Evaluation of nutritional and economic feed values of spent coffee grounds and *Artemisia princeps* residues as a ruminant feed using *in vitro* ruminal fermentation

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Much research on animal feed has focused on finding alternative feed ingredients that can replace conventional ones (e.g., grains and beans) to reduce feed costs. The objective of this study was to evaluate the economic, as well as nutritional value of spent coffee grounds (SCG) and Japanese mugwort (*Artemisia princeps*) residues (APR) as alternative feed ingredients for ruminants. We also investigated whether pre-fermentation using *Lactobacillus* spp. was a feasible way to increase the feed value of these by-products. Chemical analyses and an *in vitro* study were conducted for SCG, APR, and their pre-fermented forms. All the experimental diets for *in vitro* ruminal fermentation were formulated to contain a similar composition of crude protein, neutral detergent fiber and total digestible nutrients at 1x maintenance feed intake based on the dairy National Research Council (NRC). The control diet was composed of ryegrass, corn, soybean meal, whereas the treatments consisted of SCG, SCG fermented with *Lactobacillus* spp. (FSCG), APR, and its fermented form (FAPR). The treatment diets replaced 100 g/kg dry matter (DM) of the feed ingredients in the control. Costs were lower for the all treatments, except FAPR, than that of the control. After 24-h incubation, the NDF digestibility of the diets containing SCG and its fermented form were significantly lower than those of the other diets ($P < 0.01$); pre-fermentation tended to increase NDF digestibility ($P = 0.07$), especially for APR. Supplementation of SCG significantly decreased total gas production (ml/g DM) after 24-h fermentation in comparison with the control ($P < 0.05$); however, there were no significant differences between the control and the SCG or the APR diets in total gas production, as expressed per Korean Won (KRW). Diets supplemented with SCG or FSCG tended to have a higher total volatile fatty acid (VFA) concentration, expressed as per KRW, compared with the control ($P = 0.06$). Conversely, the fermentation process of SCG and APR significantly decreased total gas production and VFA production as expressed per KRW ($P < 0.05$). Because of their nutrient composition and relatively lower cost, we concluded that SCG and APR could be used as alternative feed sources, replacing conventional feed ingredients. However, pre-fermentation of agricultural by-products, such as SCG and APR, may be inappropriate for improving their nutritive considering the

increase in production costs.

Evaluation of nutritional and economic values of spent coffee grounds and *Artemisia princeps* residues as a ruminant feed using *in vitro* ruminal fermentation

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Abstract

Much research on animal feed has focused on finding alternative feed ingredients that can replace conventional ones (e.g., grains and beans) to reduce feed costs. The objective of this study was to evaluate the economic, as well as nutritional value of spent coffee grounds (SCG) and Japanese mugwort (*Artemisia princeps*) residues (APR) as alternative feed ingredients for ruminants. We also investigated whether pre-fermentation using *Lactobacillus* spp. was a feasible way to increase the feed value of these by-products. Chemical analyses and an *in vitro* study were conducted for SCG, APR, and their pre-fermented forms. All the experimental diets for *in vitro* ruminal fermentation were formulated to contain a similar composition of crude protein, neutral detergent fiber and total digestible nutrients at 1x maintenance feed intake based on the dairy National Research Council (NRC). The control diet was composed of ryegrass, corn, soybean meal, whereas the treatments consisted of SCG, SCG fermented with *Lactobacillus* spp. (FSCG), APR, and its fermented form (FAPR). The treatment diets replaced 100 g/kg dry matter (DM) of the feed ingredients in the control. Costs were lower for the all treatments, except FAPR, than that of the control. After 24 h incubation, the NDF digestibility of the diets containing SCG and its fermented form were significantly lower than those of the other diets ($P < 0.01$); pre-fermentation tended to increase NDF digestibility ($P = 0.07$), especially for APR. Supplementation of SCG significantly decreased total gas production (ml/g DM) after 24 h fermentation in comparison with the control ($P < 0.05$); however, there were no significant differences between the control and the SCG or the APR diets in total gas production, as expressed per Korean Won (KRW). Diets supplemented with SCG or FSCG tended to have a higher total volatile fatty acid (VFA) concentration, expressed as per KRW, compared with the control ($P = 0.06$). Conversely, the fermentation process of SCG and APR significantly

decreased total gas production and VFA production as expressed per KRW ($P < 0.05$). Because of their nutrient composition and relatively lower cost, we concluded that SCG and APR could be used as alternative feed sources, replacing conventional feed ingredients. However, pre-fermentation of agricultural by-products, such as SCG and APR, may be inappropriate for improving their nutritive considering the increase in production costs.

(Keywords: *Artemisia princeps* residues, *in vitro* rumen fermentation, *Lactobacillus* fermentation, spent coffee grounds)

1. Introduction

Research on animal feed has often focused on finding alternative feed ingredients to replace conventional ones (e.g., grains and beans) in order to reduce feed costs. This is most important in developing countries where the supplies of cereal grains and beans are not great enough to support even the human population. Historically, by-products from processing crops and food products have received much attention as feed alternatives because of their consistent and mass production. Food by-products would also likely be inexpensive because of their classification as a waste product. Many by-products, however, do not contain enough nutrients to support livestock requirements, and their palatability and digestibility would need to be enhanced, even for ruminants. Pre-fermentation of these by-products by bacteria (Han, 1975), yeast (Wanapat et al., 2011), or fungi (Salman et al., 2008) are common methods to enhance their nutritional value (see Mahesh and Mohini [2013] for a review).

Although fermentation may improve the nutritional quality of the by-products, and it is an environmentally sustainable practice, it will increase the cost for feed production. This additional cost is often ignored by researchers. To make the fermentation process feasible, the production

costs of a fermented by-product must to be competitive with conventional feed ingredients (Han, 1978). Therefore, the economic value, as well as nutritional value, of fermented by-products must be considered simultaneously.

In this study, we evaluated the economic and nutritional values of spent coffee grounds (SCG) and Japanese mugwort (*Artemisia princeps*) residues (APR), as well as their fermented products as potentially cost-effective feed ingredients for ruminants. An *in vitro* ruminal fermentation study where TMR was supplemented with SCG or APR was conducted. These by-products were chosen primarily because of the rapid increase of production, and the relatively high content of nutrients and bio-active compounds. SCG are generated during the manufacture of instant coffee. It is the residue that remains after brewing raw coffee powders with hot water or steam. Annually, 6-million tons of SCG are produced worldwide and most of it is burned as waste, resulting in greenhouse gas emissions (Tokimoto et al., 2005). There have been studies on the potential of SCG as a feed source for ruminants (Campbell et al., 1976; Bartley et al., 1978; Givens and Barber, 1986; Xu et al., 2007) and even for monogastric animals (Sikka et al., 1985; Sikka and Chawla, 1986). Xu et al. (2007) concluded that wet coffee grounds could be included up to 100 g/kg on a dry matter (DM) basis in total mixed rations (TMR) for goats. APR is a by-product from the traditional Korean medicine industry; it is produced during harvesting and processing of leaves. Positive effects were observed in growth performance of broilers supplemented by *Lactobacillus*-fermented APR (Kim et al., 2012). To the best of our knowledge, however, there has been no attempt to assess the feasibility of fermented SCG or APR as a feed alternative in ruminants based on both their nutritional and economic values.

2. Materials and methods

Two cannulated non-lactating Holstein cows at Center for Animal Science Research, Chungnam National University, Korea were used in this study. Animal use and the protocols for this experiment were reviewed and approved by the Chungnam National University Animal Research Ethics Committee (CNU-00455).

2.1. Preparation of experimental diets

The feed ingredients used in this study were ryegrass, corn, soy bean meal (SBM), SCG, APR, and the *Lactobacillus*-fermented forms of SCG and APR (FSCG and FAPR, respectively). SCG were purchased from an instant coffee manufacturer (Dongseo Food, Inc., Bupyeong, Korea), and APR was obtained from the Ganghwa Agricultural R&D Center (Incheon, Korea). The fermentation process for SCG and APR with *Lactobacillus* spp. was conducted as described by Kim et al. (2012). Briefly, four strains of *Lactobacillus* spp. (*L. acidophilus* ATCC 496, *L. fermentum* ATCC 1493 [American Type Culture Collection: Virginia, USA], *L. plantarum* KCTC 1048 [Korean Collection Type Culture, Daejeon, Korea], and *L. casei* IFO 3533 [Korea Food Research Institute, Daejeon, Korea]) were used to ferment SCG and APR. A 1-L culture medium was inoculated with a 2-mL aliquot containing 10^9 cfu/mL of each *Lactobacillus* strain (de Man, Rogosa, and Sharpe broth [Difco Laboratories, Francisco Soria Melquizo S.A., Madrid, Spain], 10 g; sucrose, 10 g) and incubated at 36ºC for 24 h. Next, 4 kg of dried SCG and APR were mixed with 400 mL of prepared *Lactobacillus* inoculum in a fermentation flask and incubated for 72 h at 36ºC. The fermented by-products were obtained after freeze-drying the cultured substrate and media for 2 days following the manufacture's recommendations (ilShinBioBase, Inc., Korea).

All of the diets that included *in vitro* fermentation were formulated to meet nutrient requirements for non-lactating dairy cows (total digestible nutrient at 1x maintenance feed intake [TDN1x], 680 g/kg; crude protein [CP], 120 g/kg; neutral detergent fiber [NDF], 420 g/kg on a DM basis) according to the National Research Council (NRC 2001). The control diet was composed of 500 g/kg of ryegrass and 500 g/kg of a corn and SBM mix. The four experimental diets (SCG, FSCG, APR, and FAPR) were formulated to contain TDN1x, CP, and NDF contents (g/kg DM) similar to the control, replacing original ingredients with 100 g/kg DM of SCG, FSCG, APR or FAPR, respectively.

2.2. In vitro incubation

Rumen fluid was collected before the morning feeding from two cannulated non-lactating Holstein cows fed a ration consisting of 600 g/kg timothy hay and 400 g/kg of a commercial concentrate mix (123 ± 8.8 g/kg CP, 35 ± 6.4 g/kg ether extract [EE], 265 ± 6.9 g/kg NDF, and 109 ± 1.2 g/kg ash) twice daily at the Center for Animal Science Research, Chungnam National University, Korea. The rumen contents were mixed and transferred into a thermos bottle, and immediately transported to the laboratory. Rumen contents were strained through 4 layers of cheesecloth and mixed with 4x volumes of *in vitro* solution (Goering and Van Soest, 1970) under strictly anaerobic conditions. Fifty mL of rumen fluid/buffer mixture was transferred into 125-mL serum bottles containing 0.5 g of experimental diets under continuous flushing with O_2-free CO_2 gas. The bottles were sealed with butyl rubber stoppers and aluminum caps, and incubated for 0, 3, 6, 9, 12, 24, and 48 h at 39°C.

140 *2.3. Analyses*

141 Contents of DM (#934.01), CP (#976.05), EE (#920.39), lignin (ADL; #973.18) and ash
142 (#942.05) in the feed samples were determined as described by AOAC (2005). NDF, analyzed
143 using a heat stable amylase and expressed inclusive of residual ash, and acid detergent fiber
144 (ADF) were determined as described by Van Soest et al. (1991). Neutral detergent insoluble
145 crude protein (NDICP) and acid detergent insoluble crude protein (ADICP) were determined as
146 described by Licitra et al. (1996). Non-fiber carbohydrate (NFC) was calculated as 1000-CP-EE-
147 Ash-(NDF-NDICP) based on NRC (2001).

148 For phenolic acid analysis, caffeic acid, *p*-coumaric acid, ferulic acid, and sinapic acid
149 (Sigma-Aldrich, Missouri, USA; # C0625, # C9008, # 12870, and # D7927, respectively) were
150 used as standards. Identification of phenolic acids in the SCG and APR samples was based
151 primarily on retention time, UV spectra obtained from high-performance liquid chromatography
152 (HPLC-DAD), and mass spectrometric data using authentic standards. Phenolic acids were
153 analyzed as described by Rochfort et al. (2006).

154 After each incubation period, total gas production was measured using a pressure transducer
155 (Sun Bee Instruments, Inc., Seoul, Korea) as described by Theodorou et al. (1994). Next, 5 mL
156 of head space gas was collected using a gas tight syringe (Hamilton, Reno, Nevada, USA) for
157 analysis of CH_4 using a gas chromatograph (Daesung Science IGC-7200, Seoul, Korea) equipped
158 with a thermal conductivity detector and HayeSep Q 80/100 column (Restek, Bellefonte,
159 Pennsylvanian, US). The pH of the cultured fluid was measured with a general purpose pH meter
160 (Istek Inc., Seoul, Korea). The cultured fluid was then centrifuged at 14,000 rpm for 10 min at
161 4°C, and the supernatant was used for the analyses of volatile fatty acid (VFA) and ammonia
162 concentrations. The remaining undegraded samples and fluid were analyzed for NDF using a

163 modified version of the micro-NDF method proposed by Pell and Schofield (1993) for
164 measuring NDF degradability. VFA concentrations were determined as described by Erwin et al.
165 (1961). Ammonia concentration was analyzed by the method of Chaney and Marbach (1962).
166 For the economic analysis, the price of each feed ingredient was estimated and expressed in
167 Korean won (KRW). The average market prices of ryegrass, corn, and SBM in 2009 were used
168 (i.e., 364, 317, and 660 KRW/kg DM, respectively). The prices of SCG and APR (i.e., 91 and
169 235 KRW/kg DM, respectively) were obtained from the respective manufacturers. Prices of
170 FSCG and FAPR (i.e., 270 and 429 KRW/kg DM, respectively) were calculated by adding SCG
171 and APR prices to the cost for the fermentation process (i.e., 200 KRW/kg as fed). The cost of
172 the fermentation process included expenses for microbial strains, culture medium, labor, use of
173 fermenter instruments, and manufacturing.
174
175 *2.4. Statistical analysis*
176 The experiment was conducted using a completely randomized design, and the data were
177 analyzed using the GLM procedure of SAS (SAS Institute Inc., Carey, North Carolina, USA) as:
178 $$y_{ij} = \mu + \tau_i + e_{ij}$$
179 Where: y_{ij} is the *j*th observation in the *i*th treatment, μ is the overall mean, τ_i is the fixed effect of
180 the *i*th treatment (*i* = 1 to 5), and e_{ij} is the unexplained random effect on the *j*th observation in
181 the *i*th treatment. Four contrasts were tested: the difference between the control and SCG
182 (control *versus* SCG and FSCG), control and APR (control *versus* APR and FAPR), SCG and
183 APR (SCG and FSCG *versus* APR and FAPR), and non-fermented and fermented groups (SCG
184 and APR *versus* FSCG and FAPR). Differences among treatments were also compared with the

185 Tukey's test when there was a significant overall treatment effect. Statistical significance was
186 defined as $P < 0.05$, and a trend was discussed at $0.05 \leq P < 0.10$.
187
188 **3. Results**
189 Both by-products had higher amounts of fiber and lower NFC compared with corn or SBM,
190 and SCG had a markedly high level (>100 g/kg DM) of EE (Table 1). Phenolic acid contents of
191 SCG and APR were 3.22 and 2.80 mg/g DM, respectively. More specifically, the concentrations
192 of caffeic acid, *p*-coumaric acid, ferulic acid, and sinapic acid were 1.07, 0.42, 1.70, and 0.04
193 mg/g DM and 1.72, 0.77, 0.32, and 0.00 in SCG and APR, respectively. The fermentation
194 process increased feed cost and decreased NFC contents in both by-products (Table 1). The
195 fermentation process also increased NDF content.
196 All the experimental diets for the *in vitro* fermentation contained similar TDN1x, CP, and
197 NDF contents (Table 2), and their prices were calculated based on the prices of each feed
198 ingredient. Inclusion of the by-products reduced the price of formulated diet (14–30 KRW/kg),
199 except for FAPR which increased the price by 4 KRW/kg (Table 2). The use of fermented by-
200 products increased the cost of diets by 5% on average.
201 Supplementation of APR or FAPR significantly reduced pH compared to other treatments (P
202 < 0.01, Table 3). Ammonia nitrogen concentration did not differ among treatments. NDF
203 digestibility of the SCG group was significantly lower than that of the other diets ($P < 0.01$) and
204 the fermentation process tended to increase NDF digestibility ($P = 0.07$), especially that of APR
205 ($P < 0.05$, Table 3). Compared to the control diet, supplementation of SCG significantly
206 decreased total gas production (ml/g DM) after 24 h fermentation ($P < 0.05$, Table 3). The
207 fermentation process of SCG and APR tended to decrease total gas production ($P = 0.10$) and

208 significantly decreased total gas production per KRW. No statistical difference in CH_4
209 production was observed among treatments; however, there was a trend for a decrease in CH_4
210 production with SCG supplementation as compared to that of APR (P = 0.07). Significant
211 differences were not detected in total VFA production (mmol/g DM), proportion of acetate,
212 proportion of propionate, or acetate:propionate ratio. The treatments supplemented with SCG or
213 FSCG; however, tended to have a higher total VFA concentration per KRW compared to the
214 control (P = 0.06). Moreover, the fermentation process of SCG and APR significantly decreased
215 total VFA production (mmol/KRW) (P < 0.05). There were significant differences among the
216 experimental diets in the concentration of butyrate (P < 0.01, Table 3).
217

218 **4. Discussion**

219 This study was designed to evaluate the nutritional and economic value of two food by-
220 products, SCG and APR, as alternatives to conventional feed ingredients, and to investigate
221 whether the pre-fermentation of these by-products using *Lactobacillus* spp. could be an
222 economically feasible practice for increasing their nutritional value. These by-products were
223 chosen primarily because of the rapid increase in their production, and their relatively high
224 content of nutrients and bio-active compounds (Wallace, 2007; Acevedo et al., 2013). It was
225 expected that the bio-active phenolic compounds contained in these by-products would modulate
226 rumen microbial activity, fermentation characteristics, and CH_4 emission (Acevedo et al., 2013;
227 Kim et al., 2013). Fermentation of selected by-products using *Lactobacillus* spp. was applied to
228 improve their nutritive values as feed ingredients. However, a dramatic increase of CP by
229 microbial proliferation was not observed in either fermented by-products (Table 1), indicating
230 that phenolic compounds, such as tannins, appear to be resistant to cell wall degradation by

231 microbial inoculants. Both NDICP and ADICP contents were high in SCG, which might be
232 caused by the Maillard reaction that occurs during beverage production under high temperature
233 (Senevirathne et al., 2012).

234 The use of SCG and APR could decrease the price of diets with similar nutrient contents.
235 Formulated experimental diets using SCG, FSCG, and APR had lower feed cost than the control
236 diet without compromising CP, NDF, or TDN1x contents; however, the experimental diet using
237 FAPR did not (Table 2). This result suggested that the residues (except those of FAPR) may be
238 applied to feed formulation to achieve economic advantages; however, lower NFC and higher EE
239 contents observed in diets having SCG compared to the control diet should be considered when
240 SCG is applied to practical feed formulation. Because of different nutrient compositions between
241 SCG and APR, SCG tends to replace energy and protein sources, whereas APR replaces forage.
242 Thus, supplementation of SCG is more favorable when the price of protein sources is high,
243 whereas APR would be a more appropriate alternative when the price of forage is high. Both
244 SCG and APR are feed ingredient alternatives for ruminants, particularly if the goal is to reduce
245 feed cost.

246 Total gas and VFA production after 24 h of *in vitro* fermentation suggested both SCG and
247 APR can be alternative feed ingredients in nutritional and economical aspects. Lower gas
248 production observed in SCG treatments than that of the control could have been caused by lower
249 NFC contents compared to the control diet, although they had similar composition in regard to
250 CP, NDF, and TDN1x. Seo et al. (2009) reported that total gas production at 48 h was primarily
251 determined by the NFC content. The diets containing SCG had significantly lower NDF
252 digestibility than the other diets. It was speculated that such phenolic compounds and resistance
253 factors from the Maillard reaction in SCG might be associated with *in vitro* digestibility (Puchala

et al., 2005; Senevirathne et al., 2012). More importantly, there was no significant difference between the control and the SCG diets in gas production as expressed by ml/KRW, which implied replacement of conventional feed ingredients by SCG could be economically beneficial without compromising ruminal fermentation.

Regarding VFA concentration, diets using APR exhibited a tendency toward increased VFA production at 24 h fermentation compared to SCG (Table 3). Meanwhile, VFA production based on price (mmol/KRW) tended to be higher in samples using SCG than the control group, indicating the use of SCG as the alternative feed source might not cause any negative effects on rumen fermentation. Xu et al. (2007) indicated that the proportion of SCG in TMR should not exceed 10% of DM. Lower nutrient digestibility and VFA production were observed when 20% of SCG was added to TMR in their study. However, since nutrient composition between treatments was not controlled in their studies, the optimal concentration of SCG that could be included in ruminant diets should be tested in terms of both nutritional and economical aspects as described in this study.

Based on the results from *in vitro* ruminal fermentation, the pre-fermentation process in this study is unlikely a feasible practice to improve the nutritional value of SCG and APR. Although pre-fermentation increased NDF digestibility of APR, there was a significant decrease in VFA production in fermented treatments when expressed based on costs (mmol/KRW). A declining trend in total gas production was also observed in the pre-fermentation of the by-products. This implies that fermented by-products may not be appropriate as feed alternatives in terms of cost-effectiveness, even though they have a potential to be a feed additive having probiotic functions that include modulation of ruminal fermentation, enhanced fiber digestion, and immune stimulation in the hind-gut (McAllister et al., 2011).

277 A previous *in vitro* study using beverage residues found that TMR using SCG had similar
278 CH_4 production to the control, whereas, both TMRs showed significantly decreased CH_4
279 production compared to the other TMR using green tea residues (Senevirathne et al., 2012). In
280 this study, CH_4 production was numerically decreased in the diet formulated with SCG compared
281 to the control; nevertheless, further research to investigate the effect of SCG usage on rumen
282 fermentation is required because it might have considerable amounts of polyphenolic compounds
283 that can modulate CH_4 emission (Puchala et al., 2005), nitrogen metabolism, and the rumen
284 protozoal population (Wallace, 2007).
285
286 **5. Conclusions**
287 The results of this study indicated that SCG and APR may be used as alternatives to
288 conventional feed ingredients because of their nutrient composition and relatively low cost. Pre-
289 fermentation of these products, however, may be inappropriate for improving their nutritional
290 content considering the increase in production costs. Further studies on *in vivo* ruminal
291 fermentation, and whole body digestion and metabolism by supplementing these by-products are
292 warranted.
293
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297
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377 Table 1. Chemical composition (g/kg, DM basis) and estimated price (KRW/kg DM) of each
378 feed ingredient.

	Feed ingredients†						
Item	Ryegrass	Corn	SBM	SCG	FSCG	APR	FAPR
DM (g/kg as fed)	954	907	900	550	925	850	932
CP	81	103	513	138	141	107	103
EE	8	34	15	136	157	18	17
Ash	54	42	62	20	18	91	95
NDF	739	116	237	656	792	649	706
ADF	481	61	105	451	526	538	523
ADL	54	22	26	142	146	99	74
NDICP	46	33	217	105	114	59	47
ADICP	22	32	139	95	100	42	23
NFC	164	738	390	155	6	194	127
Feed price‡ (KRW/kg DM)	364	317	660	91	270	235	429

379 †SBM; soybean meal, SCG; spent coffee grounds, FSCG; fermented spent coffee grounds, APR;
380 *Artemisia princeps* residues, FAPR; fermented *Artemisia princeps* residues.

381 ‡The prices for ryegrass, corn, and SBM are the average market prices in Korea in 2009. The
382 prices of SCG and APR were obtained from the respective manufacturers (Dongseo Food, Inc.,
383 and Ganghwa Agricultural R&D Center, respectively). For the prices of FSCG and FAPR, the
384 cost for the fermentation process, including the expenses for microbial strains, culture medium,
385 labor, and use of fermenter instruments was calculated and added to their raw prices.

386 Table 2. Ingredients, analyzed chemical composition (g/kg, DM basis), and estimated cost
387 (KRW/kg DM) of experimental diets.

	Experimental diets‡				
Items	Control	SCG	FSCG	APR	FAPR
Ingredients†					
Ryegrass	500	430	408	393	386
Corn	432	414	438	445	451
SBM	69	56	54	62	63
SCG	0	100	0	0	0
FSCG	0	0	100	0	0
APR	0	0	0	100	0
FAPR	0	0	0	0	100
Analyzed chemical composition					
DM	930	929	933	933	926
CP	120	120	120	120	120
EE	20	32	35	21	21
Ash	50	46	46	53	53
NDF	436	444	444	423	432
ADF	274	283	281	277	272
ADL	38	48	48	42	40
NDICP	52	56	57	52	51
ADICP	34	40	40	36	34
NFC	427	414	412	435	425
TDN1x	680	683	687	677	680
Estimated cost (KRW/kg DM)	364	334	350	348	368

388 †SBM; soybean meal, SCG; spent coffee grounds, FSCG; fermented spent coffee grounds, APR;
389 *Artemisia princeps* residues, FAPR; fermented *Artemisia princeps* residues.

390 ‡The SCG, FSCG, APR and FAPR treatments contained 100 g/kg of SCG, FSCG, APR, and
391 FAPR, respectively.

392 Table 3. Fermentation characteristics and CH_4 production after 24 h *in vitro* incubation of the experimental diets using strained
393 ruminal fluid.

Item	Treatments †					SEM	*P* - value				
	Control	SCG	FSCG	APR	FAPR		Overall	Control *vs.* SCG	Control *vs.* APR	SCG *vs.* APR	Fermen-tation‡
pH	6.58[a]	6.59[a]	6.58[a]	6.55[b]	6.55[b]	0.000	<0.01	0.07	<0.01	<0.01	0.63
NH_3-N, mg/100ml	11.9	12.1	11.5	11.7	11.5	0.24	0.39	0.79	0.27	0.30	0.20
NDF digestibility, g/kg DM	293.5[ab]	252.4[b]	242.3[b]	259.8[b]	316.9[a]	11.43	<0.01	<0.01	0.72	<0.01	0.07
Gas production, ml/g DM	193.7	184.6	180.7	192.2	184.4	3.20	0.07	<0.05	0.20	0.11	0.10
Gas production, ml/KRW	532.7[ab]	553.5[a]	516.9[ab]	551.8[a]	501.9[b]	9.03	<0.01	0.83	0.61	0.38	<0.01
CH_4, ml/g DM	19.2	18.1	18.0	18.8	20.3	0.78	0.27	0.24	0.71	0.07	0.39
VFA profiles											
Total VFA, mmol/g DM	5.99	5.87	5.82	5.97	6.06	0.090	0.41	0.24	0.80	0.09	0.80
Total VFA, mmol/KRW	16.43	17.60	16.67	17.13	16.50	0.267	0.05	0.06	0.27	0.26	<0.05
Acetate, mmol/mol	602.0	601.0	598.9	599.7	599.5	1.64	0.67	0.32	0.25	0.84	0.51
Propionate, mmol/mol	201.2	199.4	198.4	201.0	200.9	1.37	0.55	0.20	0.88	0.16	0.70
Butyrate, mmol/mol	166.6[b]	168.8[ab]	172.1[a]	168.8[ab]	169.1[ab]	0.80	0.01	<0.01	<0.05	0.08	<0.05
A/P ratio	2.99	3.02	3.02	2.98	2.98	0.028	0.81	0.43	0.89	0.26	0.91

394 † SCG; spent coffee grounds, FSCG; fermented spent coffee grounds, APR; *Artemisia princeps* residues, FAPR; fermented *Artemisia*
395 *princeps* residues. The SCG, FSCG, APR and FAPR treatments contained 100 g/kg of SCG, FSCG, APR, and FAPR, respectively.
396 ‡ Statistical difference between fermented and non-fermented substrates (SCG, APR *vs.* FSCG, FAPR).
397 [a,b] means that do not have common superscript differ significantly ($P < 0.05$).