Mcal/kg DM)	2.20	2.03	2.10	2.02	1.27	2.13	2.03	2.07	1.80
					Fatty acids				
C12:0	–	–	–	0.065	0.068	0.024	0.029	0.021	0.096
C14:0	0.084	0.012	–	0.27	0.26	0.15	0.13	0.086	0.12
C15:0	0.039	0.008	–	0.015	0.041	0.030	0.011	0.005	–
C16:0	4.82	1.66	1.71	2.77	3.13	4.06	2.15	2.05	6.06
C16:1 *c9*	0.13	0.016	–	0.045	0.16	0.096	0.029	0.014	–
C17:0	0.028	0.020	–	0.013	–	0.022	0.017	0.004	–
C18:0	0.66	0.52	0.36	0.44	0.70	0.58	0.48	0.38	1.62
C18:1 *c9*, OA	12.9	1.81	3.66	2.85	1.27	9.14	2.27	3.40	14.0
C18:1 *c11*	0.73	0.23	–	0.13	0.059	0.508	0.18	0.041	1.61
C18:2 n-6 *c9*, *c12*, LA	23.8	6.46	6.29	7.39	3.28	17.7	6.87	6.64	22.8
C18:3 n-3 *c9*, *c12*, *c15*, ALA	1.09	1.18	0.46	0.87	5.42	1.01	1.04	0.59	1.70
C20:0	0.25	0.036	0.22	0.025	0.098	0.17	0.031	0.16	0.30
C20:1 *c9*	0.20	0.017	0.21	0.13	–	0.18	0.067	0.19	0.27
C22:0	0.16	0.049	–	–	0.086	0.10	0.027	–	0.24
PUFA	24.9	7.64	6.75	8.26	8.70	18.7	7.91	7.23	24.5
Unsaturated FA	38.8	9.71	10.6	11.4	10.2	28.6	10.5	10.9	40.4

[A]MCF = mixed concentrate feed composed of: maize, wheat middlings, extruded soybean meal, extruded sunflower meal, toasted soybean seeds, maize gluten feed, ground wheat, barley, soybean hulls, extruded maize germ meal, sugar beet molasses, dried sugar beet pulp, calcium carbonate, dicalcium phosphate, sodium chloride, magnesium oxide, vitamin-mineral mix.
[B]NFC = non-fibre carbohydrates = 100 – (%NDF + %CP + %EE + %ash).

chickpea provided less fibre (NDF) but, similar to the MCF, more lipids.

Despite the differences in concentrate ingestion, the DM intake with diet (concentrate plus hay) did not significantly differ among the groups, since the ewes who received MCF were able to compensate by ingesting a greater amount of hay. Accordingly, the ewes given MCF had similar total protein intake compared with the other groups, and ingested a higher total amount of fibre (NDF), even though at a non-significant level in comparison with ewes receiving faba bean, balanced with a lower NFC intake. On the whole, NE_L intake of ewes fed MCF was slightly lower only in comparison with ewes fed faba bean.

Mainly as a result of the concentrates supply, the ewes receiving MCF showed the highest total intake of lipid, as wells as LA, OA, total PUFA and unsaturated FA, closely followed by the ewes fed chickpea.

Liveweight and BCS variation

Regardless of concentrate, the ewes showed an increase in liveweight ($P \leq 0.01$) and an improvement in BCS ($P \leq 0.01$) from the start to the end of each experimental period, but these variations were not significantly different among the groups (Table 3). However, this improvement was more limited in the ewes fed chickpea, which showed a low weight gain of only 0.5%, and a negligible increase in BCS. Thus, the final BCS of ewes fed chickpea was lower than that of ewes fed faba bean.

Milk yield and properties

The individual average milk yield was higher with faba bean and pea concentrates than with chickpea, whereas MCF resulted in an intermediate level of milk production (Table 3). In this regard, Di Grigoli *et al*. (2009) observed higher milk yield from grazing ewes fed a diet supplemented with barley and thick bean than one with maize and soybean, whereas pea was able to replace total soybean in the concentrate for dairy cows reared on both organic (Di Grigoli *et al*. 2008) and conventional farms (Tufarelli *et al*. 2012), resulting in comparable milk yield, quality, and cheese-making properties. Moreover, a pea–barley mix given to lactating ewes led to a milk yield comparable to that obtained with a mixed concentrate based on sunflower meal and soybean seeds (Renna *et al*. 2012).

In dairy sheep, Christodoulou *et al*. (2005) found that replacing soybean meal with chickpea in the diet did not influence milk yield or composition. Analogous results have been obtained in the present study where, giving a daily amount of chickpeas, which was almost double than that used

Table 2. Effects of legume grain-based concentrates on DM and nutrient intake from concentrate and hay (g/day)

MCF, mixed concentrate feed; s.e.m., standard error of the mean; n.s., not significant; OA, oleic acid; LA, linoleic acid; PUFA, polyunsaturated fatty acids. ***, $P \leq 0.001$. **, $P \leq 0.01$. *, $P \leq 0.05$. a, b, c: $P \leq 0.05$

	Concentrates				s.e.m.	*P*-value
	Chickpea	Faba bean	Pea	MCF		
Ewes (*n*)	12	12	12	12	–	–
Concentrate (% DM intake)	30.4a	29.3a	29.3a	24.8b	1.61	***
Hay (% DM intake)	69.6b	70.7b	70.7b	75.2a	1.61	***
Concentrate						
As-fed basis	780a	800a	774a	667b	27.1	**
DM	702a	702a	678a	587b	23.9	***
Crude protein	161a	162a	156a	137b	5.45	**
Ether extract	27.1b	10.9c	10.6c	29.9a	1.13	***
Ash	23.5b	22.4b	21.1b	54.1a	2.00	***
NDF	102b	133a	132a	136a	5.26	***
ADF	47.7c	77.3a	60.9b	64.4b	2.49	***
ADL	3.91bc	5.47b	2.99c	15.3a	0.561	***
NFC[A]	390a	376a	360a	230b	10.5	***
NE_L (Mcal/day)	1.50a	1.42a	1.41a	1.06b	0.045	***
C18:1 *c9*, OA	6.41b	1.59c	2.31c	8.22a	0.307	***
C18:2 n-6 *c9*, *c12*, LA	12.4a	4.82b	5.50b	13.4a	0.507	***
PUFA	13.1a	5.56b	4.90b	14.5a	1.547	***
Unsaturated FA	20.1b	7.35c	7.37c	23.8a	0.895	***
Hay						
DM	1619b	1741ab	1681b	1809a	97.1	**
Crude protein	220b	230ab	228ab	240a	11.9	**
Ether extract	30.6b	31.7ab	31.7ab	33.4a	1.62	**
Ash	171b	185a	179ab	191a	10.4	**
NDF	842b	912ab	874ab	946a	53.3	**
ADF	618b	670ab	642ab	693a	38.8	**
ADL	89.2b	94.7ab	93.5ab	97.3a	5.12	*
NFC[A]	356c	384ab	368bc	399a	20.2	***
NE_L (Mcal/day)	2.05b	2.21ab	2.12b	2.32a	0.124	**
C18:1 *c9*, OA	2.06b	2.21ab	2.13b	2.30a	0.124	**
C18:2 n-6 *c9*, *c12*, LA	5.31b	5.71ab	5.51b	5.93a	0.318	**
PUFA	14.1b	15.1ab	14.6b	15.7a	0.844	**
Unsaturated FA	16.5b	17.7ab	17.1b	18.4a	0.989	**
Diet (concentrate plus hay)						
DM	2320	2443	2359	2396	97.2	n.s.
Crude protein	380	391	384	377	12.6	n.s.
Ether extract	57.7b	42.7c	42.4c	63.3a	1.88	***
Ash	194b	207b	200b	245a	10.3	***
NDF	944b	1044a	1006ab	1082a	52.9	***
ADF	666c	748ab	703bc	757a	38.6	***
ADL	93.1c	100b	96.5bc	113a	5.09	***
NFC[A]	745b	760b	728b	630c	21.5	***
NE_L (Mcal/day)	3.55ab	3.64a	3.53ab	3.37b	0.126	*
C18:1 *c9*, OA	8.47b	3.81c	4.43c	10.5a	0.318	***
C18:2 n-6 *c9*, *c12*, LA	17.7b	10.5c	10.0c	19.3a	0.57	***
PUFA	27.2b	20.7c	19.5c	30.2a	0.96	***
Unsaturated FA	36.6b	25.1c	24.5c	42.2a	1.26	***

[A]NFC = non-fibre carbohydrate = 100 – (%NDF + %CP + %EE + %ash).

by Christodoulou *et al*. (2005), the milk yield was not different than that of ewes fed with MCF. Nevertheless, in the present study, the ewes receiving the chickpea concentrate showed a lower milk yield in comparison with the ewes fed with the other legume grains. However, these differences in production were not significant with regard to fat- and protein-corrected milk. The correction of milk yield on the basis of fat and protein percentage, which are the components that affect cheese yield, is particularly relevant for sheep milk that is entirely destined for cheese-making. The similarity of corrected milk yield among groups receiving legume grains is certainly due to the higher lipid content found in the milk of ewes fed chickpea, although at a significant

Table 3. Effect of legume grain-based concentrates on final liveweight and body condition score (BCS) of ewes, and milk yield, composition and coagulation properties

MCF, mixed concentrate feed; s.e.m., standard error of the mean; n.s., not significant. ***, $P \leq 0.001$. *, $P \leq 0.05$. a, b, c: $P \leq 0.05$

	Concentrates				s.e.m.	*P*-value
	Chickpea	Faba bean	Pea	MCF		
Final liveweight (kg)	59.1	60.2	59.8	60.0	1.62	n.s.
Liveweight variation (%)	0.53	3.04	3.59	2.00	0.916	n.s.
Final BCS (%)	2.97b	3.12a	3.03ab	3.06ab	0.047	*
BCS variation	0.03	0.15	0.12	0.06	0.042	n.s.
Milk (g/day)	654b	710a	718a	677ab	30.0	*
FPCM[A] (g/day)	735	766	781	741	30.9	n.s.
Milk samples (*n*)	36	36	36	36	–	–
Lactose (%)	4.32	4.29	4.29	4.35	0.049	n.s.
Fat (%)	7.66a	7.11b	7.22ab	7.29ab	0.374	*
Protein (total N × 6.38) (%)	6.74	6.86	6.73	6.58	0.232	n.s.
Casein (%)	5.24	5.28	5.17	5.09	0.190	n.s.
Casein N/total N	77.6a	77.0bc	76.8c	77.2ab	0.35	***
Protein/CP intake (g/kg)	111c	122ab	126a	114bc	4.70	*
Casein/CP intake (g/kg)	86.5c	93.7ab	96.6a	88.5bc	3.57	*
Urea (mg/dL)	37.6	38.8	37.5	38.3	1.23	n.s.
SCC (no. cells × 10^3/mL)	1000	652	579	954	79.9	n.s.
TBC (no. cells × 10^3/mL)	165	235	142	408	68.6	n.s.
pH	6.61	6.59	6.59	6.63	0.026	n.s.
Titratable acidity (°SH/50 mL)	4.94	4.72	5.08	4.72	0.181	n.s.
Coagulation time (r) (min)	26.8	26.5	28.2	28.5	2.69	n.s.
Curd firming time (k_{20}) (min)	1.80	1.82	2.68	1.91	0.373	n.s.
Curd firmness (a_{30}) (mm)	47.2	53.1	48.4	41.4	5.61	n.s.

[A]FPCM = milk corrected to 6.5% fat and 5.8% protein: milk kg * (0.25 + %fat + %protein) (Pulina and Nudda 2002).

level only in comparison with ewes fed faba bean, and presumably due to the concentration effect linked to a lower milk yield, rather than a direct effect of lipid intake.

The protein, casein, and urea contents of the milk (Table 3) showed no changes due to the protein source in the concentrate, whereas the ratio of casein N to total N improved with chickpea in comparison with the other legume grains. There were no significant differences among the groups with regard to SCC, TBC, or milk clotting ability (r, k_{20}, and a_{30}) (Table 3). However, it is worth noting that in all diets, milk urea levels were within normal limits for dairy ewes (Cannas *et al*. 1998), and the milk clotting parameters were close to those recorded for sheep milk (Bencini 2002).

In regard to the efficiency of dietary protein utilisation for milk protein or casein synthesis (Table 3), significantly higher values were obtained in ewes fed concentrates with faba bean and pea than in ewes receiving chickpea, whereas the differences did not reach a significant level with the MCF diet. Since the protein intake was the same, the improved utilisation of dietary N from faba bean and pea diets was probably due to the content of highly degradable NFC of these legume grains, favouring the balance between dietary energy and N in the synthesis of microbial protein in the rumen (Dewhurst *et al*. 2000).

Milk fatty acid composition

Milk FA composition varied according to protein source (Tables 4, 5). Compared with milk from faba bean and pea, those from chickpea concentrate and MCF were lower in most short- and linear medium-chain FA (from C9:0 to C17:0) (Table 4), which are entirely (linear even-chain FA from C6:0 to C14:0) o partly (linear odd-chain FA and C16:0) synthesised *de novo* within the mammary gland. Whereas the even-chain saturated FA (SFA) from C6:0 to C10:0 are of interest for human health, being used in the treatment of metabolic illness (Sanz Sampelayo *et al*. 2007), those from C12:0 to C16:0 are known for their hypercholesterolaemic effect, by increasing low density lipoprotein cholesterol (Ohlsson 2010).

With regard to the branched-chain FA (Table 4), the chickpea milk, compared with that from MCF, showed a lower content of C14:0 *iso*, tended to have a lower content of the C17:0 *iso* and, as a consequence, had a lower level of total *iso* branched-chain FA. However, in the present study, C15:0 *iso* and C16:0 *iso*, for which a certain anti-cancer activity is recognised (Parodi 2009), did not differ among groups. Among the main linear odd-chain FA, C15:0 was the lowest in chickpea milk, and C17:0 was lower in chickpea and MCF milk than in milk from the other diets. Both milk branched- and odd-chain FA are mainly derived from the biosynthesis of bacteria leaving the rumen, and thus reflect the changes in the rumen bacteria populations and are markers of the correspondent fermentation activity (Vlaeminck *et al*. 2006).

With regard to long-chain FA (Table 5), milk from the faba bean and pea groups showed lower stearic acid (C18:0), OA, and LA, as well as lower rumenic acid (RA) (C18:2 *c9 t11*), and its precursor, *trans*-vaccenic acid (VA) (C18:1 *t11*). The RA represents the most abundant among CLA isomers, and has been proposed as having anti-cancer and anti-atherogenic

Table 4. Effect of legume grain-based concentrates on short- and medium-chain fatty acid composition of milk (g/100 g FAME)

MCF, mixed concentrate feed; s.e.m., standard error of the mean; n.s., not significant. ***, $P \leq 0.001$. **, $P \leq 0.01$. *, $P \leq 0.05$. +, $P \leq 0.10$. a, b: $P \leq 0.05$

	Concentrates				s.e.m.	*P*-value
	Chickpea	Faba bean	Pea	MCF		
Milk samples (*n*)	12	12	12	12	–	–
C4:0	2.08	2.28	2.16	2.26	0.052	n.s.
C6:0	2.71	2.81	2.68	2.84	0.057	n.s.
C8:0	2.83	2.99	2.83	2.90	0.077	n.s.
C9:0	0.074b	0.090a	0.091a	0.084a	0.0048	**
C10:0	9.82b	11.15a	11.03a	9.70b	0.313	***
C11:0	0.61b	0.69a	0.69a	0.58b	0.040	**
Short-chain FA	18.12b	20.01a	19.48ab	18.35b	0.44	**
C12:0	6.16b	7.47a	7.73a	5.75b	0.259	***
C13:0	0.26b	0.34a	0.35a	0.25b	0.018	***
C14:0 *iso*	0.12b	0.14ab	0.13ab	0.15a	0.009	*
C14:0	13.59b	15.56a	15.95a	12.98b	0.41	***
C14:1 *c9*	0.40b	0.45a	0.44a	0.40b	0.042	**
C15:0 *iso*	0.27	0.30	0.30	0.31	0.014	n.s.
C15:0 *anteiso*	0.43	0.49	0.48	0.47	0.035	n.s.
C15:0	1.14b	1.36a	1.40a	1.38a	0.047	***
C16:0 *iso*	0.26	0.30	0.29	0.31	0.022	n.s.
C16:0	27.42b	31.28a	31.51a	27.91b	0.69	***
C16:1 *c9*	1.17b	1.29a	1.27a	1.32a	0.120	*
C17:0 *iso*	0.30	0.35	0.37	0.41	0.022	+
C17:0 *anteiso*	0.19	0.19	0.20	0.21	0.009	n.s.
C17:0	0.81b	0.88a	0.92a	0.83b	0.020	**
Medium-chain FA	52.74b	60.59a	61.52a	52.94b	0.73	***
Iso branched-chain FA	1.17b	1.34ab	1.35ab	1.46a	0.061	*

effects (Parodi 2009; Bauman and Lock 2010). Compared with MCF milk, the chickpea milk was higher in stearic acid and OA, but lower in VA and RA.

The protein source did not lead to differences in long chain omega-3 FA (Table 5), such as eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA), or total omega-3 FA, whereas the ALA content tended to be highest in MCF milk, in line with the level of ALA in the MCF (Table 1). These results contradict other authors who observed, on lamb meat, positive effects of a pea diet in increasing the level of omega-3 FA (Lanza *et al*. 2011; Scerra *et al*. 2011), and an increase in DPA with a chickpea diet (Priolo *et al*. 2003), whereas they are in accordance with Bonanno *et al*. (2012) who did not find differences in omega-3 FA when compared meat fat from lambs fed diets based on chickpea, faba bean, pea or soybean. These discrepancies could be related to the different unsaturated FA composition of the control diet fed to lambs in those experiments. With regard to the level of omega-6 FA and ratio of omega-6 to omega-3 FA, in the present study they were lower in milk from faba bean and pea. With all concentrates, the omega-6 to omega-3 ratio was lower and more favourable than the level ($\leq$5) recommended by FAO/WHO (1994).

Moreover, similar to milk from MCF, chickpea milk was higher in monounsaturated and total unsaturated FA, and was lower in SFA, as well as in the ratio of SFA to unsaturated and PUFA, whereas PUFA were highest in milk from MCF, followed by milk from chickpea, and then that from the other two groups. On the whole, chickpea concentrate and MCF contributed to improvements in the health-promoting index, which assigns a health value to milk fat.

The mammary Δ-9 desaturase activity was estimated by calculation of different Δ-9 desaturase ratios (DR) of the desaturase products to the sum of precursors and products (Table 5). The DR of C16:0 was highest in MCF milk, the DR of C18:0 and VA were lower in chickpea and MCF milk, whereas the DR of C14:0 did not differ among groups.

Based on these results, the comparison of the faba bean and pea concentrates with the MCF was in accordance with data of Renna *et al*. (2012) who demonstrated a higher amount of SFA and lower levels of VA and CLA in pea concentrate than in commercial concentrate containing sunflower meal and soybean seeds.

Instead, the chickpea concentrate led to several differences in the milk FA profile compared with faba bean and pea, similar to the FA composition of milk from MCF.

Although LA was the predominant FA in each of the concentrates used in the present study (Table 1), followed by OA, the MCF and chickpea-based concentrates provided higher amounts of LA and OA than the other concentrates, due to their higher lipid content. As a consequence, the LA and OA intakes for ewes fed MCF and chickpea diets were markedly higher than those for ewes fed faba bean and pea diets (Table 2).

These higher LA and OA intakes with chickpea and MCF diets can be responsible of the lower milk content in short- and medium-chain SFA, since the unsaturated FA are recognised

Table 5. Effect of legume grain-based concentrates on long-chain and grouped fatty acid composition of milk (g/100 g FAME)

MCF, mixed concentrate feed; s.e.m., standard error of the mean; n.s., not significant; VA, *trans* vaccenic acid; OA, oleic acid; LA, linoleic acid; RA, rumenic acid; GLA, γ-linolenic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; DR, Δ^9-desaturase ratio. Health-promoting index = unsaturated fatty acids/[C12:0 + (4 × C14:0) + C16:0] (Chen *et al.* 2004). ***, $P \leq 0.001$. **, $P \leq 0.01$. *, $P \leq 0.05$. +, $P \leq 0.10$. a, b, c: $P \leq 0.05$

	Concentrates				s.e.m.	*P*-value
	Chickpea	Faba bean	Pea	MCF		
C18:0 *iso*	0.23	0.25	0.26	0.27	0.012	n.s.
C18:0	6.31a	3.50c	3.51c	5.63b	0.318	***
C18:1 *t6–8*	0.122b	0.055c	0.038c	0.210a	0.0107	***
C18:1 *t9*	0.19b	0.11c	0.12c	0.25a	0.009	***
C18:1 *t10*	0.26b	0.12b	0.11b	0.51a	0.040	***
C18:1 *t*11, VA	0.66b	0.38c	0.36c	1.30a	0.033	***
C18:1 *t12–14*	0.40b	0.20c	0.19c	0.54a	0.019	***
C18:1 *c9*, OA	14.71a	9.82b	9.77b	13.06a	0.47	***
C18:1 *c11*	0.35ab	0.32b	0.29b	0.40a	0.017	**
C18:1 *c12*	0.22b	0.11c	0.11c	0.35a	0.020	***
C18:1 *c13*	0.34a	0.12b	0.11b	0.36a	0.034	**
C18:1 *c14*	0.124a	0.094b	0.088b	0.142a	0.0061	***
C18:2 n-6 *c9*, *c12*, LA	2.27a	1.55b	1.43b	2.28a	0.079	***
CLA C18:2 *c9*, *t11*, RA	0.34b	0.25c	0.23c	0.64a	0.027	***
CLA C18:2 *t9*, *c11*	0.100ab	0.104ab	0.098b	0.114a	0.0050	*
CLA C18:2 *t10*, *c12*	0.049	0.044	0.047	0.049	0.0053	n.s.
C18:3 n-6 *c6*, *c9*, *c12*, GLA	0.068	0.075	0.067	0.065	0.0063	n.s.
C18:3 n-3 *c9*, *c12*, *c15*, ALA	0.73	0.78	0.70	0.87	0.055	+
C20:0	0.24a	0.21b	0.18c	0.24a	0.009	***
C20:4 n-6	0.11	0.12	0.11	0.13	0.009	n.s.
C20:5 n-3, EPA	0.067	0.069	0.067	0.054	0.0056	n.s.
C22:0	0.15a	0.13ab	0.11b	0.17a	0.011	**
C22:5 n-3, DPA	0.11	0.11	0.11	0.12	0.005	n.s.
C22:6 n-3, DHA	0.068	0.064	0.065	0.062	0.0034	n.s.
Other FA	1.12	1.02	1.01	1.15	0.049	n.s.
Long-chain FA	29.13a	19.40b	19.00b	28.71a	0.81	***
Saturated FA	76.09b	82.83a	83.22a	75.69b	0.65	***
MUFA	19.62a	13.63b	13.45b	19.45a	0.56	***
PUFA	4.30b	3.54c	3.33c	4.86a	0.140	***
Unsaturated FA	23.91a	17.17b	16.78b	24.31a	0.66	***
Saturated/unsaturated FA	3.24b	4.86a	4.99a	3.15b	0.147	***
Saturated FA/PUFA	17.88b	23.87a	25.20a	15.75b	0.92	***
Omega-6 FA	2.82a	2.10b	1.98b	2.90a	0.086	***
Omega-3 FA	1.01	1.05	0.99	1.14	0.060	n.s.
Omega-6/omega-3	2.84a	2.04b	2.04b	2.62a	0.117	***
DR C14:1 *c*9/C14:0 + C14:1 *c*9	0.028	0.028	0.027	0.029	0.0026	n.s.
DR C16:1 *c*9/C16:0 + C16:1 *c*9	0.040b	0.040b	0.039b	0.045a	0.0034	***
DR C18:1 *c*9/C18:0 + C18:1 *c*9	0.70b	0.74a	0.74a	0.70b	0.012	***
DR RA/VA + RA	0.33b	0.40a	0.39a	0.33b	0.014	***
Health-promoting index	0.28a	0.17b	0.16b	0.28a	0.011	***

to be inhibitors of the activity of lipogenic enzymes responsible of *de novo* FA synthesis in the mammary gland (Chilliard and Ferlay 2004).

Also the changes in odd-chain FA with the chickpea diet can be linked to the higher LA and OA intakes. Since the linear odd-chain FA (C15:0 and C17:0) can be partly synthesised *de novo* in the mammary gland (Vlaeminck *et al*. 2006), their reduction in chickpea and MCF milk could be related to the effect of the higher level in unsaturated FA of chickpea and MCF concentrates in inhibiting the mammary gland synthesis, as previously mentioned. With regards to the *iso* branched-chain FA, in general, they are contained in a large amount in the cellulolytic bacteria in the rumen, and for this reason are favoured by a higher incidence of forage or fibre in the diet whereas, on the contrary, decrease with lipid-supplemented diets providing long-chain unsaturated FA (Vlaeminck *et al*. 2006). Since MCF diet was higher in lipids and long-chain FA than chickpea concentrate, the increase of milk *iso* branched-chain FA with MCF diet in comparison with chickpea diet could be linked to the effect of the higher NDF

intake (Table 2) in favouring the growth of cellulolytic bacteria in the rumen.

The higher LA and OA percentages in milk from ewes fed chickpea and MCF concentrates seem to reflect the dietary levels and intakes of these long-chain unsaturated FA. However, high intake of LA is known to inhibit the complete FA biohydrogenation (BH) in the rumen environment (Jenkins and Adams 2002). The reduced BH results in an increase of milk content in LA and other FA intermediates of the saturation process of dietary unsaturated FA to stearic acid, among which VA and RA. Accordingly, both VA and RA originate in the rumen: the VA is formed by the incomplete BH of dietary LA and ALA, and also by the isomerisation of OA, whereas the RA is formed at the first step of BH of dietary LA. However, the most part of RA is derived from the VA flowing from the rumen and transformed in the mammary gland through the activity of Δ-9 desaturase enzyme system (Chilliard *et al*. 2007; Jenkins *et al*. 2008). Therefore, in the present study, the higher LA intakes with chickpea diet and, to a greater extent, with MCF diet may have interfered with the ruminal microflora by reducing their BH activity and increasing the milk content of VA and RA. An increase of VA and RA was also observed in the intramuscular fat of meat from lambs fed chickpea (Priolo *et al*. 2003; Bonanno *et al*. 2012).

The higher milk percentage of VA and RA, and the consequent lower content of stearic acid, in milk from MCF diet than in chickpea milk can be related to the higher dietary LA intake of ewes fed MCF, that could have more strongly inhibited the BH activity of ruminal microflora.

Moreover, the higher amounts of stearic acid, as well as OA, observed with chickpea concentrate, and to a lesser extent with MCF, could be derived from the mobilisation of lipids in adipose tissues (Chilliard *et al*. 2003) as a result of the negative energy balance that the lower milk yield and the lack of improvement in body condition of ewes fed chickpea seem to suggest.

With regard to the lower mammary Δ-9 desaturase activity with chickpea and MCF diets, a similar effect was observed in dairy ewes fed a supplemented diet with soybean oil (Mele *et al*. 2006), suggesting an effect of the dietary lipid supplement also in this case. However, the DR of C14:0, that provide the best estimation of the mammary Δ-9 desaturase activity (Corl *et al*. 2001), did not differ among treatments. Therefore, the higher amounts of the substrates for the desaturation activity (C18:0 and VA) in chickpea and MCF milk could also explain the lower values of the correspondent DR in comparison with milk from the other two diets.

Conclusions

The results of this study show that it is possible to supplement the diet of lactating ewes with concentrates based on protein sources consisting of legume grains alternative to soybean, such as chickpea, faba bean, or pea. Indeed, legume grain-based concentrates were consumed by lactating ewes more than the compared commercial feed, and showed no adverse effects on milk yield, composition, or clotting ability.

Although the three protein sources did not show differences in DM and protein intake, the faba bean and pea resulted in a higher milk yield and a higher efficiency of milk casein synthesis compared with chickpea. However, chickpea increased the milk content of *trans*-vaccenic and rumenic acids in comparison with faba bean and pea and, similar to the mixed concentrate, increased the level of linoleic acid, as well as total unsaturated FA, thus improving the value of the milk health-promoting index.

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References

Antongiovanni M, Acciaioli A, Franci O, Ponzetta MP, Pugliese C, Buccioni A, Badii M (2002) Field bean (*Vicia faba* var. *minor*) as a protein feed for growing lambs with and without protected lysine and methionine supplementation. *Italian Journal of Animal Science* **1**, 229–238.

AOAC (2000) ‘Official methods of analysis.’ 17th edn. (Association of Official Analytical Chemists International: Gaithersburg, MD)

Badgley C, Moghtader J, Quintero E, Zakem E, Chappell MJ, Avilés-Vázquez K, Samulon A, Perfecto I (2007) Organic agriculture and the global food supply. *Renewable Agriculture and Food Systems* **22**, 86–108. doi:10.1017/S1742170507001640

Bauman DE, Lock AL (2010) Milk fatty acid composition: challenges and opportunities related to human health. In ‘Proceedings of the 26th world buiatrics congress, Santiago, Chile’. (Ed. F Wittwer) pp. 278–289. (World Buiatrics Association Board: Santiago)

Bencini R (2002) Factors affecting the clotting properties of sheep milk. *Journal of the Science of Food and Agriculture* **82**, 705–719. doi:10.1002/jsfa.1101

Bonanno A, Tornambè G, Di Grigoli A, Genna V, Bellina V, Di Miceli G, Giambalvo D (2012) Effect of legume grains as a source of dietary protein on the quality of organic lamb meat. *Journal of the Science of Food and Agriculture* **92**, 2870–2875. doi:10.1002/jsfa.5616

Bonomi A, Bonomi BM, Mazzotti A, Vonghia G, Caputi Iambrenghi A (2003) The use of pea meal (*Pisum sativum* L.) in milk ewe feeding. *La Rivista di Scienza dell’Alimentazione* **32**, 303–310.

Bryden WL (2012) Mycotoxin contamination of the feed supply chain: implications for animal productivity and feed security. *Animal Feed Science and Technology* **173**, 134–158. doi:10.1016/j.anifeedsci.2011.12.014

Cannas A, Pes A, Mancuso R, Vodret B, Nudda A (1998) Effect of dietary energy and protein concentration on the concentration of milk urea nitrogen in dairy ewes. *Journal of Dairy Science* **81**, 499–508. doi:10.3168/jds.S0022-0302(98)75602-4

Chen S, Bobe G, Zimmerman S, Hammond EG, Luhman CM, Boylston TD, Freeman AE, Beitz DC (2004) Physical and sensory properties of dairy products from cows with various milk fatty acid compositions. *Journal of Agricultural and Food Chemistry* **52**, 3422–3428. doi:10.1021/jf035193z

Chilliard Y, Ferlay A (2004) Dietary lipids and forages interactions 1 on cow and goat milk fatty acid composition and sensory properties. *Reproduction, Nutrition, Development* **44**, 467–492. doi:10.1051/rnd:2004052

Chilliard Y, Ferlay A, Rouel J, Lamberet G (2003) A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. *Journal of Dairy Science* **86**, 1751–1770. doi:10.3168/jds.S0022-0302(03)73761-8

Chilliard Y, Glasser F, Ferlay A, Bernard L, Rouel J, Doreau M (2007) Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. *European Journal of Lipid Science and Technology* **109**, 828–855. doi:10.1002/ejlt.200700080

Christodoulou V, Bampidis VA, Hučko B, Ploumi K, Iliadis C, Robinson PH, Mudřik Z (2005) Nutritional value of chickpeas in rations of lactating ewes and growing lambs. *Animal Feed Science and Technology* **118**, 229–241. doi:10.1016/j.anifeedsci.2004.10.017

Corl BA, Baumgard LH, Dwyer DA, Griinari JM, Phillips BS, Bauman DE (2001) The role of Δ^9-desaturase in the production of *cis*-9, *trans*-11 CLA. *The Journal of Nutritional Biochemistry* **12**, 622–630. doi:10.1016/S0955-2863(01)00180-2

Cutrignelli MI, Calabrò S, Tudisco R, Infascelli F, Piccolo V (2011) Protein sources in ruminant nutrition. In 'Recent trends for enhancing the diversity and quality of soybean products'. (Ed. D Krezhova) (InTech: Rijeka, Croatia) Available at http://www.intechopen.com/books/recent-trends-for-enhancing-the-diversity-and-quality-of-soybeanproducts/protein-sources-in-ruminant-nutrition [Verified 2 December 2014]

Dewhurst RJ, Davies DR, Merry RJ (2000) Microbial protein supply from the rumen. *Animal Feed Science and Technology* **85**, 1–21. doi:10.1016/S0377-8401(00)00139-5

Di Grigoli A, Bonanno A, Vargetto D, Tornambè G, Marchetta PF (2008) Utilizzo del pisello proteico in alternativa alla farina di estrazione di soia in un allevamento biologico di bovine da latte. In 'Atti del VI Convegno Nazionale dell'Associazione Italiana di Zootecnica Biologica e Biodinamica'. (Eds P Pignatteli, F Ferrante, S Mattiello, S Barbieri, S Lolli) pp. 19–24. (Associazione Italiana di Zootecnia Biologica e Biodinamica – ZooBioDi: Milano, Italy) Available at http://www.zoobiodi.it/doc/ATTI_2008_DEFINITIVI.pdf [Verified 2 December 2014]

Di Grigoli A, Pollicardo A, Mele M, Bonanno A, Tornambè G, Vargetto D (2009) Effect of substitution of barley and tickbean to maize and extruded soybean in the diet on milk and cheese from ewes grazing under two different stocking rates. *Options Mèditerranèennes, Séries A. Mediterranean Seminars* **85**, 459–464.

Dixon RM, Hosking BJ (1992) Nutritional value of grain legumes for ruminants. *Nutrition Research Reviews* **5**, 19–43. doi:10.1079/NRR19920005

FAO/WHO (1994) Fats and oils in human nutrition. Report of a joint FAO (Food and Agriculture Organization of the United Nations) and WHO (World Health Organization) experts consultation. FAO technical paper 57, FAO, Rome, Italy.

Friedman M (1996) Nutritional value of proteins from different food sources. A review. *Journal of Agricultural and Food Chemistry* **44**, 6–29. doi:10.1021/jf9400167

Hadjipanayiotou M (2002) Replacement of soybean meal and barley grain by chickpeas in lamb and kid fattening diets. *Animal Feed Science and Technology* **96**, 103–109. doi:10.1016/S0377-8401(01)00339-X

Holmes JHG, Dixon RM, Smith C, Entonu S, Chau H, Sanpote S, Acharyya B (1993) Resistance of trypsin inhibitors to fermentation by rumen microflora. In 'Recent advances of research in antinutritional factors in legume seeds'. EAAP publication no. 70. (Eds AFB Van der Poel, J Huisman, HS Saini) pp. 183–186. (Wageningen: Wageningen, The Netherlands)

Jenkins TC, Adams CS (2002) The biohydrogenation of linoleamide in vitro and its effects on linoleic acid concentration in duodenal contents of sheep. *Journal of Animal Science* **80**, 533–540.

Jenkins TC, Wallace RJ, Moate PJ, Mosley EE (2008) Board-invited review: recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. *Journal of Animal Science* **86**, 397–412. doi:10.2527/jas.2007-0588

Kramer JK, Cruz-Hernandez C, Deng Z, Zhou J, Jahreis G, Dugan ME (2004) Analysis of conjugated linoleic acid and trans 18: 1 isomers in synthetic and animal products. *The American Journal of Clinical Nutrition* **79**, 1137S–1145S.

Lanza M, Pennisi P, Priolo A (1999) Faba bean as an alternative protein source in lamb diet; effects on growth and meat quality. *Zootecnica e Nutrizione Animale* **25**, 71–79.

Lanza M, Bella M, Priolo A, Fasone V (2003) Peas (*Pisum sativum* L.) as an alternative protein source in lamb diet: growth performances, and carcass and meat quality. *Small Ruminant Research* **47**, 63–68. doi:10.1016/S0921-4488(02)00244-4

Lanza M, Fabro C, Scerra M, Bella M, Pagano R, Brogna DMR, Pennisi P (2011) Lamb meat quality and intramuscular fatty acid composition as affected by concentrates including different legume seeds. *Italian Journal of Animal Science* **10**, 87–94. doi:10.4081/ijas.2011.e18

Liponi GB, Casini L, Martini M, Gatta D (2007) Faba bean (*Vicia faba minor*) and pea seeds (*Pisum sativum*) as protein sources in lactating ewes' diets. *Italian Journal of Animal Science* **6**, 309–311.

Loe ER, Bauer ML, Lardy GP, Caton JS, Berg PT (2004) Field pea (*Pisum sativum*) inclusion in corn-based lamb finishing diets. *Small Ruminant Research* **53**, 39–45. doi:10.1016/j.smallrumres.2003.08.020

Loor JJ, Herbein JH, Polan CE (2002) Trans 18 : 1 and 18 : 2 isomers in blood plasma and milk fat of grazing cows fed a grain supplement containing solvent-extracted or mechanically extracted soybean meal. *Journal of Dairy Science* **85**, 1197–1207. doi:10.3168/jds.S0022-0302(02)74183-0

López-Bellido FJ, López-Bellido L, López-Bellido RJ (2005) Competition, growth and yield of faba bean (*Vicia faba* L.). *European Journal of Agronomy* **23**, 359–378. doi:10.1016/j.eja.2005.02.002

Luna P, de la Fuente MA, Juárez M (2005) Conjugated linoleic acid in processed cheeses during the manufacturing stages. *Journal of Agricultural and Food Chemistry* **53**, 2690–2695. doi:10.1021/jf048091x

Mele M, Buccioni A, Petacchi F, Serra A, Antongiovanni M, Secchiari P (2006) Effect of forage/concentrate ratio and soybean oil supplementation on milk yield, and composition from Sarda ewes. *Animal Research* **55**, 273–285. doi:10.1051/animres:2006019

Nabradi A, Popp J (2011) Economics of GM crop cultivation. *Applied Studies in Agribusiness and Commerce* **5**, 7–15.

National Research Council (NRC) (2001) 'Nutrient requirements of dairy cattle.' 7th rev. edn. (National Academy Press: Washington, DC)

Ohlsson L (2010) Dairy products and plasma cholesterol levels. *Food Nutrition Research* **54**, 5124. doi:10.3402/fnr.v54i0.5124

Parodi PW (2009) Milk fat nutrition. In 'Dairy fats and related products'. (Ed. AY Tamime) pp. 28–51. (Wiley-Blackwell: Oxford)

Priolo A, Lanza M, Galofaro V, Fasone V, Bella M (2003) Partially or totally replacing soybean meal and maize by chickpeas in lamb diets: intramuscular fatty acid composition. *Animal Feed Science and Technology* **108**, 215–221. doi:10.1016/S0377-8401(03)00166-4

Pulina G, Nudda A (2002) Milk production. In 'Dairy sheep feeding and nutrition'. (Ed. G Pulina) pp. 11–27. (Avenue Media: Bologna, Italy)

Ramos Morales E, Molina Alcaide E, Sanz Sampelayo MR (2008) Milk production of dairy goats fed diets with different legume seeds: effects of amino acid composition of the rumen undegradable protein fraction. *Journal of the Science of Food and Agriculture* **88**, 2340–2349. doi:10.1002/jsfa.3355

Renna M, Cornale P, Lussiana C, Malfatto V, Fortina R, Mimosi A, Battaglini LM (2012) Use of *Pisum sativum* (L.) as alternative protein resource in diets for dairy sheep: effects on milk yield, gross composition and fatty acid profile. *Small Ruminant Research* **102**, 142–150. doi:10.1016/j.smallrumres.2011.07.007

Russel AJF, Doney JM, Gunn RG (1969) Subjective assessment of body fat in live sheep. *The Journal of Agricultural Science* **72**, 451–454. doi:10.1017/S0021859600024874

Sanz Sampelayo MR, Chilliard Y, Schmidely P, Boza J (2007) Influence of type of diet on the fat constituents of goat and sheep milk. *Small Ruminant Research* **68**(1-2), 42–63. doi:10.1016/j.smallrumres.2006.09.017

SAS (2010) 'SAS/STAT qualification tools user's guide (version 9.2).' (Statistical Analysis System Institute Inc.: Cary, NC)

Scerra M, Caparra P, Foti F, Cilione C, Zappia G, Motta C, Scerra V (2011) Intramuscular fatty acid composition of lambs fed diets containing alternative protein sources. *Meat Science* **87**, 229–233. doi:10.1016/j.meatsci.2010.10.015

Sinclair TR, Vadez V (2012) The future of grain legumes in cropping systems. *Crop and Pasture Science* **63**, 501–512. doi:10.1071/CP12128

Sukhija PS, Palmquist DL (1988) Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. *Journal of Agricultural and Food Chemistry* **36**, 1202–1206. doi:10.1021/jf00084a019

Tufarelli V, Khan RU, Laudadio V (2012) Evaluating the suitability of field bean as a substitute for soybean meal in early-lactating dairy cow: production and metabolic responses. *Animal Science Journal* **83**, 136–140. doi:10.1111/j.1740-0929.2011.00934.x

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**, 3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2

Vasta V, Nudda A, Cannas A, Lanza M, Priolo A (2008) Alternative feed resources and their effects on the quality of meat and milk from small ruminants. *Animal Feed Science and Technology* **147**, 223–246. doi:10.1016/j.anifeedsci.2007.09.020

Vlaeminck B, Fievez V, Cabrita ARJ, Fonseca AJM, Dewhurst RJ (2006) Factors affecting odd- and branched-chain fatty acids in milk: a review. *Animal Feed Science and Technology* **131**, 389–417. doi:10.1016/j.anifeedsci.2006.06.017

Zannoni M, Annibaldi S (1981) Standardization of the renneting ability of milk by Formagraph. *Scienza e Tecnica Lattiero Casearia* **32**, 79–94.