Valorisation of spent coffee grounds as functional feed ingredient improves productive performance of Latxa dairy ewes

X. Díaz de Otálora[a*], R. Ruiz[a], I. Goiri[a], J. Rey[a], R. Atxaerandio[a], D. San Martin[b], M. Orive[b], B. Iñarra[b], J. Zufia[b], J. Urkiza[c], A. García-Rodríguez[a].

[a] *Department of Animal Production, NEIKER-Tecnalia, Granja Modelo de Arkaute, 46. 01080, Vitoria-Gasteiz (Spain).*

[b] *Department of Efficient and Sustainable Processes, AZTI, Parque Tecnológico de Bizkaia,. Astondo Bidea Edificio 609, E-48160, Derio (Spain)*

[c] *Cooperativa Agraria MIBA, Polígono Industrial Galartza, 48277, Etxebarria (Spain)*

* Corresponding author. Xabier Díaz de Otálora Aguirre. Email: xdiaz@neiker.eus

Declarations of interest: none

Abstract

Spent coffee grounds (SCG) represent one of the main residues derived from restoration and hostelry. The aim of this study was to evaluate the effect of SCG, included in the concentrate at different concentrations (0, 30, 50 and 100 g/kg), on milk yield and quality, feeding behaviour, dry matter intake, apparent digestibility and ruminal short chain fatty acid profile. In this trial of 51 days of duration, 48 Latxa dairy ewes were used. The ewes were blocked in quartets according to milk yield (1918 ± 287g) and days in milk (35.7 ± 8.9 days). All of the concentrates were formulated to be isoenergetic (1.01 UFL), isoproteic (166 g/kg), isofat (76 g/kg) and to meet the production needs. The concentrate was given in two doses of 450 g of dry matter during the morning and afternoon milkings, and fescue hay was offered *ad libitum*. Milk production was recorded and samples were taken for fat, protein and lactose composition analysis. Dry matter intake and apparent dry matter digestibility were

estimated using two markers, and feeding behaviour data was recorded. Increasing doses of SCG in the concentrate up to 100g/kg resulted in a linear (P<0.001) increase in the rumen of the isovaleric and isobutyric acid contents which could explain the observed quadratic response (P<0.001) in milk yield and a linear increase (P<0.001) in milk protein. A linear increase (P<0.001) in milk fat was found which could be explained by the observed linear increase (P<0.001) in ruminal acetic and butyric acid contents. Increasing doses of SCG in the concentrate linearly decreased ruminal (P<0.001) propionic acid content, resulting in a concomitant linear increase (P<0.001) in acetic:propionic ratio. Furthermore, no differences were found in intake, apparent dry matter digestibility and feeding behaviour. In conclusion, inclusion of SCG up to 100g/kg in the concentrate modified ruminal fermentation pattern towards an increase in isoacids and acetic and butyric acid contents in the rumen with a concomitant improvement in milk production and composition without impairing feeding behaviour or apparent digestibility.

Keywords: melanoidins, phenolic compounds, antimicrobial, circular economy, efficiency, by-products

Abbreviations: ADFom, acid detergent fiber; aNDFom, neutral detergent fiber; BCVFA, branched chain volatile fatty acids; BCS, body conditioning score; BW, body weight; CP, crude protein; DM, dry matter; DMD, apparent dry matter digestibility; DMI, dry matter intake; FPCM, fat and protein corrected milk; GAE: gallic acid equivalent; SEM, standard error of the mean; SCG: spent coffee grounds; UFL, feed units for lactation

1. Introduction

Coffee is one of the most valuable primary products in world trade due to the high consumption of coffee beverage; it is after water the second most popular beverage

50 worldwide, and the second largest commodity in stock exchange after oil (Mussatto et
51 al., 2011; Girotto et al., 2018). According to the International Coffee Organization
52 statistics, coffee world production in 2018 was around 9.5 million tons, of which EU
53 countries consume about 2.52 million tons, with an increasing tendency.
54 Coffee consumption leads to amounts of organic waste; the primary by-product from
55 coffee production is spent coffee grounds (SCG). SCG is the insoluble residue that
56 remains after coffee beans are roasted, milled and brewed. For every kilogram of coffee
57 consumed, two kilograms of SCG are generated (Mussatto et al., 2011). The generation
58 of SCG is distributed in soluble coffee industry, which uses about 50% of the global
59 coffee harvest, and in coffee shops and the consumers, accounting for the remaining
60 50% (Scully et al., 2016).
61 In Europe, most of the SCG is currently being incinerated or disposed of in landfills
62 (Mata et al., 2018), despite its potential interest for different applications such as
63 cosmetic, nutraceutical or even pharmaceutical, based on its chemical composition:
64 cellulose, hemicelluloses, proteins, fat, polyphenols, minerals (Esquivel and Jimenez,
65 2012; Mussatto et al., 2011). Besides, in addition to its interesting nutritional profile,
66 SCG also contains a wide range of components formed through the Maillard reactions
67 during the roast, such as melanoidins (Borrelli et al., 2004), which are supposed to have
68 antimicrobial properties and play an important role in preventing oxidative damage and
69 diseases related to free radicals (Wang et al., 2011).
70 In this regard, considering the large amount of SCG produced annually all over the
71 word and their potential pollution hazard affecting especially the soil ecosystem
72 (Mussatto et al., 2011), the reutilization of this by-product is a relevant subject in the
73 search of the circular economy. Some alternative uses have been proposed to revalorize
74 this by-product, like pellet (Silva et al., 1998), biodiesel production (Kondamudi et al.,

2008), its use as substrate for fermentation technology (Ramalakshmi et al., 2009) or in wastewater treatment (Franca et al., 2009). In addition, its use as functional ingredient in human diets has also been proposed due to the antioxidant, antihypertensive and antimicrobial activities in gut microbiota associated to some of their chemical constituents like coffee melanoidins and phenolic compounds (Rufian-Henares and Morales, 2007).

These by-products have been suggested as alternative feed source for livestock. In this context, some studies in the late 70s have been focused on the use of SCG as feed ingredient in ruminant's rations. *In vitro* studies observed that dry matter digestibility was decreased at a dose higher than 100 g/kg of SCG in the ration (Bartley et al, 1978). In addition, production trials with dairy cows and beef including SCG in the ration at doses between 50 and 200 g/kg of total diet concluded that some undesirable consequences, like reduced grain intake in dairy cows and reduced average daily gain in beef cattle, can appear possibly related to reduced ration palatability and digestibility (Bartley et al, 1978). More recent studies also concluded that SCG at a dose between 100 and 200 g/kg reduced linearly the *in vitro* digestibility of dry matter (DM), protein, fiber and energy (Xu et al., 2007). In all the reported studies, SCG have been used as a feed ingredient replacing nutritionally more rich-feed ingredients and therefore reducing the energy and nutrient content of the ration. However, to our knowledge, there is no report about the potential use of SCG at lower doses as a functional ingredient in the diet. In this regard, different compounds with antimicrobial properties, like monensin (Richardson et al., 1976), plant extracts (Busquets et al., 2006) and chitosan (Goiri et al., 2009, 2010) among others, have shown positive effects manipulating ruminal ecosystem and enhancing production. In this context, some chemical constituents of SCG like coffee melanoidins and phenolic compounds have been proposed as

100 antimicrobials (Jimenez-Zamora et al., 2015) and hypothetically could exert antibiotic-
101 like growth promoter action resulting, if provided in adequate doses, in increased
102 production performance.
103 Therefore, the objective of the present study was to evaluate the use of the SCG as a
104 functional ingredient in the concentrate of dairy ewes and its effects on digestibility,
105 production performance, milk quality and feeding behaviour.

106 **2. Material and methods**

107 All experimental procedures were performed in accordance with the European
108 Union Directive (2010/63/EU) and Spanish Royal Decree (RD 53/2013) for the
109 protection of animals used for experimental and other scientific purposes, and approved
110 by the ethics committee (NEIKER-OEBA-2018-004).

111 *2.1.Spent coffee grounds collection and drying*

112 This study was focused on the SCG produced by hotels, restaurants and coffee
113 shops located in the north of Spain (Basque Country and Navarre) and south of France
114 (Aquitaine). About 0.5 tons of SCG were stored and collected during a week. This
115 amount of SCG was transported to a drying plant for its processing as an ingredient for
116 dairy ewes feed. SCG were dried using the flash dryer technology (RINA-JET 1008,
117 Riera Nadeu S.A.), in order to prevent biodegradation due to the microbial activity.
118 Chemical composition and nutritional value of SCG can be seen on Table 1.

119 *2.2.Animals, experimental design and diets*

120 The study was conducted with 48 Latxa ewes of the experimental flock of
121 NEIKER-Tecnalia in Arkaute (Spain) at the beginning of lactation. Ewes were blocked
122 in quartets based on their milk production (1918 ± 287 g) and days in milk (35.7 ± 8.9
123 days). Each ewe in the quartet was assigned a treatment based on SCG inclusion level in
124 the concentrate supplied (0, 30, 50 and 100 g/kg DM). Table 1 shows the composition

125 and the chemical analysis of the concentrates and forage supplied. The concentrate was
126 supplied in individual feeders in the milking parlour in two doses of 450g of DM during
127 the morning (7:30) and afternoon (18:00) milkings. Fescue (*Festuca pratensis*) hay was
128 group fed *ad libitum* in a feed bunk (0.5 m/ewe). The quantity of fescue hay offered was
129 based on morning bunk readings, and the amount of feed offered was adjusted daily to
130 allow 10% refusals. The experimental period lasted for 51 days, of which the first 7
131 days were for covariate determination, the following 7 for adaptation to diets and the
132 last 37 days were used for measurements and samplings.

133 *2.3.Productive performance*

134 Ewes were milked daily at 07:30 and 18:00 during the experimental period, and
135 milk production was recorded individually 7 d/wk electronically (MM25 SG, DeLaval,
136 Madrid, Spain). On days 17, 31 and 43, a portion of milk (am and pm milkings) from
137 each ewe was stored with azidiol at 4ºC until further analysis of fat, protein and lactose.
138 Body weight (BW) and body conditioning score (BCS) were measured on the first and
139 last day of the trial.

140 *2.4.Dry matter intake, apparent dry matter digestibility and feeding behaviour*

141 The quantity of concentrate offered and rejected was determined individually on
142 a daily basis. Dry matter intake (DMI) and apparent dry matter digestibility (DMD)
143 were estimated as proposed by Cochran et al. (1986) using chromium sesquioxide
144 (Cr_2O_3) as an external marker and acid-insoluble ash as an internal marker. Ewes
145 received one gram of Cr_2O_3, stored in gelatine capsules at 07:30 h and 18:00 h during
146 10 days. The quantity of chromium received daily was 1.37 g per ewe. After the 7-day
147 standardization period, faecal samples were taken on three consecutive days. Samples
148 were obtained from the rectum, composited by ewe and stored at -20ºC for the analysis
149 of chromium and insoluble ash.

For the feeding behaviour data a close visual observation of the sheep was carried out during 48 consecutive hours. The predominant behaviour (eating, rumination, and idling) was recorded within intervals of 10 minutes. Behavioural activities were averaged by ewe.

2.5.Ruminal samples

The last day of the experimental period rumen fluid samples were taken for short chain fatty acid (SCFA) analysis. Ewes were fasted since the previous afternoon milking. Ruminal samples were collected from each dairy ewe after the morning milking using an esophageal tube (0.9 cm in diameter and 150 cm in length) connected to a mechanical pumping unit (Vacuubrand ME 2SI, Wertheim, Germany). Ruminal samples were filtered through four layers of sterile cheese cloths, then about 10 ml of each ruminal extraction were placed into a container and were immediately frozen and stored at -20±5C until further analysis of SCFA.

2.6.Chemical analyses

Milk fat, protein and lactose contents were analysed by near-infrared spectroscopy (Foss System 4000, Foss Electric, Hillerød, Denmark; Instituto Lactológico Lekunberri, Lekunberri, Spain).

Roughage and concentrate were dried in a forced-air oven and fecal grab samples were freeze-dried (Christ Alpha 1-4 LD Plus, Fisher Bioblock Scientific, Madrid, Spain). All samples including SCG were ground to pass a 1-mm screen. DM content (method 934.01) was determined following (AOAC, 2007). Nitrogen content (method 941.04) was determined using the macro-Kjeldahl procedure on a Kjeltec Auto 1030 (Foss, Hillerød, Denmark). Neutral detergent fibre (aNDFom) was determined with use of an alpha amylase, but without sodium sulphite, and was expressed free of ash (Van Soest et al., 1991). Acid detergent fibre (ADFom) was determined and expressed exclusive of

175 residual ash (Robertson and Van Soest, 1981). Acid detergent lignin was determined
176 (Method 973.18) following (AOAC, 2007). Fat content (Method 2005.5) was
177 determined without hydrolysis by the automated soxhlet method (Selecta S.A.,
178 Barcelona, Spain) using hexane for 6 h as solvent. Acid-insoluble ash contents of feeds
179 and faeces were determined gravimetrically after drying, ashing, boiling of ash in
180 hydrochloric acid, filtering and washing of the hot hydrolysate, and re-ashing (Van
181 Keulen and Young, 1977). Starch content was measured by polarimetry (MAPA, 1995).
182 Faeces were analysed for Cr by atomic absorption spectrometry (Williams et al., 1962).
183 Concentrates were analysed for caffeine (ISO 20481, 2008), total phenolic compounds
184 (Singleton and Rosi, 1965) and melanoidins (Pérez-Hernández et al., 2012).
185 The analysis of SCFA (acetic, propionic, butyric, isobutyric, valeric and isovaleric) of
186 rumen samples was performed by gas chromatography: a volume of 4 mL of rumen
187 liquor mixed with 1 mL of a solution of 20 g/L of ortophosphoric acid and 4 g/L of
188 isocaproic acid as an internal standard was centrifuged (15000 × g for 15 min at 4°C) to
189 separate the liquid phase from the feed residuals. After, the liquid phase was
190 microfiltered (premium syringe filter regenerated cellulose, 0.45um 4mm, Agilent
191 Technologies, Madrid, Spain), and 1 mL of liquid phase was directly injected in the
192 apparatus (Perkin-Elmer Inc., Boston, MA) using a semicapillary column (300 mm ×
193 7.8 mm; 9-µm particle size; TR-FFAP, Supelco, Barcelona, Spain) kept at 250°C in the
194 injector with a helium flow rate of 13 mL/min. The analyses were carried out applying
195 an isocratic elution (flux 0.6 mL/min) with a 0.008 N H_2SO_4 solution as mobile phase;
196 the injection loop was 20 µL. Individual SCFA were identified using a standard solution
197 of 4.50 g/L of acetic acid, 5.76 g/L of propionic acid, 7.02 g/L of butyric acid and
198 isobutyric acid, 8.28 g/L of valeric acid and isovaleric acid in 0.1 N H_2SO_4 (A6283,
199 P1386, B103500, I1754, 240370, 129542, respectively; Sigma-Aldrich, Madrid, Spain).

Quantification expressed in mmol/L was done using an external calibration curve based on the standards described above. Data were expressed in mmol/mol.

2.7.Calculations and statistical analysis

Average daily weight gain was calculated by subtracting the final to the initial BW and divided by the total days of the trial. Body conditioning score was assessed according to the five-point scale described by Russel et al. (1984). Milk fat, protein and lactose concentrations were calculated as weighted average of the morning and afternoon data. Milk production was adjusted to 6.5% fat and 5.8% protein in order to determinate de fat and protein corrected milk (FPCM) following the equation developed by Pulina et al. (2005). Individual concentrate intake was calculated as the difference between the quantities offered and refused. Branched-chain volatile fatty acid (BCVFA) values were calculated as isovaleric plus isobutiric acids.

For the statistical analysis, each dairy ewe (n=48) was considered as the experimental unit. Milk yield, FPCM, milk fat and protein concentration and yields were analyzed using the MIXED procedure (SAS, 2017) for repeated measures (Littell et al., 1998) and assuming a covariance structure fitted on the basis of Schwarz's Bayesian information model fit criterion, according to the following statistical model, including the fixed effects of block (*B*), concentrate (*Con*) and week, their interaction and the initial record measured at week 0 as covariate,

$$Y_{ijklm} = \mu + Cov_i + Con_j + B_k + W_l + (W_l x Con_j)_m + \varepsilon_{ijklm}$$

Where Y is the dependent variable, μ is the mean value, Cov is the covariate data used, Con is the fixed effect of the concentrate, B is the fixed effect of the block, W is the fixed effect of the week, $WxCon$ is the fixed effect of the interaction between the concentrate and the week and ε the residuals. The effect of the sheep within the block was considered as random effect.

Total, hay and concentrate DMI, DMD, SCFA, average daily weight gain, BCS and feeding behavior were analyzed using the GLM procedure (SAS, 2017), according to the following model:

$$Y_{jk} = \mu + Con_j + B_k + \varepsilon_{jk}$$

Where Y is the dependent variable, μ is the mean values for each treatment, Con is the fixed effect of the concentrate used, B is the fixed effect of the block and ε are the residuals

Due to the unequal doses, spaced coefficients for orthogonal polynomials were calculated using the ORPOL function in PROC IML in SAS (SAS, 2017), in order to determinate linear and quadratic trends.

3. Results

3.1.Ruminal fermentation

Regarding ruminal fermentation, increasing doses of SCG resulted in similar total SCFA production (Table 2). However, increasing doses of SCG resulted in a linear/quadratic increase effect in the acetic acid content (P=0.002 and P=0.012 respectively) and a linear increase (P=0.028) in the butyric acid content (Table 2). Concomitantly, increasing doses of SCG linearly decreased propionic acid content (P<0.001), and increased acetic:propionic ratio (P<0.001). The BCVFA content were affected quadratically (P=0.003) as the concentration of SCG was increased in the concentrate. Valeric acid content, however, was not affected.

3.2.Productive performance and milk quality

The effect of increasing doses of SCG in the concentrate on milk yield and composition can be seen in Table 3. Increasing doses of SCG resulted in a quadratic effect (P<0.001) in milk yield and in a linear increase in the FPCM (P= 0.099). Fat yield (P=0.002) and concentration (P<0.001) experimented a linear increase, whereas protein

and lactose yields (P=0.043 and P=0.007, respectively) and concentrations (P=0.038 and P=0.012, respectively) were affected quadratically. Productive efficiency measured as milk yield per kg of DMI showed a quadratic response (P=0.011) and productive performance measured as FPCM per kg of DMI showed a linear increase (P=0.077) to SCG inclusion in the concentrate. Inclusion of SCG did affect neither average daily weight gain nor BCS (Table 3). Inclusion of SCG in the concentrate did not affect either forage or total DMI, or DMD at any of the studied doses.

3.3.Feeding behaviour

The effect of increasing doses of SCG in the concentrate on sheep's feeding behaviour can be seen in Table 4. It was observed that increasing doses of SCG resulted in a linear decrease in time spent eating (P= 0.019) and in a tendency for a quadratic effect in the rumination time (P= 0.064). However, SCG did not affect rumination time per kg of DMI.

4. Discussion

Research on animal nutrition has often been focused on finding alternative feed ingredients to replace edible ones in order to reduce feed costs and competition with human consumption. In addition, with the aim of promoting the circular economy, the use of agro industrial by-products as feed resources has been prompted bringing benefits both for the economy and the environment. In this context, there have been studies on the potential use of SCG as a feed source for ruminants (Campbell et al., 1976; Bartley et al., 1978; Givens and Barber, 1986; Xu et al., 2007). The common conclusion of these authors was that SCG do not contain enough nutrients to support livestock requirements. It has been reported that diets containing SCG had significantly lower aNDFom DMD, and it was speculated that phenolic compounds and some components derived from the Maillard reaction in SCG might be associated with this

lower DMD (Puchala et al., 2005; Senevirathne et al., 2012) and palatalability. However, no studies have tackled the use of SCG at lower doses, taking advantage of the potential beneficial effect on rumen fermentation of these active compounds present in SCG. Therefore, the hypothesis tested in this trial was that the inclusion of SCG as a functional ingredient in the concentrate for ruminants could represent a way of valorisation of this by-product that is usually managed as a residue.

The SCG DMI observed in the current trial (between 1.2 and 4% of total DMI) was lower than that reported in other studies (Abate et al., 1986), but it was enough to cause a shift in the ruminal fermentation pattern towards an increased accumulation of BCVFA. SCG are characterized by high valine, leucine and isovaline concentrations (Campos-Vega et al., 2015) whose natural rumen degradation and decarboxylation could explain the observed BCVFA accumulation (Andries et al., 1987).

These isoacids are essential nutrients for certain rumen microorganisms, and have been reported to enhance the growth of fiber-digesting microorganisms in the rumen (Liu et al., 2018). Further research analysing the effect of SCG on microbial populations is necessary to elucidate if the observed shift towards a greater acetic and butyric acid content is explained by an increase in the abundance of fibre digesting microorganisms. These results agree with Seo et al. (2015) and Senevirathne et al. (2012), and are consistent with the concomitant reduced propionic acid content. On the other hand, the observed change in fermentation pattern towards more acetic and butyric acid contents could result in the observed greater total fat content in milk (Folley et al., 1950). Because the concentration of fat in milk is normally positively correlated with body fat mobilization and the concentration of aNDFom in the diet (Nudda et al., 2002), it is important to point out that these results were obtained without a BW or BCS loss, and with a similar aNDFom DMI. In this sense, although some studies relate certain coffee

300 components, such as caffeine, with fat mobilization process in humans (Bellet et al.,
301 1968; Acheson et al., 2004), results from the present study do not point out that a
302 significant fat mobilization process was taking place in terms of BW or BCS loss. In
303 addition the aNDFom level in the ration ranged between 33 and 40% that is above the
304 30-32% threshold recommended by Nudda et al. (2002) beyond which a further increase
305 in milk fat content is not observed.
306 The increased response in milk yields cannot be associated to a greater digestible DMI,
307 since DMI or DMD remained unchanged between treatments. Cook et al. (1985)
308 proposed that isoacid receptors are present in the rumen and/ or the liver, and that
309 interaction between the acid and the receptor results in an increase in plasma growth
310 hormone and a decrease in insulin levels. Growth hormone is considered to possess
311 lactogenic activity, whereas insulin decreases transport of nutrients to the mammary
312 gland in favour of other organs. This effect of isoacids outside the rumen may be related
313 to the increase in milk production observed in SCG-fed ewes.
314 According to Bravo et al. (2012), caffeine concentration in SCG range from 3.59 to 8.09
315 mg/g of SCG. However, in the present study caffeine concentration of SCG used to
316 formulate experimental concentrates seemed to be widely under this range
317 (approximately 1.6 mg/g SCG), probably because SCG were obtained from hostelry and
318 could contain a mix of caffeinated and decaffeinated coffee residues. The effects of
319 caffeine have been studied with animals showing either an increase in the anxiogenic
320 (Maximino et al., 2011) or anxiolytic (Garcia et al., 2011) effect depending on the
321 timing of administration and dosage (Kulkarni et al., 2007). In the current trial feeding
322 SCG did not result in an adverse or stressed behavioral pattern measured as time spent
323 eating or concentrate DMI agreeing with previous results described in the current trial
324 which indicate that dosage was not excessive.

In summary any nutritional strategy resulting in improved milk yields and/or composition without impairing animal behaviour, such as feeding SCG as functional ingredient, would be interesting for farmers because most milk payment systems are based on volume but with a differential for variation in milk composition (De Wet, 1998). However, further research is needed to shed light on the effects of SCG on ruminal microbiome.

5. Conclusions

In conclusion, inclusion of SCG up to 100 g/kg in the concentrate modified ruminal fermentation pattern towards increased isoacids, acetic and butyric acid contents in the rumen with a concomitant improvement in milk production and composition without impairing feeding behaviour or apparent digestibility.

Acknowledgements

This work was supported by SOSDIE project from the European Regional Development Fund and the Department of Economic Development and Competitiveness of the Basque country. The first author of this paper was granted a scholarship from the Department of Agriculture of the Basque country.

References

Abate, A., Pfeffer, E., 1986. Changes in nutrient intake and performance by goats fed coffee pulp-based diets followed by a commercial concentrate. Anim. Feed Sci. Technol. 14, 1–10. https://doi.org/10.1016/0377-8401(86)90002-7

Acheson, K.J., Gremaud, G., Meirim, I., Montigon, F., Krebs, Y., Fay, L.B., Gay, L.J., Schneiter, P., Schindler, C., Tappy, L.2004. Metabolic effects of caffeine in humans: lipid oxidation or futile cycling?. Am J Clin Nutr. 9, 40-46 https://doi.org/10.1093/ajcn/79.1.40

AOAC, 1996. Official methods of analysis. Association of Official Analytical Chemists. AOAC International, Gaithersburg, USA.

AOAC, 1999. Official methods of analysis. Association of Official Analytical Chemists. AOAC International, Gaithersburg, USA.

Andries, J.I., Buysse, F.X., De Brabander, D.L., Cottyn, B.G., 1987. Isoacids in ruminant nutrition: Their role in ruminal and intermediary metabolism and possible influences on performances - A review. Anim. Feed Sci. Technol. 18, 169–180. https://doi.org/10.1016/0377-8401(87)90069-1

Bartley, E.E., Ibbetson, R.W., Chyba, L.J., Dayton, A.D., 1978. Coffee Grounds. II. Effects of coffee grounds on performance of milking dairy cows and feedlot cattle, and on rumen fermentation and dry matter removal rate. J. Anim. Sci. 47, 791–799. https://doi.org/10.2527/jas1978.474791x

Bellet S, Kershbaum A, Finck EM. 1968. Response of free fatty acids to coffee and caffeine. Metabolism 17, 702-707 https://doi.org/10.1016/0026-0495(68)90054-1

Borrelli, R.C., Esposito, F., Napolitano, A., Ritieni, A., Fogliano, V., 2004. Characterization of a new potential functional ingredient: coffee silverskin. J. Agric. Food Chem. 52, 1338–1343. https://doi.org/10.1021/jf034974x

Bravo, J., Juániz, I., Monente, C., Caemmerer, B., Kroh, L.W., De Peña, M.P., Cid, C., 2012. Evaluation of spent coffee obtained from the most common coffeemakers as a source of hydrophilic bioactive compounds. J. Agric. Food Chem. 60, 12565–12573. https://doi.org/10.1021/jf3040594

Busquet, M., Calsamiglia, S., Ferret, A., Kamel, C., 2006. Plant extracts affect in vitro rumen microbial fermentation. J. Dairy Sci. 89, 761–771. https://doi.org/10.3168/jds.S0022-0302(06)72137-3

373 Campbell, T.W., Bartley, E.E., Bechtle, R.M., Dayton, A.D., 1976. Coffee Grounds. I.
374 Effects of coffee grounds on ration digestibility and diuresis in cattle, on in vitro
375 rumen fermentation, and on rat growth. J. Dairy Sci. 59, 1452–1460.
376 https://doi.org/10.3168/jds.S0022-0302(76)84384-6
377 Campos-Vega, R., Loarca-Piña, G., Vergara, H., and Oomah, D., 2015. Spent coffee
378 grounds: A review on current research and future prospects. Trends Food Sci.
379 Technol. 45, 24-36. https://doi.org/10.1016/j.tifs.2015.04.012
380 Cochran, R. C., Adam,D.C., Wallace, J.D., and Galyean, M. L., 1986. Predicting
381 digestibility of different diets with internal markers: evaluation of four potential
382 markers. J. Anim. Sci. 63, 1476–1483. https://doi.org/10.2527/jas1986.6351476x
383 Cook, R., 1985. Isoacids. A new technology for dairy cows. Anim. Health Nutr. 40, 14.
384 Esquivel, P., Jiménez, V.M., 2012. Functional properties of coffee and coffee by-
385 products. Food Res. Int. 46, 488–495.
386 https://doi.org/10.1016/j.foodres.2011.05.028
387 Folley, S. J., and French, T. H., 1950. The intermediary metabolism of the mammary
388 gland; acetate metabolism of lactating mammary gland slices with special
389 reference to milk fat synthesis. Biochem. J. 46, 465-473.
390 https://doi.org/10.1042/bj0460465
391 Franca, A.S., Oliveira, L.S., 2009. Coffee processing solid wastes: Current uses and
392 future perspectives. Agricultural Wastes. 9, 155-189
393 Garcia, A.M.B., Cardenas, F.P., Morato, S., 2011. The effects of pentylenetetrazol,
394 chlordiazepoxide and caffeine in rats tested in the elevated plus-maze depend on
395 the experimental illumination. Behav. Brain Res. 217, 171–177.
396 https://doi.org/10.1016/j.bbr.2010.09.032

Girotto, F., Pivato, A., Cossu, R., Nkeng, G.E., Lavagnolo, M.C., 2018. The broad spectrum of possibilities for spent coffee grounds valorisation. J. Mater. Cycles Waste Manag. 20, 695–701. https://doi.org/10.1007/s10163-017-0621-5

Givens, D.I., Barber, W.P., 1986. In vivo evaluation of spent coffee grounds as a ruminant feed. Agric. Wastes 18, 69–72. https://doi.org/10.1016/0141-4607(86)90108-3

Goiri, I., Garcia-Rodriguez, A., Oregui, L.M., 2009. Effect of chitosan on mixed ruminal microorganism fermentation using the rumen simulation technique (Rusitec). Anim. Feed Sci. Technol. 152, 92–102. https://doi.org/10.1016/j.anifeedsci.2009.04.005

Goiri, I., Indurain, G., Insausti, K., Sarries, V., Garcia-Rodriguez, A., 2010. Ruminal biohydrogenation of unsaturated fatty acids in vitro as affected by chitosan. Anim. Feed Sci. Technol. 159, 35–40. https://doi.org/10.1016/j.anifeedsci.2010.05.007

De Wet, H., 1998. Payment Systems for Ex-Farm Milk – Results of IDF Questionaire 2296/a, Bulletin International Dairy Federation, 6-6.

International Organization for Standarization (ISO) 20481. Coffee and coffee products Determination of the caffeine content using high performance liquid chromatography (HPLC). Reference method. IDF 2005 2008. ISO, Geneva, Switzerland.

Jiménez-Zamora, A., Pastoriza, S., Rufián-Henares, J.A., 2015. Revalorization of coffee by-products. Prebiotic, antimicrobial and antioxidant properties. LWT - Food Sci. Technol. 61, 12–18. https://doi.org/10.1016/j.lwt.2014.11.031

Kondamudi, N., Mohapatra, S.K., Misra, M., 2008. Spent coffee grounds as a source of bioactive compounds. J. Agric. Food Chem. 56, 11757–11760. https://doi.org/10.1166/jbmb.2013.1369

422 Kulkarni, S.K., Singh, K., Bishnoi, M., 2007. Involvement of adenosinergic receptors in
423 anxiety related behaviours. Indian J. Exp. Biol. 45, 439–443.
424 Littell, R.C., Henry, P.R., Ammerman, C.B., 1998. Statistical analysis of repeated
425 measures data using SAS procedures. J. Anim. Sci. 76, 1216–1231.
426 https://doi.org/10.2527/1998.7641216x.
427 Liu, Q., Wang, C., Guo, G., Huo, W.J., Zhang, Y.L., Pei, C.X., Zhang, S.L., Wang, H.,
428 2018. Effects of branched-chain volatile fatty acids supplementation on growth
429 performance, ruminal fermentation, nutrient digestibility, hepatic lipid content and
430 gene expression of dairy calves. Anim. Feed Sci. Technol. 237, 27–34.
431 https://doi.org/10.1016/j.anifeedsci.2018.01.006
432 MAPA. Métodos oficiales del MAPA. BOE 52, 7167, 1995
433 Mata, T.M., Martins, A.A., Caetano, N.S., 2018. Bio-refinery approach for spent coffee
434 grounds valorization. Bioresource Technol. 247, 1077-1084.
435 https://doi.org/10.1016/j.biortech.2017.09.106
436 Maximino, C., da Silva, A.W.B., Gouveia, A., Herculano, A.M., 2011. Pharmacological
437 analysis of zebrafish (Danio rerio) scototaxis. Prog. Neuro-Psychopharmacology
438 Biol. Psychiatry 35, 624–631. https://doi.org/10.1016/j.pnpbp.2011.01.006
439 Mussatto, S.I., Machado, E.M.S., Martins, S., Teixeira, J.A., 2011. Production,
440 composition, and application of coffee and its industrial residues. Food Bioprocess
441 Technol. 4, 661-672. https://doi.org/10.1007/s11947-011-0565-z
442 Nudda, A., Battacone, G., Bencini, R., Pulina, G. 2002. Nutrition and Milk Quality. Ch.
443 8. Dairy Sheep Feeding and Nutrition. G. Pulina, ed. Avenue media Ed., Bologna,
444 Italia, pp. 197–228.
445 Pérez Hernández, L.M., Chávez-Quiroz, K., Medina-Juárez, L.A., Gámez-Meza, N.,
446 2012. Phenolic characterization, melanoidins, and antioxidant activity of some

commercial coffees from *coffea arabica* and *coffea canephora*. J. Chem. Mex. Soc. 56, 430-435.

Puchala, R., Min, B.R., Goetsch, A.L., Sahlu, T., 2005. The effect of a condensed tannin-containing forage on methane emission by goats. J. Anim. Sci. 83, 182–186. https://doi.org/10.2527/2005.831182x

Pulina, G., Macciotta, N., Nudda, A., 2005. Milk composition and feeding in the Italian dairy sheep. Ital. J. Anim. Sci. 4, 5–14. https://doi.org/10.4081/ijas.2005.1s.5

Ramalakshmi, K., Rao, L.J.M., Takano-Ishikawa, Y., Goto, M., 2009. Bioactivities of low-grade green coffee and spent coffee in different in vitro model systems. Food Chem. 115, 79–85. https://doi.org/10.1016/j.foodchem.2008.11.063

Richardson, L.F., Raun, A.P., Potter, E.L., Cooley, C.O., Rathmacher, R.P., 1976. Effect of monension on rumen fermentation in vitro and in vivo. J. Anim. Sci. 43, 657-664. https://doi.org/10.2527/jas1976.433657x

Robertson, J.B., Van Soest, P., 1981. The detergent system of analysis, in James, W.P.T and Theander, O. (Eds), The Analysis of Dietary Fibre in Food. Marcel Dekker, New York, USA, pp. 123–158.

Rufián Henares, J. A., Morales, F. J., 2007. Functional properties of melanoidins: In vitro antioxidant, antimicrobial and antihypertensive activities. Food Res. International 40, 995-1002. http://dx.doi.org/10.1016/j.foodres.2007.05.002

Russel, A., 1984. Body condition scoring of sheep. In Practice 6, 91-93.

SAS 2017. Enterprise's Guide. Cary, NC, USA.

Senevirathne, N.D., Okamoto, T., Takahashi, J., Umetsu, K., Nishida, T. 2012. Effect of Mixed Microbial Culture on Fermentation of Beverage Residues and the Effect of the Fermented Beverage Residues on in Vitro Rumen Fermentation and Methane

Production. Int. J. Biosci. Biochem. Bioinform. 2, 3 https://doi.org/10.17706/IJBBB

Seo, J., Jung, J.K., Seo, S. 2015. Evaluation of nutritional and economic feed values of spent coffee grounds and Artemisia princeps residues as a ruminant feed using in vitro ruminal fermentation. PeerJ 3:e1343 https://doi.org/10.7717/peerj.1343

Scully, D.S., Jaiswal, A.K., Abu-Ghannam, N., 2016. An investigation into spent coffee waste as a renewable source of bioactive compounds and industrially important sugars. Bioengineering 3, 33. https://doi.org/10.3390/bioengineering3040033

Silva, M.A., Nebra, S.A., Machado Silva, M.J., Sanchez, C.G., 1998. The use of biomass residues in the Brazilian soluble coffee industry. Biomass Bioenergy 14, 457–467. https://doi.org/10.1016/S0961-9534(97)10034-4

Singleton, V. L., Rossi, J. A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144-158.

Van Keulen, J., Young, B.A., 1977. Evaluation of Acid-Insoluble Ash as a Natural Marker in Ruminant Digestibility Studies. J. Anim. Sci. 44, 282–287. https://doi.org/10.2527/jas1977.442282x

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Wang, H.Y., Qian, H., Yao, W.R., 2011. Melanoidins produced by the Maillard reaction: Structure and biological activity. Food Chem. 128, 573–584. https://doi.org/10.1016/j.foodchem.2011.03.075

Williams, C.H., David, D.J., Iismaa, O., 1962. The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. J. Agric. Sci. 59, 381–385. https://doi.org/10.1017/S002185960001546X

Xu, C.C., Cai, Y., Zhang, J.G., Ogawa, M., 2007. Fermentation quality and nutritive value of a total mixed ration silage containing coffee grounds at ten or twenty percent of dry matter. J. Anim. Sci. 85, 1024–1029. https://doi.org/10.2527/jas.2005-628

Table 1

Ingredients and chemical composition of experimental concentrates and fescue hay.

Item	SCG inclusion level (g/kg DM)				Fescue hay	SCG
	0	30	50	100		
Ingredients (g/kg DM)						
Barley	200	250	250	250	—	
Oats	300	170	150	50	—	
Corn	170	220	220	270	—	
Rapeseed meal	150	150	150	150	—	
Hydrogenated palm fat	20	20	20	20	—	
Molasses	30	30	30	30	—	
Vitamin-mineral premix	30	30	30	30	—	
DDGs	100	100	100	100	—	
Coffee grounds	0	30	50	100	—	
Chemical composition (g/kg DM)						
Dry matter	894	912	896	909	943	
Organic matter	936	943	937	940	—	985
Crude protein	161	165	167	170	123	125
aNDFom	257	217	227	213	468	730
ADFom	115	96	100	97	231	480
Fat	78	77	75	74	—	164
Starch	333	370	354	355	—	30
Caffeine (mg/kg DM)	0	50	96	120	—	

GAE(mg/g DM)	0	3.7	3.8	4.0	—
Melanoidins (g/100g DM)	0	3.5	3.7	4.2	—
UFL	1.0	1.0	1.0	1.0	—

SCG= spent coffee ground; DM= dry matter; DDGs: distillers dried grains with solubles; aNDFom= neutral detergent fiber; ADFom= acid detergent fiber; GAE= gallic acid equivalents; UFL= feed units for lactation

Table 2

Ruminal SCFA profile of dairy ewes fed with increasing levels of coffee grounds in the concentrate.

Item	SCG inclusion level (g/kg DM)					Polynomial contrast	
	0	30	50	100	SEM	*L*	Q
SCFA (mmol/L)	42.2	43.2	44.3	47.1	14.94	0.51	0.78
Individual SCFA (mmol/mol)							
Acetic	648	662	666	667	11.2	0.002	0.012
Propionic	197	176	179	160	11.7	<0.001	0.30
Butyric	123	131	126	135	11.0	0.028	0.82
Valeric	13.7	13.3	13.4	12.8	1.30	0.16	0.92
Isovaleric	11.9	9.66	8.79	15.2	2.401	0.007	<0.001
Isobutyric	7.21	6.93	6.5	10.1	2.231	0.002	0.020
BCVFA	18.4	16.6	15.3	25.3	4.37	0.003	0.004
Acetic: Propionic	3.31	3.78	3.73	4.19	0.269	<0.001	0.30

SCG= spent coffee ground; *L*= linear effect; *Q*= quadratic effect; SEM= standard error of the mean; SCFA=short chain fatty acid; BCVFA=branched-chain volatile fatty acids

Table 3

Milk yield and composition, intake, dry matter digestibility, average daily weight gain and body conditioning score of dairy ewes fed with increasing levels of coffee grounds in the concentrate.

Item	SCG inclusion level (g/kg DM)					Polynomial contrast	
	0	30	50	100	SEM	*L*	*Q*
Yield (g/day)							
Milk	1982	1984	2203	1987	12.5	0.14	<0.001
FPCM	1650	1741	1831	1828	48.8	0.099	0.14
Fat	101	108	114	119	3.9	0.002	0.36
Protein	86.0	92.2	95.8	93.2	2.00	<0.001	0.043
Lactose	100	103	110	101	2.4	0.66	0.007
Efficiency							
Milk yield per kg DMI (g/kg)	924	957	1042	921	32.5	0.95	0.011
FPCM per kg DMI (g/kg)	761	804	846	833	28.7	0.077	0.18
Milk composition (g/kg)							
Protein	44.0	45.4	45.9	46.8	0.49	<0.001	0.038
Fat	50.7	57.4	53.4	60.0	1.13	<0.001	0.89
Lactose	50.3	50.8	52.4	51.5	0.40	0.021	0.012
Intake (kg DM/day)							
Forage	1.39	1.31	1.32	1.34	0.319	0.80	0.60
Total	2.28	2.26	2.22	2.23	0.319	0.80	0.60

DMD	0.62	0.61	0.61	0.61	0.055	0.82	0.78
Average daily weight gain (g/day)	78.4	80.3	81.9	56.9	10.11	0.28	0.51
Body conditioning score	2.44	2.25	2.54	2.52	0.571	0.93	0.76

SCG= spent coffee ground; *L*= linear effect; *Q*= quadratic effect; SEM= standard error of the mean; SEM= standard error of the mean; FPCM= fat and protein corrected milk; DM= dry Matter; DMI= dry matter intake; DMD= apparent dry matter digestibility coefficient

566 **Table 4**

567 Feeding behaviour of dairy ewes fed with increasing levels of coffee grounds in the
568 concentrate.

Item	SCG inclusion level (g/kg DM)					Polynomial contrast	
	0	30	50	100	SEM	*L*	*Q*
Intake (min/day)	391	356	374	349	21.1	0.019	0.52
Rumination (min/day)	539	588	568	548	18.6	0.95	0.064
Chewing (min/day)	929	943	945	897	33.0	0.21	0.22
Idling (min/day)	509	497	498	543	32.9	0.15	0.16
Rumination per kg of DMI (min/kg)	253	280	263	260	16.6	0.99	0.41

569 SCG= spent coffee ground; *L*= linear effect; *Q*= quadratic effect; SEM= standard error
570 of the mean; DM=dry matter; DMI=dry matter intake

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