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PAPER

Effects of *BLG* polymorphism and dietary supplementation with carob pulp on ewe milk traits and fatty acid composition

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ABSTRACT

The current study examined the effect on milk traits and composition of the replacement of barley grain with carob pulp as a supplement for grazing ewes with different genotypes at the β-lactoglobulin (*BLG*) locus. Forty-six Valle del Belìce lactating ewes were chosen for the feeding trial and split into control and carob groups based on the BLG p.Tyr38His polymorphism. The carob group received a supplement of 250 g/d of carob pulp, whereas the control group received 250 g/g of a barley whole grain-based concentrate. There were no milk yield or gross composition variations related to the *BLG* genotype. Nevertheless, milk fatty acid composition was influenced by the *BLG* polymorphism. Compared to the AB genotype, milk from BB sheep had higher concentrations of linoleic, linolenic acids, and total polyunsaturated fatty acids. The addition of carob pulp did not significantly modify fat and protein-corrected milk, whereas reduced protein and urea concentration, increased the fat content but worsened the milk quality in terms of fatty acid composition, increasing saturated and medium chain fatty acids. The genotype × feed interaction had no appreciable effect on milk composition or quality.

HIGHLIGHTS

- Diet, genotype, and their interaction affect the milk traits and quality.
- Carob pulp reduced protein, urea and worsened fat quality.
- The genotype positively influenced the milk fat quality.

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β-lactoglobulin polymorphism; carob pulp; milk fatty acid; milk traits

Introduction

Several genetic polymorphisms in ovine major milk proteins (caseins and whey proteins) have been described in the literature. Over the years, a strong association between milk protein polymorphisms and milk yield, composition, and technological aspects was observed, leading to increasing interest in investigating the local sheep breed's genetic potential in milk production.

β-lactoglobulin (β-LG) represents about 60% of whey protein in sheep (Moatsou et al. 2005) β-LG is a globular protein that belongs to the lipocalin family, small proteins with the capacity to bind small hydrophobic molecules. It is encoded by the *BLG* gene, localised on ovine chromosome 3 (Hayes and Petit 1993), and expressed in the mammary gland during lactation. In sheep, three genetic polymorphisms (A, B, and C) of β-LG have been reported differing each other in amino acid sequence differences. Variant C was only detected in a few breeds, whilst variants A and B are the most common allelic variants (Selvaggi et al. 2015).

As reviewed by Selvaggi et al. (2015), it is not yet clear the role, in sheep, of this polymorphism on milk traits, coagulation properties, and composition, as result in different experimental conditions are often contradictory. Some significant relationships are highlighted by Mele et al. (2007) between β-LG polymorphism and fatty acid composition, as this protein seems to be involved in the transport and metabolism of dietary lipids (Pérez and Calvo 1995; Le Maux et al. 2014).

Carob tree (*Ceratonia siliqua* L.) (Fabaceae) is native to the Mediterranean area. Despite the world production of carob in the last few years has decreased, in

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Italy, it remained constant (FAO 2020; Feng et al., 2004). According to the Food and Agriculture Organisation of the United Nations (FAO), the main carob fruit-producing countries are Portugal (39,935 tons annually), Italy (30,734 tons), Morocco (22,421 tons), Greece (14,696 tons), and Turkey (14,103 tons). In Mediterranean countries, the economic importance of carob production is linked to the carob tree's ability to adapt to drought, poor soils, and to climate changes. Carob is cultivated for human and animal nutrition, nowadays the carob fruit pulp and seeds have a wide application as food additives and stabiliser agents in food products and are used to produce syrups, ice creams, and a great variety of bakery products (Rodríguez-Solana et al. 2021). Several studies investigated the use of carob in animal nutrition in sheep (Karabulut et al. 2006; Obeidat et al. 2012), lambs (Priolo et al. 1998, Pelegrin-Valls et al. 2022), and pigs (Kotrotsios et al. 2012).

The use of barley grain as a supplement for grazing lactating ewes represents the common practice in the Mediterranean dairy sheep system. The high feed cost in semi-arid countries and the lower price of animal products make concentrate supplementation challenging. The local agro-industrial by-products represent reasonable alternatives for ruminant feeding, as they are available at competitive costs compared to commercial feed. Due to its very high sugar content, carob pulp could replace barley, reducing feeding costs (Richane et al. 2022).

Carob pulp is rich in soluble sugars and tannins. For these reasons, in different ways, these components may influence some microbial functions in the rumen. Soluble sugars meet the microbes' needs for rapidly degradable energy and could lead to a synchronisation of the speed of degradation of protein and carbohydrate sources (Miller et al. 2001; Pagano et al. 2011). Condensed tannins generally reduce protein degradability by binding forage proteins, thus protecting them from rapid degradation in the rumen (Valenti et al. 2019; Correddu et al. 2020). Tannins may also affect the ruminal biohydrogenation activity of dietary fatty acids (Vasta and Luciano 2011; Valenti et al. 2019).

In livestock, milk production traits are influenced by many factors, including the genetic polymorphism at several genes involved in milk yield and composition, whose regulation is often affected by the environment and feeding composition.

In previous studies on different ruminant species, it has been highlighted the possible interaction between diet and genotype at loci involved in milk yield and composition: different diets seem to cause a resizing of genotype effects due to different nutrient availability (Pagano et al. 2010; Puppel et al. 2016; Valenti et al. 2019; Tumino et al. 2021).

The aim of the study was to investigate the effects on milk traits and fat composition of substituting barley grain with carob pulp as a supplement for grazing ewes with different genotypes at the *BLG* locus.

Materials and methods

Genetic characterisation

Genetic characterisation was conducted on seventy-one Valle del Belìce lactating ewes. Genomic DNA was extracted from somatic milk cells (MSCs) using a salting-out method (Miller et al. 1988) with modifications. The MSCs pellet was obtained by adding 50 μL of 0.5 M EDTA to 50 mL of fresh milk and centrifuging at 2000 g at 4 °C for 20 min. The fat layer was removed, the milk was discarded, and the obtained cell pellet was rinsed using 20 mL of PBS (pH 7.2) and 0.5 mM EDTA. After centrifugation at 2000 g at 4 °C for 15 min, the supernatant was discarded, and the pellet was washed with 10 mL of PBS-EDTA and centrifuged at 2000 g at 4 °C for 10 min. After the last centrifugation, the MSCs pellet was mixed and incubated overnight at 56 °C with 600 μl of Proteinase K solution (1 mg of Proteinase k in 2 mM-EDTA and 1% SDS). After incubation, 600 μL of NaCl 5 M was added, and the proteins were pelleted by centrifugation at 2000 g at 4 °C for 5 min. The DNA in the aqueous layer was recovered and precipitated by adding 3 mL of absolute ethanol. The precipitated DNA was recovered and washed twice with 70% ethanol. DNA was then redissolved in 500 μL of TE buffer. DNA concentration was quantified with NanoDrop 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

BLG variants A and B were identified by the PCR-RFLP method according to Corral et al. (2010). The PCR reaction was performed in a total volume of 30 μL, containing 1X DreamTaq Buffer, 0.25 μM each primer, 200 μM dNTP, 1.25 U DreamTaq DNA polymerase (Thermo Fisher Scientific Inc. Waltham, Massachusetts, U.S.) and 50 ng of genomic DNA. Amplification was carried out under the following thermal profile: an initial denaturation step at 95 °C for 1 min followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 65 °C for 30 s, and extension at 72 °C for 1 min, and a final extension of 72 °C for 15 min. The 236 bp amplified fragments were subjected to digestion for 2 h with 5 units of RsaI restriction enzyme (New England BioLabs Inc., Ipswich, MA,

USA). Digests separated by electrophoresis on 3% agarose gel stained with GelRed Nucleic Acid Stain (Biotium, Inc., Fremont, CA, USA).

Animals and feeding management

Among the seventy-one characterised animals, forty-six multiparous (2nd to 3rd lactation) Valle del Belìce lactating ewes, homogeneous for milk yield (830 ± 224 g/day) and days of lactation (110 ± 15 days), were selected based on their genotype at the BLG p.Tyr38His locus, which discriminates among *BLG* A and *BLG* B alleles as follows: 16 ewes homozygous (BB genotype) and 30 heterozygous ewes (AB genotype). The ewes were divided into two blocks (a and b). In each block, the two *BLG* genotypes were equally distributed (15 AB and 8 BB) and arranged in an experimental design consisting of two simultaneous changeover designs for the two genotypes (AB and BB) with one block for each diet (control diet, carob diet) in a 2 × 2 factorial arrangement of treatments. All the animals, managed according to the guidelines of the Animal Ethics Committee (O.P.B.A.) of the University of Catania (prot. No. 158467), received the experimental diets as follows:

- Control group: 8 h on a mixed pasture and 250 g/d of barley whole grain.
- Carob group: 8 h on a mixed pasture and 250 g/d of carob pulp.

The supplements were divided into two equal portions and individually administered during the two daily milkings. Table 1 reports the chemical composition of the administrated supplements.

The pre-experimental period consisted of a 12-day during which the animals received 250 g of a mix of the two experimental diets. The experiment lasted 40 days, from 21 March to 30 April. Each experimental-diet period lasted 20 days, consisting of 12 days for progressive adaptation and 8 days for data and sample collection, during which the ewes received the scheduled diet.

Table 1. Chemical composition of supplements and pasture.

	Control	Carob	Mixed pasture
Dry matter (DM), g/kg	870.0	817.0	151.0
CP, g/Kg DM	129.0	55.0	168.0
Ether extract, g/kg DM	28.0	8.0	26.8
NDF, g/kg DM	251.0	322.0	332.0
ADF, g/kg DM	121.0	286.0	280.0
WSC, g/kg NFC	52.0	485.0	129.0
Starch, g/kg NFC	522.0	–	18.0
SP, g/100 g CP	37.8	35.8	26.6
ADIP, g/100 g CP	3.5	25.2	8.0
NDIP, g/100 g CP	27.1	39.0	17.5
Total polyphenols, mg/g DM	2.8	17.9	4.4
Tannins, mg/g DM	1.5	12.5	2.5
Fatty acids, g/100 g TFA			
16:0	22.1	25.0	21.7
18:0	1.7	5.2	4.8
18:1 *c*9	14.0	21.4	6.7
18:2	53.8	32.7	25.5
18:3	3.12	7.0	39.5

CP: crude protein; WSC: water soluble carbohydrate; SP: soluble protein; ADIP: acid detergent insoluble CP; NDIP: neutral detergent insoluble CP; NFC: non-fibre carbohydrates; TFA: total fatty acids

Measurements and analysis

During the pre-experimental period, individual milk production from the two milkings was measured, and milk samples were collected. During each 8-d data and sample collection period the morning and afternoon milk productions were recorded and individual milk samples from both milkings were collected at the end of each experimental diet period (at day 7 and day 8).

Pasture biomass and herbage height were measured at the beginning of each experimental period cutting herbage on eight plots of 1 × 1 m randomly distributed over the pasture. Herbage height was also measured in the same plots.

Feedstuffs chemical composition

Three samples for each supplement and six pasture samples for each experimental period were analysed for ether extract, crude protein, ash (AOAC 1990), and structural carbohydrates (Van Soest et al. 1991). The crude protein (CP) was partitioned into fractions, according to the Cornell Net Carbohydrate and Protein System, as modified by Licitra et al. (1996). The water soluble carbohydrate (WSC) contents were determined following the modified anthrone method (Deriaz 1961). Starch contents were determined according to Hall et al. (2015). The analysis of total phenolic compounds and total tannins were performed according to Makkar et al. (1993), tannic acid standard solution with concentrations between 0 and 100 mg/mL were used to prepare a calibration curve. Feeds fatty acids were extracted from freeze-dried sample and converted to fatty acid methyl esters (FAMEs) with a one-step procedure using chloroform and 2% (v/v) sulphuric acid in methanol (Shingfield et al. 2003) and nonadecanoic acid (Sigma–Aldrich, St. Louis, MO, USA) as internal standard. Individual FAME separation and quantification were performed by gas-chromatography according to Campione et al. (2020).

Milk analyses

Individual milk samples, consisting of proportional volumes of the morning and afternoon milking according to the milk amount recorded at respective milkings, were collected and subdivided into aliquots for analyses. One of the two milk subsamples was analysed immediately after the collection for fat, lactose, protein, casein, and urea content by infra-red method (Milkoscan FT1 supplied by Foss S.r.l., Padova, Italy). Milk yield was normalised at 6.5% of fat as FCM (6.5%) and at 5.8% of protein contents as FPCM (6.5%; 5.8%) according to the equations developed by Pulina et al. (2005).

The second aliquots were collected and treated using Sodium Azide 99% (Sigma-Aldrich, St. Louis, MO, USA) to reduce and block the coagulation of milk and were stored at −30 °C pending analysis for milk fatty acid profile performed as follows: milk fat was extracted according to Feng et al. (2004) and converted to fatty acid methyl esters (FAME) by base-catalyzed transesterification, using 1 mL of sodium methoxide in methanol 0.5 N and 1 mL of hexane. Nonadecanoic acid was used as an internal standard. FAME were analysed on a Trace Thermo Finnigan GC equipped with a flame ionisation detector (FID) and a 100 m × 0.25 mm i.d. fused-silica capillary column (SP-2560, Supelco, Inc., Bellefonte, PA, USA). Helium was the carrier gas at a constant flow of 1 mL/min. The total FAME profile in a 1 μL sample volume at a split ratio of 1:50 was determined using the GC conditions reported by (Valenti et al. 2019).

Statistical analysis

Individual data for milk yield and composition, and fatty acids were analysed using the GLM procedure for repeated measures (SPSS for Windows, Inc., Chicago, IL, USA). The model included diet, *BLG* genotype, blocks (a, b), periods, and *BLG* genotype x diet. Pre-experimental milk production and composition were used as covariates for milk production and gross composition, respectively. When the covariate was not significant, it was removed from the model.

Results

Table 1 reports feed chemical composition. Carob pulp was characterised by much higher levels of sugars, acid detergent insoluble protein, total polyphenols and tannins, and lower levels of crude protein, compared to barley grain. The pasture, a mixture of sulla, oat, barley, and compositae, had good protein level e a moderate fibre content.

Milk yield and gross composition are reported in Table 2. Milk yield and composition were not affected by genotype. Carob pulp significantly decreased milk yield, protein, and urea content whereas increased milk fat. No significant effect of diet was evident for FCM, FPCM, and fat, protein, and lactose yields. No parameter was significantly affected by the genotype × diet interaction.

Table 3 reports the main effects of milk fatty acid composition. *BLG* genotype significantly influenced some fatty acids: BB ewes milk was richer in 18:2 c9c12, 18:3 α, total trans FA and PUFA, and had lower levels of 18:1 c9. Diet strongly affected milk fatty acid composition. Milk from ewes fed with carob pulp was richer in different cis fatty acids (18:1 c11, 18:1 c12, c18:2 c9c12), odd FA (C17:0), 14:0, total SFA and total MCFA and had lower levels of 4:0, branched chain FA (17:0 iso), cis fatty acid (16:1 c7), trans-FA (18:1 t11), total trans fatty acids, total MUFA and 18:2 c9t11. Atherogenic and thrombogenic indices were

Table 2. Milk yield and gross composition. Main effects.

	BLG genotype (G)		Diet (D)		Significance			
	AB	BB	Control	Carob	G	D	G × D	SEM
Milk yield, g/d	848.50	798.40	882.70	764.20	0.400	0.049	0.712	29.390
FCM (6.5%), g/d[a]	826.40	745.00	785.40	786.00	0.184	0.993	0.993	28.820
FPCM (6.5%; 5.8%), g/d[a]	846.20	764.30	814.40	796.10	0.183	0.764	0.978	28.580
Fat, g/100 g	6.46	6.39	6.00	6.76	0.756	0.001	0.462	0.157
Fat, g/d	53.12	47.72	49.12	51.72	0.168	0.504	0.874	1.990
Protein, g/100 g	6.34	6.28	6.53	6.13	0.853	0.029	0.510	0.069
Protein, g/d	52.70	48.02	53.80	47.00	0.211	0.071	0.990	1.730
Lactose, g/100 g	4.58	4.64	4.63	4.60	0.383	0.972	0.933	0.039
Lactose, g/d	39.02	36.04	39.04	36.02	0.417	0.411	0.844	1.590
Urea, mg/dl	26.30	29.40	29.50	26.80	0.180	0.048	0.829	1.015
Casein, g/100 g	5.05	5.03	5.20	4.92	0.854	0.082	0.529	0.063
Casein, g/d	41.95	38.20	42.64	37.51	0.210	0.087	0.983	1.380

FCM: fat-corrected milk yield; FPCM: fat and protein corrected milk yield.
[a]Calculated according to Pulina et al. (2005).

Table 3. Milk fatty acid composition (% total fatty acids). Main effects.

	BLG genotype (G)		Diet (D)		Significance			
	AB	BB	Control	Carob	G	D	G × D	SEM
4:0	2.45	2.28	2.51	2.21	0.227	0.044	0.543	0.078
6:0	2.33	2.19	2.33	2.19	0.102	0.141	0.369	0.048
8:0	2.47	2.34	2.42	2.39	0.156	0.724	0.270	0.047
10:0	7.56	7.57	7.42	7.71	0.973	0.334	0.279	0.143
11:0	0.34	0.33	0.33	0.34	0.627	0.302	0.052	0.001
12:0	4.20	4.30	4.10	4.40	0.561	0.105	0.228	0.083
13:0	0.16	0.17	0.16	0.17	0.133	0.174	0.099	0.004
14:0 *iso*	0.14	0.14	0.14	0.14	0.912	0.987	0.956	0.004
14:0	10.70	10.5	10.20	11.00	0.429	0.002	0.302	0.114
15:0 *iso*	0.28	0.28	0.28	0.28	0.619	0.428	0.933	0.006
15:0 *anteiso*	0.56	0.59	0.58	0.56	0.319	0.510	0.670	0.013
14:1 *c9*	0.22	0.20	0.18	0.24	0.620	0.088	0.938	0.014
15:0	1.16	1.23	1.17	1.22	0.106	0.230	0.335	0.019
16:0 *iso*	0.36	0.39	0.38	0.37	0.085	0.351	0.958	0.006
16:0	22.10	21.80	21.40	22.50	0.648	0.113	0.413	0.286
17:0 *iso*	0.36	0.36	0.37	0.35	0.813	0.005	0.103	0.005
16:1 *c7*	0.29	0.31	0.32	0.28	0.105	0.002	0.353	0.005
17:0 *anteiso*	0.55	0.56	0.56	0.55	0.640	0.640	0.352	0.009
16:1 *c9*	0.89	0.91	0.87	0.94	0.705	0.129	0.426	0.021
17:0	0.77	0.80	0.76	0.81	0.058	<0.001	0.883	0.008
17:1 *c9*	0.23	0.25	0.23	0.25	0.238	0.220	0.768	0.006
18:0	8.80	9.04	9.08	8.76	0.497	0.380	0.568	0.167
18:1 *t6-8*	0.21	0.25	0.23	0.22	0.088	0.702	0.121	0.013
18:1 *t9*	0.32	0.35	0.38	0.29	0.629	0.112	0.657	0.024
18:1 *t10*	0.44	0.50	0.50	0.44	0.107	0.091	0.680	0.144
18:1 *t11*	2.98	3.45	3.63	2.80	0.071	0.002	0.676	0.018
18:1 *c9*	15.70	14.90	15.40	15.30	0.045	0.799	0.577	0.209
18:1 *c11*	0.65	0.69	0.59	0.74	0.271	<0.001	0.488	0.030
18:1 *c12*	0.20	0.21	0.19	0.23	0.285	<0.001	0.706	0.006
18:2 *c9c12*	1.71	1.81	1.68	1.85	0.044	0.001	0.683	0.028
20:0	0.26	0.28	0.27	0.26	0.157	0.642	0.853	0.009
18:3 α	1.78	1.99	1.94	1.83	0.013	0.224	0.737	0.040
18:2 *c9t11*	1.48	1.68	1.75	1.42	0.192	0.033	0.985	0.070
21:0	0.14	0.15	0.14	0.15	0.251	0.107	0.470	0.004
22:0	0.16	0.17	0.17	0.16	0.244	0.273	0.219	0.004
SFA	63.60	62.60	61.90	64.30	0.340	0.014	0.513	0.453
SCFA	7.27	6.80	7.27	6.81	0.103	0.114	0.357	0.154
MCFA	22.40	22.40	21.70	23.00	0.971	0.044	0.183	0.301
MUFA	24.60	24.60	25.20	24.10	0.921	0.034	0.509	0.263
OBCFA	4.71	4.84	4.82	4.73	0.208	0.388	0.792	0.049
Trans FA	4.91	5.56	5.78	4.69	0.048	0.001	0.787	0.177
PUFA	4.96	5.49	5.38	5.08	0.004	0.099	0.998	0.082
AI	2.38	2.27	2.19	2.46	0.236	0.004	0.755	0.043
TI	2.15	2.05	2.01	2.20	0.109	0.008	0.807	0.031

SFA: saturated fatty acids; SCFA: short-chain fatty acid; MCFA: medium-chain fatty acid; MUFA: monounsaturated fatty acids; OBCFA: odd and branched-chain fatty acid; PUFA: polyunsaturated fatty acids; SEM: standard error of mean; Atherogenic index (AI) = [C12:0 + 4 (C14: 0) + C16:0]/(sum of unsaturated FA); Thrombogenic index (TI) index = (C14:0 + C16:0 + C18:0)/[(0.5 × ΣMUFA) + (0.5 × Σ*n* − 6 PUFA) + (3 × Σ*n* − 3 PUFA) + (*n* − 3/*n* − 6)] (Ulbricht and Southgate 1991).

significantly higher in ewes fed with carob pulp. No effect of genotype x diet interaction was evident.

Discussion

In our condition, only two *BLG* genotypes (AB and BB) were found, with the predominance of the heterozygous genotype (16 BB ewes *vs.* 30 AB sheep). The largest share of individuals with heterozygous AB observed, albeit limited to a small number of individuals, has been reported in several breeds with different purposes, such as the Chios breed (Triantaphyllopoulos et al. 2017), Polish breeds (Mroczkowski et al. 2004; Rozbicka-Wieczorek et al. 2015) Awassi and Morkaraman sheep (Çelik and Özdemir 2006; Jawasreh et al. 2019).

The observed predominance of the B allele resulted in an agreement with the results previously shown in Valle del Belìce and Sarda breeds (Giaccone et al. 2000; Pietrolà et al. 2000), which are genetically connected breeds (Portolano 1987).

The effect of *BLG* genotype on milk traits in different species is not yet clearly defined. In our condition, no significant effect of *BLG* was evident on milk yield and gross composition. In the same breed, Giaccone et al. (2000) found higher milk yield and lower fat and protein levels in AA ewes. In other sheep breeds contrasting results are reported. The reasons for these

different results are probably due to breed, environment, and sample size, as reported by Özdemir and Esenbuğa (2020). These authors, through a meta-analysis, evidential that, in the dairy type sheep, *BLG* AA genotype was superior in term of total milk yield, and AB genotype was superior in terms of protein and casein content. Another meta-analysis conducted on sheep and goats showed that the B allele positively affected milk fat percentage, whereas no associations of the *BLG* polymorphism were evident for milk protein level (Razmkabir et al. 2021).

Carob pulp has been recognised as a valid alternative in different species of ruminants and with different productive attitudes when supplied in partial replacement of cereal grains. The total replacement of cereals with this by-product as a supplement for grazing animals could represent a further push towards a sustainable feeding, also in relation to its peculiar dietary and nutritional characteristics.

In the present experiment, the use of carob pulp for animals fed on pasture arises from the hypothesis that a sugar-rich feed associated with moderate levels of tannins could positively interfere with ruminal protein efficiency (Giovanetti et al. 2019). In fact, taking into account that herbage protein is generally characterised by high degradability, it is often associated with high non-protein nitrogen losses.

In our conditions, the contents of total polyphenol, sugars, and crude protein of carob falls within the range of values reported by Avallone et al. (1997) on samples collected in the same area of our trial.

A slight detrimental effect of carob pulp was highlighted in our conditions on milk yield and protein level. The lower crude protein level, also associated to a much higher percentage of acid detergent insoluble protein could explain the reduction in productivity. Only fat percentage was positively associated with carob pulp. For this reason, FCM and the FPCM calculated as reported by Pulina et al. (2005) were not significantly affected by diet. The fat gain with carob pulp could be associated with its high sugar content. The positive relationship between dietary sugars and milk fat has already been found by Broderick et al. (2008) and by Razzaghi et al. (2016). The significant decrease of milk urea in the carob group seems to support a possible effect of this by-product in improving protein utilisation in the rumen, as this parameter represents a good indicator of protein efficiency (Spek et al. 2013).

Most studies on carob pulp feeding have been carried out on lambs or kids, whereas few studies are available on milking animals. Louca and Papas (1973) fed lactating goats with 15% and 30% of carob pod in a concentrate mixture. Only the higher level of inclusion significantly reduced milk yield, whereas no effect was evident with 15%. Aloueedat et al. (2019) did not find significant variations in milk yield and composition as the effect of the inclusion of 20 or 40% of carob pulp in a total mixed ration for Awassi ewes. Hassan et al. (2016) report that in Zaraibi goats' milk yield, fat and protein levels increased by supplying up to 50 g of carob pulp inclusion in a diet based on a mixed concentrate and corn silage, whereas performance decreased with 100 g of carob inclusion. In our conditions, assuming a pasture dry matter intake of 1300 g per day, obtained according to an equation developed within the same grazing system and in the same geographical area (Avondo et al. 2002), the level of carob inclusion should be about 15% of the diet, a rather low value, compared with data from literature.

The interaction between diet and genotype did not yield significant results for any parameter. The thesis of a possible different role of the diet on the two genotypes arose from the results obtained in previous papers on the genetic polymorphism at the lactoprotein locus of alpha s1 casein in goats: a different intake of energy with the diet led to an increase in production and a decrease in urea only in the milk of animals with strong genotype (AA), demonstrating that a high energy intake with the diet increased the feeding efficiency only in the strong genotype (Pagano et al. 2010). It was also found that a higher energy input worsened the qualitative characteristics of milk fat (more medium-chain fatty acids) only in the weak genotype but not in the strong one (Valenti et al. 2010). Therefore we believed that carob, mainly due to its high sugar content, could exert a different effect in relation to the two genotypes under study.

Although the physiological functions of β-lactoglobulin in the organism of ruminants are not yet well-understood, it would seem to have a role in the transport of fatty acids (Pérez and Calvo 1995; Le Maux et al. 2014). In particular, palmitic and oleic acids together would represent 75% of the FA bounds (Le Maux et al. 2014).

A preliminary study on the association between polymorphism at the *BLG* locus and acidic composition of Massese ewes milk was conducted by Mele et al. (2007), finding some significant differences between genotypes. In particular, they found that milk from heterozygous ewes (AB) was richer in MUFA, LCFA, different trans FA, CLA, and poorer in MCFA compared to milk from AA and BB ewes. Differently, Rozbicka-Wieczorek et al. (2015) reported that the B

allele was associated with ewe milk richer in MUFA, oleic acid, PUFA n-3, and LCFA.

Results from our conditions are not in line with these previous data: only a few FA were significantly affected by *BLG* genotype: oleic acid was higher in AB milk, whereas linoleic, alpha-linolenic acid, total trans FA, and total PUFA were higher in BB milk.

The different results are probably attributed to different breeds raised in distinct environmental conditions. Several genes are known to be involved in FA synthesis (e.g. *DGAT1*, *FASN*, and *SCD*). Numerous studies observed that genetic variants identified at the lipogenic loci and associated with different milk FA profiles had different frequencies depending on the breed (Crisà et al. 2010; Pecka-Kiełb et al. 2021).

Based on the limited knowledge of the functional role of β-lactoglobulin, we are not able to associate our results with particular structural differences of β-lactoglobulin, due to the different genotypes. However, at least limited to oleic acid, which in our conditions was influenced by the genotype, we can refer to the role of beta-lactoglobulin in the prevailing transport of oleic acid, together with palmitic acid.

The fatty acid composition was strongly affected by carob pulp supplementation. A worsening in fat composition was found in milk from carob-fed ewes: total SFA were higher, and CLA and trans-FA were lower in milk from ewes fed with carob pulp, compared to barley. Similar differences were found between diets with different sugar content for CLA (Avondo et al. 2008) and for trans fatty acids (Razzaghi et al. 2016). The atherogenic (AI) and thrombogenic indexes (TI) values were higher in raw milk of ewes fed carob. However, the AI index value was found to be similar to those reported by Soják et al. (2013) and lower than values reported by Mierlita et al. (2011) for Spanca sheep raw milk fat and by Sinanoglou et al. (2015) for Karagouniko and Chios sheep breeds in early and middle lactation stages, while the TI index values resulted higher than those reported by Sinanoglou et al. (2015).

Saturated fatty acids, in particular medium-chain fatty acids, have been demonstrated to have cholesterol increasing properties and, as a consequence, seem associated with increased coronary heart disease risk (Davis et al. 2022). Trans fatty acids have either not been associated or have been negatively associated with an increased risk of coronary heart diseases (Stender et al. 2008). IA and IT are the most commonly used indexes to assess the diet fatty acid composition effect on cardiovascular health however no recommended values have yet been provided (Chen and Liu 2020; Corral et al., 2010). The positive effect of CLA on health is well-known (Koba and Yanagita 2014).

Results on fatty acid profile are reported only for growing animals whereas, to our knowledge, no data on the role of carob feeding are reported on the fatty acid profile of milk.

A positive effect on fat nutritional quality is reported in the literature: in fact, carob supplied to lambs at 24 and 35% of the diet or growing pigs at 8 and 15% of the diet caused increases in PUFA n3, MUFA, CLA, and decreases of SFA and PUFA n6 (Gravador et al. 2015; Inserra et al. 2015). On the contrary, in line with our data, Vasta et al. (2007) with a higher level of carob inclusion (450 g/kg), found a worsening of lambs fat quality, with lower CLA and trans FA and higher SFA and C14 levels, compared to a control diet based on a commercial concentrate. This detrimental effect on fat quality was probably due to too high a level of carob inclusion.

The level of carob pulp in our feeding trial was much lower than that reported by Gravador et al. (2015) and by Inserra et al. (2015) in pigs. Nonetheless, 250 g/d of carob pulp reduced performance and milk quality (apart from an increase in milk fat content and a decrease in urea level).

The hypothesis that in dairy animals, the level of tannins that can be tolerated without adverse effects on fat composition could be lower than in growing animals seems to be withdrawn by results reported by Valenti et al. (2019), which supplying a sugar and tannins rich by-product, such as pomegranate pulp, found that lactating ewes, even reaching much higher levels of tannins intake, showed an improvement in milk fat quality. However, as suggested by Min et al. (2003), the effects produced by condensed tannins depend not only on concentration but also on their structure.

Another hypothesis could be that the presence of tannins in carob fed ewes may have caused a decrease in pasture intake, which could be associated with the less healthy milk fatty acid profile compared to the control milk. However, it should be taken into account that total tannins from carob pulp were very low (12.5 g/kg DM) and much lower than 55 g/kg DM, which is reported by Min et al. (2003) as the level above which ingestion tends to decrease. It is therefore unlikely that such a low level could have negatively affected the intake capacity of the grazing animals.

The great variability of the results reported in the literature, therefore, suggests that it is more likely that milk traits and composition, as often happens, are

closely associated with a combination of factors, such as the particular chemical-physical structure of the feeds used, their interaction with the available forage, the breed, the physiological state of the animals and the environmental conditions that together may contribute to the determination of results.

Conclusion

Interaction between *BLG* genotype and carob supplement to grazing ewes was not evident. *BLG* was not determinant on milk yield and gross composition but influenced fatty acids profile, as BB genotype showed a reduction (5%) of oleic acid but increases in linoleic (6%) and linolenic (12%) acids, thus denoting higher healthy value, compared to AB genotype. Carob pulp, at relatively moderate levels, such as that utilised in our conditions, decreased milk urea probably due to an improved ruminal protein efficiency associated with its sugar and tannins content; it increased fat concentration but worsened its quality as demonstrated by the higher total saturated fatty acids, compared to barley. Although the carob pulp supplementation resulted in a slight reduction in milk quality, it did not affect the FCM and FPCM. Moreover, the observed reduction of urea content and the low cost of carob pulp could make its use still convenient in terms of environmental and economic sustainable feeding management.

Ethical approval

The study was analysed and approved by the Animal Welfare Committee (OPBA) of the University of Catania (prot. No. 158467).

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

Derived data supporting the findings of this study are available from the corresponding author (S.T.) on request.

References

Aloueedat MK, Obeidat BS, Awawdeh MS. 2019. Effects of partial replacement of conventional with alternative feeds on nutrient intake, digestibility, milk yield and composition of Awassi ewes and lambs. Animals. 9(9):684.
AOAC. 1990. Official methods of analysis. 15th ed. Arlington, VA: AOAC Washington, DC.
Avallone R, Plessi M, Baraldi M, Monzani A. 1997. Determination of chemical composition of carob (*Ceratonia siliqua*): protein, fat, carbohydrates, and tannins. J Food Compos Anal. 10(2):166–172.
Avondo M, Bonanno A, Pagano RI, Valenti B, Di Grigoli A, Alicata ML, Galofaro V, Pennisi P. 2008. Milk quality as affected by grazing time of day in Mediterranean goats. J Dairy Res. 75(1):48–54.
Avondo M, Bordonaro S, Marletta D, Guastella AM, D'Urso G. 2002. A simple model to predict the herbage intake of grazing dairy ewes in semi-extensive Mediterranean systems. Livest Prod Sci. 73(2–3):275–283.
Broderick GA, Luchini ND, Reynal SM, Varga GA, Ishler VA. 2008. Effect on production of replacing dietary starch with sucrose in lactating dairy cows. J Dairy Sci. 91(12): 4801–4810.
Campione A, Natalello A, Valenti B, Luciano G, Rufino-Moya PJ, Avondo M, Morbidini L, Pomente C, Krol B, Wilk M, et al. 2020. Effect of feeding hazelnut skin on animal performance, milk quality, and rumen fatty acids in lactating ewes. Animals. 10(4):588.
Çelik Ş, Özdemir S. 2006. β-Lactoglobulin variants in Awassi and Morkaraman sheep and their association with the composition and rennet clotting time of the milk. Turk J Vet Anim Sci. 30(6):539–544.
Chen J, Liu H. 2020. Nutritional indices for assessing fatty acids: a mini-review. Int J Mol Sci. 21(16):5695.
Corral JM, Padilla JA, Izquierdo M. 2010. Associations between milk protein genetic polymorphisms and milk production traits in Merino sheep breed. Livestock Science. 129(1-3):73–79.
Correddu F, Lunesu MF, Buffa G, Atzori AS, Nudda A, Battacone G, Pulina G. 2020. Can agro-industrial by-products rich in polyphenols be advantageously used in the feeding and nutrition of dairy small ruminants? Animals. 10(1):131.
Crisà A, Marchitelli C, Pariset L, Contarini G, Signorelli F, Napolitano F, Catillo G, Valentini A, Moioli B. 2010. Exploring polymorphisms and effects of candidate genes on milk fat quality in dairy sheep. J Dairy Sci. 93(8):3834–3845.
Davis H, Magistrali A, Butler G, Stergiadis S. 2022. Nutritional benefits from fatty acids in organic and grass-fed beef. Foods. 11(5):646.
Deriaz RE. 1961. Routine analysis of carbohydrates and lignin in herbage. J Sci Food Agric. 12(2):152–160.

FAO. 2020. https://www.fao.org/faostat/en/#data/QCL
Feng S, Lock AL, Garnsworthy PC. 2004. Technical note: a rapid lipid separation method for determining fatty acid composition of milk. J Dairy Sci. 87(11):3785–3788.
Giaccone P, Di Stasio L, Macciotta NP, Portolano B, Todaro M, Cappio-Borlino A. 2000. Effect of beta-lactoglobulin polymorphism on milk-related traits of dairy ewes analysed by a repeated measures design. J Dairy Res. 67(3): 443–448.
Giovanetti V, Boe F, Decandia M, Bomboi GC, Atzori AS, Cannas A, Molle G. 2019. Milk urea concentration in dairy sheep: accounting for dietary energy concentration. Animals. 9(12):1118.
Gravador RS, Luciano G, Jongberg S, Bognanno M, Scerra M, Andersen ML, Lund MN, Priolo A. 2015. Fatty acids and oxidative stability of meat from lambs fed carob-containing diets. Food Chem. 182:27–34.
Hall MB, Arbaugh J, Binkerd K, Carlson A, Doan T, Grant T, Heuer C, Inerowicz HD, Jean LB, Johnson R, et al. 2015. Determination of dietary starch in animal feeds and pet food by an enzymatic-colorimetric method: collaborative study. J AOAC Int. 98(2):397–409.
Hassan AA, Yacout MH, Khalel MS, Adrian TR, Dorina M, Magdalena TR. 2016. The economic effect of a daily supplementation of carob pods (*Ceratonia siliqua* L.) on rumen fermentation and lactating goats performance. Cellulose. 124:224–225.
Hayes HC, Petit EJ. 1993. Mapping of the β-lactoglobulin gene and of an immunoglobulin M heavy chain-like sequence to homoeologous cattle, sheep, and goat chromosomes. Mamm Genome. 4(4):207–210.
Inserra L, Luciano G, Bella M, Scerra M, Cilione C, Basile P, Lanza M, Priolo A. 2015. Effect of including carob pulp in the diet of fattening pigs on the fatty acid composition and oxidative stability of pork. Meat Sci. 100:256–261.
Jawasreh K, Amareen AA, Aad P. 2019. Effect and interaction of β-lactoglobulin, kappa casein, and prolactin genes on milk production and composition of Awassi sheep. Animals. 9(6):382.
Karabulut A, Canbolat O, Kamalak A. 2006. Evaluation of carob, *Ceratonia siliqua* pods as a feed for sheep. Livest Res Rural Dev. 18(7):277.
Koba K, Yanagita T. 2014. Health benefits of conjugated linoleic acid (CLA). Obes Res Clin Pract. 8(6):e525–e532.
Kotrotsios N, Christaki E, Bonos E, Florou-Paneri P. 2012. Dietary carob pods on growth performance and meat quality of fattening pigs. Asian Australas J Anim Sci. 25(6): 880–885.
Le Maux S, Bouhallab S, Giblin L, Brodkorb A, Croguennec T. 2014. Bovine β-lactoglobulin/fatty acid complexes: binding, structural, and biological properties. Dairy Sci Technol. 94(5):409–426.
Licitra G, Hernandez TM, Van Soest PJ. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim Feed Sci Technol. 57(4):347–358.
Louca A, Papas A. 1973. The effect of different proportions of carob pod meal in the diet on the performance of calves and goats. Anim Sci. 17(2):139–146.
Makkar HPS, Blümmel M, Borowy NK, Becker K. 1993. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. J Sci Food Agric. 61(2):161–165.
Mele M, Conte G, Serra A, Buccioni A, Secchiari P. 2007. Relationship between beta-lactoglobulin polymorphism and milk fatty acid composition in milk of Massese dairy ewes. Small Ruminant Research. 73(1-3):37–44.
Miller LA, Moorby JM, Davies DR, Humphreys MO, Scollan ND, MacRae JC, Theodorou MK. 2001. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.): milk production from late-lactation dairy cows. Grass Forage Sci. 56(4):383–394.
Miller SA, Dykes DD, Polesky HF. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16(3):1215.
Min B, Barry T, Attwood G, McNabb W. 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. Anim Feed Sci Technol. 106(1–4):3–19.
Moatsou G, Hatzinaki A, Samolada M, Anifantakis E. 2005. Major whey proteins in ovine and caprine acid wheys from indigenous Greek breeds. Int Dairy J. 15(2):123–131.
Mroczkowski S, Korman K, Erhardt G, Piwczynski D, Borys B. 2004. Sheep milk protein polymorphism and its effect on milk performance of Polish Merino. J Archiv Fur Tierzucht. 47(6):114–121.
Obeidat BS, Alrababah MA, Alhamad MN, Gharaibeh MA, Ishmais MA. 2012. Effects of feeding carob pods (*Ceratonia siliqua* L.) on nursing performance of Awassi ewes and their lambs. Small Rumin Res. 105(1–3):9–15.
Özdemir M, Esenbuğa N. 2020. Associations among beta-lactoglobulin genotypes and some production traits in sheep: A systematic review and meta-analysis. Journal of Animal and Plant Sciences. 30(5):1092–1097.
Pagano RI, Pennisi P, Valenti B, Lanza M, Di Trana A, Di Gregorio P, De Angelis A, Avondo M. 2010. Effect of CSN1S1 genotype and its interaction with diet energy level on milk production and quality in Girgentana goats fed *ad libitum*. J Dairy Res. 77(2):245–251.
Pagano RI, Valenti B, De Angelis A, Avondo M, Pennisi P. 2011. Morning versus afternoon cutting time of Berseem clover (*Trifolium alexandrinum* L.) affects feed intake, milk yield and composition in Girgentana goats. J Dairy Res. 78(4):500–504.
Pecka-Kiełb E, Kowalewska-Łuczak I, Czerniawska-Piątkowska E, Króliczewska B. 2021. FASN, SCD1 and ANXA9 gene polymorphism as genetic predictors of the fatty acid profile of sheep milk. Sci Rep. 11(1):1–11.
Pelegrin-Valls J, Serrano-Pérez B, Villalba D, Molina E, Espinal J, Joy M, Álvarez-Rodríguez J. 2022. Is the inclusion of carob (*Ceratonia siliqua* L.) pulp in the concentrate of weaned light lambs worth it? Anim Feed Sci Technol. 293: 115452.
Pérez MD, Calvo M. 1995. Interaction of β-lactoglobulin with retinol and fatty acids and its role as a possible biological function for this protein: a review. J Dairy Sci. 78(5):978–988.
Pietrolà E, Carta A, Fraghi A, Piredda G, Pilla F. 2000. Effect of β-lactoglobulin locus on milk yield in Sarda ewes. Zoot Nutr Anim. 26(3):131–135.
Portolano N. 1987. La pecora della Valle Del Belìce, Edagricole 1987. In Pecore e Capre Italiane.
Priolo A, Lanza M, Biondi L, Pappalardo P, Young OA. 1998. Effect of partially replacing dietary barley with 20% carob

pulp on post-weaning growth, and carcass and meat characteristics of Comisana lamb. Meat Sci. 50(3):355–363.
Pulina G, Macciotta N, Nudda A. 2005. Milk composition and feeding in the Italian dairy sheep. Ital J Anim Sci. 4(sup1): 5–14.
Puppel K, Kuczyńska B, Nałęcz-Tarwacka T, Gołębiewski M, Sakowski T, Kapusta A, Budziński A, Balcerak M. 2016. Effect of supplementation of cows diet with linseed and fish oil and different variants of β-lactoglobulin on fatty acid composition and antioxidant capacity of milk. J Sci Food Agric. 96(6):2240–2248.
Razmkabir M, Mahmoudi P, Rashidi A, Rostamzadeh J, Idrees S. 2021. Effect of BLG gene variants on milk-related traits in small ruminants: a meta-analysis. Ital J Anim Sci. 20(1): 1410–1422.
Razzaghi A, Valizadeh R, Naserian AA, Mesgaran MD, Carpenter AJ, Ghaffari MH. 2016. Effect of dietary sugar concentration and sunflower seed supplementation on lactation performance, ruminal fermentation, milk fatty acid profile, and blood metabolites of dairy cows. J Dairy Sci. 99(5):3539–3548.
Richane A, Ismail HB, Darej C, Attia K, Moujahed N. 2022. Potential of Tunisian carob pulp as feed for ruminants: chemical composition and *in vitro* assessment. Trop Anim Health Prod. 54(1):58.
Rodríguez-Solana R, Romano A, Moreno-Rojas JM. 2021. Carob pulp: a nutritional and functional by-product worldwide spread in the formulation of different food products and beverages. A review. Processes. 9(7):1146.
Rozbicka-Wieczorek A, Radzik-Rant A, Rant W, Puppel K. 2015. The effect of breed, β-lactoglobulin variants and somatic cell count on yield, chemical components and whey protein composition in milk of non-dairy sheep. J Anim Plant Sci. 25(3):633–639.
Selvaggi M, Laudadio V, Dario C, Tufarelli V. 2015. β-Lactoglobulin gene polymorphisms in sheep and effects on milk production traits: a review. Adv Anim Vet Sci. 3(9):478–484.
Shingfield KJ, Ahvenjärvi S, Toivonen V, Ärölä A, Nurmela KVV, Huhtanen P, Griinari JM. 2003. Effect of dietary fish oil on biohydrogenation of fatty acids and milk fatty acid content in cows. Anim Sci. 77(1):165–179.
Sinanoglou VJ, Koutsouli P, Fotakis C, Sotiropoulou G, Cavouras D, Bizelis I. 2015. Assessment of lactation stage and breed effect on sheep milk fatty acid profile and lipid quality indices. Dairy Sci Technol. 95(4):509–531.
Soják L, Blaško J, Kubinec R, Górová R, Addová G, Ostrovský I, Margetín M. 2013. Variation among individuals, breeds, parities and milk fatty acid profile and milk yield of ewes grazed on pasture. Small Rumin Res. 109(2–3):173–181.
Spek J, Dijkstra J, Van Duinkerken G, Bannink A. 2013. A review of factors influencing milk urea concentration and its relationship with urinary urea excretion in lactating dairy cattle. J Agric Sci. 151(3):407–423.
Stender S, Astrup A, Dyerberg J. 2008. Ruminant and industrially produced trans fatty acids: health aspects. Food Nutr Res. 52(1):1651.
Triantaphyllopoulos KA, Koutsouli P, Kandris A, Papachristou D, Markopoulou KE, Mataragka A, Massouras T, Bizelis I. 2017. Effect of β-lactoglobulin gene polymorphism, lactation stage and breed on milk traits in Chios and Karagouniko sheep breeds. Ann Anim Sci. 17(2):371–384.
Tumino S, Criscione A, Moltisanti V, Marletta D, Bordonaro S, Avondo M, Valenti B. 2021. Feeding system resizes the effects of DGAT1 polymorphism on milk traits and fatty acids composition in Modicana cows. Animals. 11(6):1616.
Ulbricht TLV, Southgate DAT. 1991. Coronary heart disease: seven dietary factors. Lancet. 338(8773):985–992.
Valenti B, Criscione A, Moltisanti V, Bordonaro S, De Angelis A, Marletta D, Di Paola F, Avondo M. 2019. Genetic polymorphisms at candidate genes affecting fat content and fatty acid composition in Modicana cows: effects on milk production traits in different feeding systems. Animal. 13(6):1332–1340.
Valenti B, Luciano G, Morbidini L, Rossetti U, Codini M, Avondo M, Priolo A, Bella M, Natalello A, Pauselli M. 2019. Dietary pomegranate pulp: effect on ewe milk quality during late lactation. Animals. 9(5):283.
Valenti B, Pagano R, Pennisi P, Lanza M, Avondo M. 2010. Polymorphism at αs1-casein locus. Effect of genotype × diet interaction on milk fatty acid composition in Girgentana goat. Small Rumin Res. 94(1–3):210–213.
Van Soest P, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci. 74(10): 3583–3597.
Vasta V, Pennisi P, Lanza M, Barbagallo D, Bella M, Priolo A. 2007. Intramuscular fatty acid composition of lambs given a tanniniferous diet with or without polyethylene glycol supplementation. Meat Sci. 76(4):739–745.
Vasta V, Luciano G. 2011. The effects of dietary consumption of plants secondary compounds on small ruminants' products quality. Small Rumin Res. 101(1–3):150–159.