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Effects of spent coffee grounds on production traits, haematological parameters, and antioxidant activity of blood and milk in dairy goats

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ABSTRACT

Spent coffee ground (**SCG**) is a byproduct of coffee beverage preparation and a potential source of carbohydrate, protein, and phenolic compounds for livestock feeding. In this study, the effects of SCG supplementation in the diet of lactating goats on milk production traits and health status were studied. The antioxidant status of blood and milk was also evaluated. Twenty-four Saanen goats were fed a total mixed ration containing commercial concentrate, soybean, and haylage; they were divided into three groups: control diet (**CON**), **SCG50** (50 g/d SCG), and **SCG100** (100 g/d SCG). The experiment lasted 6 weeks. Linear and quadratic contrasts were used to evaluate the effects of the byproduct doses. SCG supplementation did not affect milk production, but influenced some milk fatty acids. SCG supplementation increased the contents of C18:1, cis-9, trans-11 C18:2, odd and branched-chain fatty acids, and total conjugated linoleic acid. Most of the haematological and biochemical parameters were within the physiological range for goats. The basophil, eosinophil, and glucose contents were quadratically affected by SCG, whereas platelet count increased linearly with the SCG dose. The SCG supplementation had a positive effect on the blood antioxidant status, as evidenced by an increase in ferric reducing antioxidant power and a decrease in malondialdehyde. The SCG supplementation had no effect on the milk antioxidant status. The results show that SCG (up to 100 g/d) did not negatively affect milk production and health status in goats. However, quadratic effects on some antioxidant and biochemical parameters suggest that further investigations are necessary, especially with regard to the optimisation of the supplement dose.

Implications

Spent coffee ground is a byproduct of the global coffee industry, which can be conveniently recycled as a supplement for animal feeding. Spent coffee contains bioactive components that can positively affect animal productive performances and health status. This work showed that this byproduct did not have negative effects on animal performance and milk quality. Also, spent coffee ground decreased the oxidative stress in goat blood, improving the animal welfare. Spent coffee ground can replace some conventional feed ingredients, thus improving profitability of farms and reducing wastes from industry.

Introduction

Coffee is one of the most consumed beverages worldwide. Several byproducts are generated during its various processing stages. Among these byproducts, spent coffee ground (**SCG**) is the main byproduct of coffee beverage preparation. Approximately, 1 kg of green coffee generates 0.65 kg of SCG, and 1 kg of instant coffee produces approximately 2 kg of wet SCG. Most of this byproduct is incinerated or disposed of in landfills (San Martin et al., 2020). However, SCG contains bioactive compounds that could be recovered and reused, increasing the coffee industry's sustainability. SCG is a source of NDF (64–68%, Choi et al., 2018), protein (15.4%), and lipids (9.3–16.2%, Cruz et al., 2012), with a considerable amount of polyunsaturated fatty acids. In addition, SCG and other coffee byproducts are a good source of polyphenols (1–1.5% total polyphenols, Campos-Vega et al., 2015) that could exert a potential antioxidant effect. The literature has investigated the use of byproducts in livestock feeding (Nudda et al., 2017; Buffa et al., 2020a; Salami et al., 2021); however, further research on this

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byproduct is necessary. The effects of SCG on milk composition have been investigated in cows (San Martin et al., 2020) and ewes (de Otálora et al., 2020). According to our literature review, no information is available regarding the effects of SCG on animal production performances, and health status of dairy goats. Thus, the aim of this study was to investigate the effects of SCG supplementation in the diet of lactating goats on milk yield, composition, fatty acid profile, and antioxidant stability. Blood antioxidant activity and animal health status were also studied to evaluate the healthiness of this byproduct. The doses used for this study were chosen, while considering the results of a recent study on sheep by de Otálora et al. (2020) along with the polyphenols and methylxanthines contents of SCG collected and used in this trial.

Preliminary results have been presented at the 24th Congress of the Animal Science and Production Association, and the abstract has been published in the conference proceedings (Carta et al., 2021).

Material and methods

Experimental design

Twenty-four Saanen dairy goats were randomly allocated to three experimental groups (eight animals per group) homogeneous for milk yield (4.31 ± 0.06 kg/d per head, mean ± SD), BW (61.6 ± 0.87 kg, mean ± SD), body condition score (**BCS**, 2.84 ± 0.05, mean ± SD), and days in milk (70 ± 15 days, mean ± SD). The goats were machine milked twice daily at 08:00 and 20:00, and the individual milk production for the morning and evening milking was recorded weekly. The BCS (1–5 scale) and BW were recorded at the beginning and at the end of the experiment. The trial lasted 6 weeks, with 1 week of adaptation to the byproduct and 5 weeks of experimental sampling.

Each goat was fed a total mixed ration (**TMR**) as a control diet, containing 2.3 kg/d haylage, 160 g/d soybean meal, 200 g/d beet pulp, and 1.2 kg/d commercial concentrate. Animals of each group were housed together in a barn divided into three adjacent pens to ensure normal social behaviour. However, they were individually fed with the TMR offered four times per day: two times during milking and two times at 14:00 and 24:00 in individual self-capturing feeders. Fresh, clean water was available for ad libitum consumption. The experimental groups received the control diet supplemented with 0 (Control group, **CON**), 50 g (**SCG50**), and 100 g (**SCG100**) of SCG, respectively. The SCG dose was administered in two aliquots offered to animals during morning and evening milking. The used byproduct was collected from different coffee shops in northern Sardinia and dried at 65 °C to prevent microbial deterioration. The chemical composition and the polyphenolic profile of SCG are reported in Tables 1 and 2, respectively.

Table 2
Polyphenols and methylxanthines of spent coffee ground offered to dairy goats.

Items	mg/kg of SCG
Ferulic acid	12 393
Caffeic acid	885
5-Caffeoylquinic acid	158
Catechol	141
Trigonellin	12.5
1,2,4 benzenetriol	<0.1
Caffeine	894
Theobromine/Theophylline	283

Abbreviation: SCG = spent coffee ground.

Sampling and analysis

All dietary ingredients were collected at the beginning and at the end of the trial and were analysed for DM content by drying at 105 °C for 24 h. Feed samples were also analysed for NDF according to the method developed by Mertens (2002), using heat-stable amylase and expressed exclusive of residual ash (aNDFom). ADL were analysed according to the Association of official Analytical Chemists (**AOAC**) method 973.18 (AOAC, Official Method of Analysis, 2000; method 973.18); the ADL using the Robertson and Van Soest (1981) method and using sulfuric acid.

The CP was determined by using the Kjeldahl method (AOAC, 2000; method 988.05), and ash was determined using a muffle at 550 °C (AOAC, 2000; method 942.05). All these parameters were expressed as % of DM.

Individual milk samples were collected weekly, at the morning and afternoon milking for a total of five samplings. The samples were analysed separately for fat, protein, casein, lactose, and urea content (Milkoscan 6000, Foss Electric, Hillerød, Denmark) and for somatic cell count (**SCC**) (Fossomatic 360, Foss Electric). The weighted average of the morning and afternoon data was calculated for each considered parameter. Individual milk samples were

Table 1
Ingredients and chemical composition of diets offered to dairy goats.

Items	Commercial concentrate	SCG	Diet		
			CON	SCG50	SCG100
Ingredients mixed meal (kg/d per head, as fed)					
Spent coffee ground			-	0.05	0.10
Beet pulp			0.20	0.20	0.20
Soybean			0.16	0.16	0.16
Commercial concentrate			1.2	1.2	1.2
Haylage			2.3	2.3	2.3
Total offered (kg/d, as fed)			3.86	3.91	3.96
Total offered (kg/d, on DM)			2.30	2.35	2.39
Total measured intake (kg/d, as fed)			3.80	3.83	3.90
Chemical composition (% of DM)					
NDF	27.79	55.48	40.05	40.36	40.64
ADF	12.31	33.15	23.55	23.74	23.92
CP	16.93	15.03	15.95	15.93	15.91
Ash	5.64	91.35	7.56	7.45	7.34

Abbreviations: SCG = spent coffee ground; CON = diet without supplementation; SCG50 = diet supplemented with 50 g/d per head of spent coffee ground; SCG100 = diet supplemented with 100 g/d per head of spent coffee ground.

also collected every week and stored at −20 °C for fatty acid methyl ester (**FAME**) analysis (Supplementary Material S1). Finally, milk samples for antioxidant analysis were collected at weeks 1, 2, and 5 of the trial and stored at −20 °C and then analysed for ferric reducing antioxidant power (**FRAP**), 2,2′-azino-di(3-ethylbenzthia zoline-6-sulforic acid; **ABTS**), malondialdehyde (**MDA**), and protein carbonyls (Supplementary Material S2).

Blood samples were collected by jugular venipuncture after the morning milking at the beginning and at the end of the experiment. Vacutainers with clotting accelerator were used to collect blood for serum, whereas vacutainers with dipotassium-ethylenediamine tetraacetic acid, as an anticoagulant, were used to have the whole blood, respectively. For the antioxidant analysis, blood samples were collected with a heparinised vacuum tube and then centrifuged at 3 000*g*, at 4 °C, for 10 min to separate plasma from the corpusculate fraction and were stored at −80 °C. Serum samples were also analysed by an automatic spectrophotometer to determine the quantity of albumin, alkaline phosphatase, bilirubin, calcium, creatinine, phosphorus, protein, gamma-glutamyl transferase, serum glutamic oxaloacetic transaminase, serum glutamic-pyruvic transaminase, urea, cholesterol, creatine kinase, glucose, and triglycerides. Using an electronic particle counter, the following measurements were conducted: red blood cells, white blood cells, platelets, haemoglobin, mean corpuscular haemoglobin concentration and proportion, corpuscular haemoglobin haematocrit, mean corpuscular volume, lymphocytes, monocytes, granulocytes neutrophils, eosinophils, and basophils.

The antioxidant capacity in milk and blood was evaluated using different assays. In both matrices, the total antioxidant capacity was evaluated by FRAP and ABTS, whereas the lipid and protein oxidative biomarkers were determined by MDA and protein carbonyls assays (Supplementary Material S2).

Statistical analysis

All data (i.e. animal performance, milk composition, and haematological parameters) were analysed as a completely randomised design with repeated measures by using the PROC MIXED procedure of SAS software (version 9.2; SAS Institute, 2008). The model included the fixed effects of diet, sampling date, and diet × sampling date interaction, and the random effect of the animal (Supplementary Material S3). The differences among the levels of the considered effects were tested using orthogonal polynomial contrasts (linear and quadratic). Effects were considered significant at $P < 0.05$ and to have a tendency to difference at $P < 0.10$.

Results

Dry matter intake, body weight, and milk yield and composition

The SCG was completely consumed by the goats of both supplemented groups, and it did not influence the feed intake ($P = 0.49$). The latter was 3.80, 3.83, and 3.90 kg/d per head for CON, SCG50, and SCG100, respectively. BW was not affected by the SCG supplementation ($P = 0.64$), but it decreased significantly from the beginning to the end of the experiment in all animals (from 61.6 ± 8.6 kg to 58.5 ± 8.4 kg, overall mean ± SD). The effect of dietary supplementation of SCG on milk yield and composition is reported in Table 3. Milk yield was not affected by SCG supplementation (P = 0.73), but it decreased throughout the experimental period ($P < 0.001$; Supplementary Fig. S1). SCG supplementation did not influence the milk protein, fat, and lactose contents. Urea yield (g/d) tended to decrease linearly ($P = 0.08$) with SCG supplementation. The SCG affected the SCC quadratically, because the low dose decreased the SCC ($P < 0.001$), whereas the highest dose did not change the cellular count compared with that of the CON group. The content of NaCl linearly increased with the SCG supplementation ($P = 0.05$).

Milk fatty acid profile

The effect of SCG on milk fatty acid profile is reported in Table 4. The short-chain fatty acids C6:0 ($P = 0.05$) and C8:0 ($P = 0.09$) tended to decrease linearly. Branched-chain fatty acids, as isoC14:0 ($P = 0.001$) and anteisoC15:0 ($P = 0.04$), increased linearly, and isoC15:0, isoC16:0, and anteisoC17:0 tended to increase linearly with SCG supplementation. The SCG supplementation resulted in a linear increase in C15:0 ($P = 0.01$). The SCG supplementation affected the concentrations of C18:1 trans-11 that increased quadratically ($P = 0.005$), and of CLA cis-9 trans-11 that increased linearly.

Blood and milk oxidation

Blood oxidation capacity improved after using SCG in the diet (Table 5). Notably, the coffee byproduct supplementation increased the FRAP ($P = 0.02$) and decreased the MDA ($P = 0.01$). The antioxidant capacity of SCG on blood plasma is confirmed by the MDA assays. However, ABTS and protein carbonyls were not influenced by the byproduct. The SCG supplementation did not affect any antioxidant parameters tested in goat milk (Table 6).

Table 3
Effect of dietary supplementation of two doses of spent coffee ground on goat milk yield and composition.

Items	Diet			SEM	*P*-value		
	CON	SCG50	SCG100		L	Q	Time
Yield (g/d)							
Milk	3 680.9	3 592.0	3815.5	58.12	0.669	0.508	<0.001
Fat	115.65	107.70	114.21	1.88	0.900	0.343	0.009
Protein	111.08	103.98	110.10	1.63	0.910	0.333	<0.001
Lactose	162.05	156.45	164.25	2.66	0.866	0.549	<0.001
Urea	1.37	1.30	1.29	0.02	0.082	0.615	<0.001
Milk composition							
Fat (%)	3.16	3.01	3.02	0.04	0.521	0.740	0.029
Protein (%)	3.03	2.92	2.89	0.02	0.273	0.688	0.488
Lactose (%)	4.41	4.36	4.29	0.02	0.205	0.848	0.020
Urea (mg/dl)	37.53	36.61	33.73	0.53	0.411	0.671	<0.001
Log SCC (1 000 cell/ml)	2.66	2.46	2.89	0.04	0.074	<0.001	<0.001
NaCl	302.50	325.57	334.16	3.08	0.051	0.666	0.015

Abbreviations: CON = diet without supplementation; SCG50 = diet supplemented with 50 g/d per head of spent coffee ground; SCG100 = diet supplemented with 100 g/d per head of spent coffee ground; SCC = somatic cell count; L = linear effect of the diet; Q = quadratic effect of the diet; Time = sampling time effect.

Table 4
Fatty acid profile and nutritional indices of fatty acids of milk of goat fed with control diet (CON), 50 g spent coffee ground (SCG50) and 100 g of spent coffee ground (SCG100).

Fatty acid (g/100 g of FAME)	Diet			SEM	*P*-value		
	CON	SCG50	SCG100		L	Q	Time
C4:0	1.70	1.68	1.75	0.03	0.778	0.267	0.064
C6:0	1.82	1.79	1.73	0.02	0.051	0.093	<0.001
C8:0	2.08	1.97	1.95	0.02	0.089	0.750	<0.001
C9:0	0.06	0.07	0.08	0.002	0.059	0.801	<0.001
C10:0	7.59	7.22	7.33	0.10	0.155	0.999	<0.001
C10:1	0.04	0.04	0.04	0.001	0.363	0.473	<0.001
C11:0	0.22	0.22	0.20	0.004	0.620	0.157	<0.001
C12:0	3.38	3.27	3.32	0.05	0.985	0.729	<0.001
iso C13:0	0.02	0.02	0.02	0.0004	0.458	0.990	0.631
anteiso C13:0	0.03	0.03	0.03	0.001	0.548	0.107	<0.001
iso C14:0	0.09	0.10	0.11	0.002	0.001	0.826	<0.001
C14:0	10.06	9.86	10.18	0.08	0.719	0.334	<0.001
C14:1cis-9	0.11	0.11	0.10	0.003	0.380	0.422	<0.001
iso C15:0	0.22	0.22	0.24	0.003	0.063	0.565	<0.05
anteiso C15:0	0.34	0.35	0.36	0.005	0.038	0.688	<0.001
C15:0	0.93	0.94	1.00	0.01	0.013	0.525	<0.001
C15:1	0.05	0.05	0.05	0.001	0.516	0.912	<0.05
iso C16:0	0.29	0.31	0.34	0.005	0.058	0.928	<0.05
C16:0	25.84	26.77	27.03	0.22	0.586	0.386	<0.05
C16:1trans-4	0.04	0.05	0.04	0.001	0.996	0.130	<0.001
C16:1trans-5	0.04	0.04	0.03	0.001	0.776	0.351	<0.001
C16:1 trans-6 + trans-7	0.05	0.05	0.05	0.001	0.214	0.632	0.003
isoC17_0	0.40	0.40	0.40	0.01	0.793	0.919	0.734
C16:1 trans-9	0.10	0.08	0.09	0.01	0.906	0.243	0.103
C16:1 trans-10	0.02	0.02	0.02	0.0004	0.336	0.829	<0.001
C16:1trans-11 + trans-12	0.04	0.04	0.04	0.001	0.339	0.505	<0.001
C16:1cis-7	0.30	0.29	0.29	0.0003	0.711	0.217	<0.001
anteiso C17:0	0.43	0.42	0.45	0.005	0.088	0.343	0.048
C16:1cis-9	0.53	0.52	0.48	0.01	0.771	0.500	<0.001
C16:1cis-10	0.02	0.02	0.02	0.0004	0.584	0.955	<0.001
C16:1cis-11	0.01	0.01	0.01	0.0003	0.671	0.910	<0.001
C17:0	0.82	0.81	0.83	0.01	0.215	0.713	<0.001
C17:1cis-9	0.28	0.28	0.25	0.01	0.651	0.893	<0.001
C18:0 (SA)	11.44	11.30	10.82	0.17	0.508	0.343	<0.001
C18:1trans-4	0.01	0.01	0.01	0.0003	0.456	0.741	<0.001
C18:1trans-5	0.01	0.01	0.01	0.0004	0.823	0.169	<0.001
C18:1trans-6 + trans-8	0.15	0.15	0.15	0.002	0.376	0.594	<0.001
C18:1trans-9	0.18	0.19	0.19	0.0002	0.093	0.558	0.010
C18:1trans-10	0.21	0.22	0.22	0.004	0.606	0.887	0.102
C18:1 trans-11(VA)	0.73	0.68	0.74	0.01	0.870	0.005	0.042
C18:1trans-12	0.20	0.21	0.21	0.003	0.280	0.657	<0.001
C18:1trans-13 + trans-14	0.32	0.33	0.35	0.01	0.328	0.996	0.386
C18:1cis-9	22.02	22.16	21.58	0.22	0.731	0.456	<0.001
C18:1cis-11	0.54	0.54	0.52	0.01	0.914	0.370	<0.001
C18:1 cis-12	0.21	0.21	0.21	0.003	0.405	0.830	<0.001
C18:1cis-13	0.06	0.05	0.05	0.001	0.826	0.679	<0.001
C18:1 trans-16 + cis-14	0.23	0.24	0.24	0.004	0.102	0.014	<0.001
C18:2trans-9, trans-12	0.01	0.01	0.01	0.0004	0.955	0.533	0.115
C18:2 cis-9, trans-12	0.071	0.072	0.073	0.001	0.033	0.509	<0.001
C18:2 trans-9, cis-12	0.02	0.02	0.02	0.0003	0.591	0.583	0.010
C18:2 trans-11, cis-15	0.049	0.044	0.05	0.001	0.037	0.319	0.003
C18:2n-6 (LA)	2.71	2.69	2.70	0.03	0.945	0.386	0.006
C18:3n-6	0.03	0.03	0.03	0.0006	0.612	0.683	<0.001
C18:3n-3 (LNA)	0.39	0.35	0.40	0.01	0.813	0.686	<0.001
CLA cis-9, trans-11 (RA)	0.411	0.413	0.444	0.01	0.049	0.018	0.004
C18:4n-3	0.01	0.01	0.01	0.0002	0.756	0.586	<0.001
C20:0	0.26	0.29	0.28	0.004	0.139	0.249	0.006
CLAtrans-9, cis-11 + C21:0	0.06	0.06	0.06	0.001	0.064	0.126	<0.001
CLA trans-10, cis-12	0.01	0.01	0.01	0.0004	0.191	0.480	0.002
CLA trans-11, trans-13	0.02	0.02	0.02	0.0003	0.131	0.664	<0.001
CLAtrans-9, trans-11	0.01	0.01	0.01	0.0002	0.922	0.445	<0.001
C20:1cis-9	0.03	0.03	0.03	0.001	0.376	0.358	0.048
C20:2n-9	0.0088	0.0097	0.0098	0.0002	0.027	0.393	0.003
C20:2n-6	0.02	0.02	0.02	0.0005	0.970	0.423	<0.001
C20:3n-9	0.05	0.04	0.05	0.001	0.401	0.068	<0.001
C20:3n-6	0.03	0.02	0.03	0.0005	0.889	0.300	0.42
C20:4n-6	0.19	0.20	0.20	0.002	0.341	0.900	<0.001
C20:3n-3	0.01	0.01	0.01	0.0002	0.733	0.969	0.258
C22:0	0.08	0.08	0.08	0.001	0.200	0.337	<0.05
C20:4n-3	0.01	0.01	0.01	0.0003	0.368	0.373	0.42
C22:1n-9	0.0111	0.0121	0.0124	0.0002	0.009	0.421	<0.001
C22:5n-3 (EPA)	0.04	0.03	0.04	0.001	0.887	0.579	0.01
C22:2n-6	0.04	0.04	0.05	0.001	0.108	0.680	<0.001

Table 4 (*continued*)

Fatty acid (g/100 g of FAME)	Diet			SEM	*P*-value		
	CON	SCG50	SCG100		L	Q	Time
C22:4n-6	0.19	0.20	0.20	0.001	0.444	0.986	0.033
C24:0	0.03	0.03	0.03	0.001	0.973	0.937	0.336
C22:5n-3 (DPA)	0.08	0.07	0.09	0.002	0.149	0.326	<0.001
C22:6n-3 (DHA)	0.02	0.02	0.02	0.0005	0.356	0.341	0.103
Groups							
SCFA	13.29	12.76	12.88	0.14	0.154	0.895	<0.001
MCFA	44.84	45.44	46.18	0.24	0.621	0.541	<0.001
LCFA	41.86	41.79	40.93	0.32	0.593	0.553	<0.001
SFA	68.40	68.40	68.83	0.27	0.656	0.399	<0.001
MUFA	26.89	26.96	26.39	0.25	0.687	0.404	<0.001
PUFA	4.70	4.62	4.76	0.04	0.452	0.365	<0.05
UFA	31.59	31.58	31.15	0.27	0.664	0.404	<0.001
OCFA	2.09	2.10	2.16	0.02	0.026	0.534	<0.001
BCFA	1.89	1.91	2.00	0.02	0.037	0.882	0.050
OBCFA	3.98	4.01	4.17	0.03	0.017	0.897	<0.001
PUFA n-6	3.05	3.03	3.05	0.03	0.979	0.425	<0.001
PUFA n-3	0.55	0.50	0.56	0.01	0.998	0.863	<0.001
n6:n3	5.69	6.21	5.53	0.09	0.738	0.387	<0.001
Total CLA	0.52	0.52	0.55	0.01	0.044	0.041	0.002
TFA	2.81	2.78	2.88	0.03	0.188	0.295	<0.001
Δ9- desaturase indices							
C10:1	0.49	0.51	0.54	0.02	0.373	0.629	0.003
C14:1	1.09	1.11	1.00	0.02	0.176	0.165	<0.001
C16:1	2.04	1.94	1.77	0.04	0.652	0.482	<0.001
C18:1	65.85	66.23	66.63	0.39	0.449	0.293	0.064
CLA cis-9, trans-11	36.02	37.70	37.30	0.38	0.022	0.418	<0.001
AI	2.22	2.24	2.30	0.03	0.886	0.559	<0.001
TI	2.16	2.24	2.26	0.03	0.788	0.407	<0.001
h:H	0.73	0.72	0.70	0.01	0.862	0.492	<0.001

Abbreviations: FAME = fatty acid methyl ester; SA = stearic acid; VA = vaccenic acid; LA = linoleic acid; LNA = linolenic acid; RA = rumenic acid; EPA = eicosapentaenoic acid; DPA = docosapentaenoic acid; DHA = docosahexaenoic acid; SFA = sum of the individual saturated fatty acids; UFA = sum of the individual unsaturated fatty acids; MUFA = sum of the individual monounsaturated fatty acids; PUFA = sum of the individual polyunsaturated fatty acids; TFA = trans fatty acids, sum of the individual trans fatty acids, except CLA isomers; OCFA = odd-chain fatty acids; BCFA = branched-chain fatty acids, sum of iso- and anteiso-FA; OBCFA = odd- and branched-chain fatty acids, sum of odd-, iso-, and anteiso-FA; SCFA = short-chain fatty acids, sum of the individual fatty acids from C4:0 to C10:0; MCFA = medium-chain fatty acids, sum of the individual fatty acids from C11:0 to C17:0; LCFA = long-chain fatty acids, sum of the individual fatty acids from C18:0 to DHA; PUFA n-3 and PUFA n-6 = sum of individual n-3 and n-6 fatty acids, respectively; CLA = sum of individual conjugated linoleic acids; TI = thrombogenic index; AI = atherogenic index; h:H = hypocholesterolemic to hypercholesterolemic ratio; CON = control diet; SCG50 = diet containing 50 g/d per head of spent coffee ground; SCG100 = diet containing 100 g/d per head of spent coffee ground; L = linear effect of the diet; Q = quadratic effect of the diet; Time = sampling time effect.

Table 5
Total antioxidant capacity, malondialdehyde, and protein carbonyls content from goat blood plasma for each treatment.

Items	Diet			SEM	*P*-value		
	CON	SCG50	SCG100		L	Q	Time
FRAP (μmol ascorbic acid)	0.72	0.71	0.82	0.02	0.016	0.063	<0.001
ABTS (% inhibition)	34.21	31.99	31.86	0.51	0.096	0.387	0.006
MDA (μM)	0.78	0.68	0.66	0.02	0.010	0.396	0.023
PC (nmol/ml)	8.89	8.81	8.72	0.21	0.607	0.981	<0.001

Abbreviations: CON = control diet; SCG50 = diet containing 50 g/d per head of spent coffee ground; SCG100 = diet containing 100 g/d per head of spent coffee ground; FRAP = Ferric Reducing Ability of Plasma; ABTS = 2,2′-azino-di(3-ethylbenzthiazoline-6-sulforic acid); MDA = Malondialdehyde; PC = Protein carbonyls; L = linear effect of the diet; Q = quadratic effect of the diet; Time = sampling time effect.

Blood analysis

The results of haematological and biochemical parameters are reported in Tables 7 and 8. The eosinophils concentration and percentage ($P = 0.03$ and 0.02, respectively) and basophils percentage ($P = 0.01$) quadratically decreased with the SCG supplementation. In addition, platelets count was markedly increased by both doses of SCG ($P = 0.02$), but its values remained within the physiological range.

Regarding biochemical parameters, the SCG did not cause the alteration of enzymatic activities in the liver and the kidney. The glucose concentration was decreased quadratically by SCG. The SCG supplementation decreased linearly with the creatine kinase (Table 8). Table 9 shows the effects of SCG on the electrophoresis profile of serum protein fractions. The SCG supplementation did not affect the content of serum blood protein and albumin. However, this byproduct tended to decrease α_1-globulins ($P = 0.08$) and to increase γ-globulins ($P = 0.09$) but always within the physiological range. However, the total globulins and the ratio of globulins to albumin did not change with the SCG inclusion in the diet.

Discussion

Dry matter intake, body weight, and milk yield and composition

In this study, the effect of SCG supplementation on a goat diet was investigated. This byproduct is not commonly used in animal feeding, probably because some its compounds (e.g. melanoidines, alkaloids, or polyphenols) could reduce diet palatability and

Table 6
Total antioxidant capacity, malondialdehyde, and protein carbonylis content from goat milk for each treatment.

Items	Diet			SEM	*P*-value		
	CON	SCG50	SCG100		L	Q	Time
FRAP (μmol ascorbic acid)	2.98	2.78	2.76	0.07	0.354	0.655	0.002
ABTS (% inhibition)	32.90	31.16	30.77	0.65	0.326	0.728	0.121
MDA (μM)	0.23	0.20	0.21	0.01	0.170	0.233	0.009
PC (nmol/ml)	4.53	4.07	4.22	0.12	0.147	0.117	<0.001

Abbreviations: CON = control diet; SCG50 = diet containing 50 g/d per head of spent coffee ground; SCG100 = diet containing 100 g/d per head of spent coffee ground; FRAP = Ferric Reducing Ability of Plasma; ABTS = 2,2′-azino-di(3-ethylbenzthiazoline-6-sulforic acid); MDA = Malondialdehyde; PC = Protein carbonyls; L = linear effect of the diet; Q = quadratic effect of the diet; Time = sampling time effect.

Table 7
Effect of dietary supplementation of two doses of spent coffee ground on haematological parameters in dairy goats.

Items	Reference values	Diet			SEM	*P*-value		
		CON	SCG50	SCG100		L	Q	Time
WBC (10^3/μl)	6.43–10.93	11.72	12.72	13.40	0.37	0.283	0.922	0.730
Neu (10^3/μl)	2.09–4.8	5.38	5.63	6.57	0.24	0.191	0.606	0.718
Lym (10^3/μl)	3.26–5.42	5.54	6.51	6.04	0.18	0.487	0.262	0.352
Mon (10^3/μl)	0.06–0.38	0.37	0.31	0.39	0.03	0.796	0.414	0.462
Eos (10^3/μl)	0.16–0.34	0.25	0.11	0.20	0.02	0.417	0.029	0.013
Bas (10^3/μl)	0.03–0.1	0.06	0.06	0.08	0.004	0.324	0.178	<0.001
LUC (10^3/μl)	-	0.10	0.11	0.12	0.01	0.445	0.963	0.081
Neu (%)	27–53	45.82	43.97	48.00	0.92	0.534	0.269	0.888
Lym (%)	39.2–63	47.50	51.48	46.02	0.93	0.680	0.095	0.405
Mon (%)	0.7–4.1	2.91	2.42	3.05	0.20	0.77	0.23	0.43
Eos (%)	1.8–4.5	2.36	0.85	1.44	0.16	0.089	0.021	0.034
Bas (%)	0.3–1.1	0.58	0.44	0.60	0.02	0.768	0.014	0.002
LUC (%)	-	0.84	0.86	0.90	0.05	0.724	0.833	0.094
RBC (10^6/μl)	12.48–16.03	14.16	14.04	13.93	0.13	0.615	0.973	0.003
HGB (g/dl)	8.3–10.8	9.01	8.96	9.06	0.07	0.841	0.764	0.001
HCT (%)	24.7–32.2	27.20	26.32	27.30	0.28	0.902	0.287	0.057
MCH (pg)	5.6–7.6	6.40	6.40	6.53	0.05	0.533	0.775	<0.0001
MCHC (g/dl)	31.5–35.9	33.21	33.50	33.26	0.08	0.911	0.359	<0.0001
MCV (fl)	15.5–24.3	19.26	19.10	19.65	0.19	0.575	0.649	<0.0001
PLT (10^3/μl)	130–624	151.48	307.13	345.67	21.92	0.023	0.413	<0.0001

Abbreviations: WBC = white blood cell count; Neu = neutrophil granulocytes; Lym = lymphocytes; Mon = monocytes; Eos = eosinophils granulocytes; Bas = basophiles granulocytes; LUC = Large unstained cells; RBC = red blood cell; HGB = haemoglobin; HCT = haematocrit; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume; PLT = platelet; CON = control diet; SCG50 = diet containing 50 g/d per head of spent coffee ground; SCG100 = diet containing 100 g/d per head of spent coffee ground; L = linear effect of the diet; Q = quadratic effect of the diet; Time = sampling time effect.

Table 8
Effect of dietary supplementation of two doses of spent coffee ground on biochemical parameters in dairy goats.

Items	Reference values	Diet			SEM	*P*-value		
		CON	SCG50	SCG100		L	Q	Time
Albumin (g/dl)	2.5–3.5	2.87	2.90	2.73	0.05	0.104	0.146	<0.0001
ALP (u/l)	35–300	196.17	176.54	264.75	44.43	0.730	0.754	<0.05
Total bilirubin (mg/dl)	0.3–0.6	0.22	0.20	0.22	0.01	0.894	0.448	<0.05
Calcium (mg/dl)	6–9.5	8.26	8.03	8.03	0.18	0.459	0.680	<0.0001
Creatinine (mg/dl)	0.3–0.8	0.59	0.62	0.57	0.02	0.605	0.341	<0.0001
Phosphorus (mg/dl)	3.5–12	6.88	7.15	6.59	0.22	0.683	0.497	<0.0001
Total protein (g/dl)	6.5–8.5	8.32	8.22	7.99	0.18	0.312	0.818	<0.0001
GGT (u/l)	40–135	53.75	50.67	64.58	2.45	0.252	0.298	<0.0001
GOT/AST (u/l)	170–180	135.67	119.21	124.21	4.47	0.492	0.458	<0.0001
GPT/ALT (u/l)	25–50	32.25	32.75	26.00	1.41	0.805	0.994	<0.0001
Urea (mg/dl)	50–80	38.71	37.54	36.29	0.87	0.243	0.981	<0.0001
Cholesterol (mg/dl)	50–95	86.96	88.54	91.58	2.66	0.594	0.923	<0.0001
CK (u/l)	35–150	268.00	215.29	222.96	12.97	0.074	0.164	<0.0001
Glucose (mg/dl)	25–60	30.00	34.71	27.25	1.29	0.356	0.022	<0.0001
Triglyceride (mg/dl)	10–50	16.3	21.3	25.4	0.37	0.326	0.959	<0.05

Abbreviations: CON = control diet; SCG50 = diet containing 50 g/d per head of spent coffee ground; SCG100 = diet containing 100 g/d per head of spent coffee ground; ALP = alkaline phosphatase; GGT = gamma-glutamyl transferase; GOT/AST = serum glutamic oxaloacetic transaminase; GPT/ALT = serum glutamic-pyruvic transaminase; CK = creatine kinase; L = linear effect of the diet; Q = quadratic effect of the diet; Time = sampling time effect.

acceptability. A depressive effect on diet palatability and daily intake in sheep supplemented with a 10% level of SCG was reported by Choi et al. (2018). However, in this study, no adverse effects on goat DM intake were observed using 50 or 100 g/d of SCG.

Milk yield decreased during the experimental period (Supplementary Fig. S1) partly due to the high temperature recorded during the trial (T_{mean} 23.3 ± 2.4 °C, T_{max} 31.3 ± 3.6 °C; RH_{mean} 57.5 ± 8.7, RH_{max} 89.9 ± 8.1; Supplementary Fig. S2). However,

Table 9
Effect of dietary supplementation of two doses of spent coffee ground on serum protein fractions in dairy goats.

Items	Reference values	Diet			SEM	*P*-value		
		CON	SCG50	SCG100		L	Q	Time
Total protein (g/dl)	6.5–8.5	7.68	7.81	7.51	0.23	0.679	0.542	<0.001
Albumin (%)	32.8–42.3	43.52	41.93	40.23	1.11	0.229	0.983	0.027
α_1-globulins (%)	4.5–7.6	6.06	5.63	5.17	0.18	0.085	0.983	0.024
α_2-globulins (%)	11.6–16.1	12.81	13.39	12.64	0.26	0.815	0.289	0.379
β-globulins (%)	5.2–8.9	6.68	6.52	6.88	0.34	0.780	0.685	<0.001
γ-globulins (%)	21–36.7	30.96	32.54	35.08	0.94	0.090	0.811	<0.001
Total globulins (%)	-	56.51	58.08	59.77	1.11	0.232	0.978	0.028
Globulins/albumin	-	1.37	1.43	1.59	0.06	0.157	0.737	0.041

Abbreviations: CON = control diet; SCG50 = diet containing 50 g/d per head of spent coffee ground; SCG100 = diet containing 100 g/d per head of spent coffee ground; L = linear effect of the diet; Q = quadratic effect of the diet; Time = sampling time effect.

the SCG supplementation did not influence milk protein, fat, and lactose contents, in accordance with results in cows (San Martin et al., 2020), but contrasted with observations in dairy sheep, in which an increase in milk fat and protein contents was found with the ingestion of a similar dose of spent coffee (de Otálora et al., 2020). The high temperatures recorded during the experimental period may have covered the potential impacts of this supplement on milk components.

The observed depressive effect of SCG on milk urea is in accordance with an observation in dairy cows supplemented with coffee hulls in combination with soybean oil (Santos et al., 2014). This result could be partly due to the presence of caffeine, which can affect urea metabolism (Jorda et al., 1989), and ferulic acid, which is the most abundant polyphenolic compound present in SCG. Notably, polyphenols can reduce dietary protein degradation in the rumen and, consequently, ammonia production (Correddu et al., 2020). A study in sheep reported that the inclusion of a 10% level of SCG could reduce protein digestibility and nitrogen absorption (Choi et al., 2018). In spite of the high content of ferulic acid in SCG (12 g/kg of SCG), animals ingested a low amount per kilogram of BW because of the reduced amount of byproduct offered. The ingested amounts were 10.5 and 20.1 mg/kg BW in groups SCG50 and SCG100, respectively. A study conducted on laboratory animals, treated with 25 mg/kg BW of ferulic acid, showed a significant decrease in the levels of urea, uric acid, and creatinine (Manikandan et al., 2014). This result could support the role of ferulic acid in nitrogen metabolism in goats. The SCC was quadratically affected by SCG, which was reduced only by the low dose of the byproduct. A study on dairy cows affected by subclinical mastitis reported that feeding coffee ground silage containing large amounts of polyphenols decreased the milk SCC (Kawai et al., 2018), suggesting a positive role on the immune activity.

Milk fatty acid profile

The fatty acid profile of ruminants' products is gaining attention from consumers, as demonstrated by the studies that have attempted to decipher the phenotypic and genetic background of this trait (Mele et al., 2016; Chikwanha et al., 2018; Cesarani et al., 2019; Correddu et al., 2021). Thus, in this study, the effect of SCG on the milk fatty acid profile was investigated. The SCG supplementation resulted in small variations in the milk fatty acid profile. In particular, a linear increase in some branched chain fatty acids, as isoC14:0 and anteisoC15:0, and C15:0 has been observed. These fatty acid are considered biomarkers of rumen microbial fermentation and microbial de novo lipogenesis. Their increase with SCG supplementation suggests that polyphenols may have affected the rumen microbial profile (Salami et al., 2021). The increase in C15:0 is also notable from a nutritional view because of the beneficial effects on human health: the potential reduction of inflammation and incidence of cardiovascular and liver diseases (Venn-Watson et al., 2020). The increase in C18:1 trans-11 and CLA cis-9 trans-11 with SCG supplementation suggests a slowing of rumen biohydrogenation of dietary polyunsaturated fatty acids, probably due to the presence of polyphenols in the byproduct. However, there was no substantial change in the overall contents of unsaturated and saturated fatty acid in milk, despite the results in the literature that the inclusion of polyphenols in ruminant diets modifies the fatty acid profile of milk (Correddu et al., 2020).

Blood and milk oxidation

The antioxidant capacity of blood was improved by the SCG supplementation, as demonstrated by the increase in FRAP and decrease in MDA. The effect on FRAP, together with MDA values, suggested that the higher the dose the better the antioxiandat capacity. An improvement in blood antioxidant capacity due to coffee consumption measured with FRAP assay was reported in humans (Martínez-López et al., 2019). The same improvement, measured by the MDA assays, was also reported in sheep fed coffee pulp (Salinas-Rios et al., 2015), in cows fed ground coffee silage (Kawai et al., 2018), and in laboratory animals fed coffee (Vitaglione et al., 2010). The antioxidant compounds, determined by the ABTS assay, might be responsible for the reduction of MDA, one of the main products of lipid oxidation. The antioxidant potential of SCG could be related to the ability of caffeine, ferulic acid (4-hydroxy-3-methoxycinnamic acid), and secondary metabolites (e.g. melanoidins) to trap free radicals (Vitaglione et al., 2010; Salinas-Rios et al., 2015). The antioxidant capacity of milk was not modified by the SCG supplementation. Similar results were found in dairy ewes (Buffa et al., 2020b) by using other byproducts rich in polyphenols, such as grape marc and exhausted myrtle berries. These byproducts did not modify milk ABTS and FRAP but reduced milk protein carbonyls. In our trial, the protein carbonyls were not affected by the SCG supplementation; thus, its polyphenols did not show a protective role against milk protein oxidation.

Blood analysis

The results of haematological and biochemical parameters showed an effect of SCG on goat immune response, demonstrated by the quadratic effect on eosinophils concentration and for basophils percentage, that achieved the lowest values in the group fed with the low SCG dose. The eosinophils plays a crucial role in the inflammatory response and in the modulations of acute and chronic hyperimmune reactions, via the release of inflammatory mediators into tissue sites such as specific granule proteins. Eosinophils are also involved in the regulation of the immune response

against helminth infections (pulmonary, intestinal, or hepatic) in sheep, causing their increase above the upper limit of the physiological range. Even if all animals appeared healthy and no parasite search in faeces was assessed during the experiment, the reduction of eosinophils suggests a positive effect of SCG. The increase in platelets with both doses of SCG could be related to the function of caffeine and other compounds in blood homeostasis. Commonly, phenolic compounds of coffee reduce blood platelet aggregation in humans (Olas and Bryś, 2019), but the increase in platelets in goat blood with SCG consumption is difficult to explain, even if it remains within the physiological range. Regarding biochemical parameters, the SCG did not alter enzymatic activities in the liver and kidney. Only the glucose concentration was decreased quadratically by SCG. An opposite pattern was observed in humans: several studies have demonstrated an increase in glucose after coffee consummation, probably due to the implication of caffeine in glucose metabolism (Johnston et al., 2003; Moisey et al., 2008). Notably, ferulic acid has been found to exert antidiabetic properties by stimulating glucose uptake (Sompong et al., 2015) and improving insulin sensitivity (Narasimhan et al., 2015). Unfortunately, little is known of the effects of bioactive compounds specifically present in coffee on blood parameters and on glucose metabolism in ruminants. However, different studies conducted using polyphenols in the diet showed contrasting results: Chedea et al. (2017) did not find an effect on plasma glucose in dairy cows after ingestion of grape pomace; by contrast, Zhong et al. (2011) reported increased glucose levels in goats fed tea catechin. The different results of these studies could be attributed to the different phenolic composition and to the different amounts of consumed phenols. In our study, creatine kinase decreased linearly in the plasma of goats supplemented with SCG. High creatine kinase activity is associated with cardiac and skeletal muscle damages or with muscle intense activity after exercise (Rider and Miller, 1995; Cechella et al., 2014). The reduction of creatine kinase in blood during exercise has been also observed in rats fed caffeine, which can reduce the damaged muscle fibres, probably due to its strong activity against oxidative stress (Cechella et al., 2014).

No effect was found for serum protein and albumin, even if there was a numerical decrease in α_1-globulins and an increase in γ-globulins. Albumin, α- and β-globulins are synthesised in the liver; γ-globulins are produced by B lymphocytes or plasma cells and are responsible for the humoral response of the immune system. Usually, α- and γ-globulins are involved in the immune response of the mammary gland; notably, α-globulins production tends to decrease, whereas γ-globulins tend to increase after an inflammatory stimulus. In our study, the total globulins and the ratio of globulins to albumin did not change with SCG inclusion in the diet, suggesting a natural variation in globulin fractions in response to a specific treatment, the inclusion of a supplement.

Conclusion

The inclusion of up to 100 g/d of SCG into the diet of dairy goats did not affect feed intake, milk yield, and almost all milk components. The analyses of blood oxidation markers suggest that SCG can be used as a putative dietary ingredient to modulate oxidative stress in goats. The responses of animals to this byproduct were associated with the given doses; thus, an assessment of the most optimum level of SCG in animal diet is necessary. Moreover, it is relevant to explore the effects of longer periods of treatments with bioactive substances on animal production traits and health and oxidative statuses. Finally, the hormetic response of some parameters suggests that further studies on dose–response effects of the bioactive compounds present in several agro-industrial byproducts are necessary for dairy animals.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2022.100501.

Ethics approval

The experiment was approved by the Ethics Committee of the University of Sassari (no. 87140/2020).

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study findings are available upon request.

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Declaration of interest

None.

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