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Full Length Research Paper

In situ rumen degradability characteristics of rice straw, soybean curd residue and peppermint (*Mentha piperita*) in Hanwoo steer (*Bos Taurus coreanae*)

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This study was intended to evaluate *in situ* rumen degradability characteristic of soybean curd residue (SCR) and peppermint compared to rice straw, which are used as a functional feed source for beef cattle for high quality beef production. Two steers were fitted with rumen and duodenum cannulae and *in situ* degradable and nylon bags were used to assess digestion of rice straw, SCR and peppermint in three replicated experiments. The highly effective dry matter (DM) degradability of peppermint was attributable to the high rate of the *a* and *b* fractions. Dry matter disappearance rate of SCR after 48 h was higher ($p < 0.05$) than that of peppermint. Based on DM disappearance, SCR showed the highest digestibility ($p < 0.05$). Crude protein disappearance (%) was higher for SCR and peppermint than rice straw ($p < 0.05$), and neutral detergent fibre disappearance (%) was higher for SCR ($p < 0.05$). Soybean curd residue and peppermint have great nutritive values as feed sources for ruminants, and using SCR and peppermint would have possibilities for efficient and functional livestock production.

Key words: Beef cattle, digestibility, effective degradability, soybean curd residue, peppermint.

INTRODUCTION

Concentrate and rice straw are widely used as the main feed sources in Korean beef cattle industry. However, because of low productivity due to the inadequate nutritive value of rice straw (Abaza et al. 1981), high ash content and low crude protein and digestibility, total mixed rations including several agricultural by-products are currently used in the beef industry and thus there is increasing interest in the nutritive value of agricultural by-product feeds. Soybean curd residue (SCR) is a by-product which is produced in the process of making tofu or soymilk from soybeans.

It has been reported that soybean has essential nutrients such as protein, genistein, isoflavones, essential amino acids, and tocopherol which have several active functions (Ryoo et al., 2004; Kim and Kang, 2009). Abe (2001) reported that feeding soybean curd residue to Japanese black wagyu steer resulted in good fattening performances. Therefore, it indicates that the use of SCR in the ruminants would increase productivity and improve beef quality. Soybean curd residue has been widely used as a feed for Korean beef cattle, Hanwoo (*Bos Taurus coreanae*), especially when tofu factories are located nearby. Despite its high nutritive value, palatability and low price (Kwak and Yoon, 2003), it is hard to find a study on a method of SCR utilization and its impact on rumen physiological characteristics of Hanwoo. Thus, it is necessary to evaluate its rumen digestive characteristics

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Abbreviations: SCR, Soybean curd residue; **HPLC,** high-performance liquid chromatography; **CP,** crude protein; **NDF,** neutral detergent fibre; **CF,** crude fibre; **EE,** ether extract; **AD,** acid detergent; **NPN,** non-protein nitrogen.

and nutritive value for efficient use.

It has also been reported that peppermint has several active functions, including antiseptic, antibacterial, disinfectant and antioxidative activities (Han, 2002; Campeol et al., 2003). Ushid et al. (2002) reported that volatile sulphur-complex production by colonic bacterial activity in pigs was significantly decreased by including peppermint in the animals' feed. Feeding peppermint to Holstein steers resulted in low concentrations of ammonia and protozoa in the rumen (Ando et al., 2003). Also, peppermint decreased microorganisms that can synthesis methane in the rumen, and thus it was suggested that peppermint may regulate rumen fermentation (Agarwal et al., 2009). Therefore, this study was aimed at evaluating *in situ* rumen degradability characteristics of SCR and peppermintin compared to rice straw, which are expected to serve as a functional feed source of beef cattle for high quality beef production.

MATERIALS AND METHODS

The dry matter, crude protein, crude fat, crude fiber, crude ash, and neutral detergent fiber contents of experimental diet samples were measured in accordance with the AOAC (1990) and Van Soest et al. (1991) method. Isoflavone was revealed by high-performance liquid chromatography (HPLC) assay, and its concentration expressed as µg/g. Isoflavone was detected as daizein and genistein. Menthol was detected with GC (Hewlett-Packard, HP0 5890II, silica capillary column 50 cm × 0.32 mm × 0.33 µm). Measurement of *in situ* degradability was undertaken in two Hanwoo steers (*Bostauruscoreanae*), each weighing approximately 500 ± 2.0 kg, that were fitted with rumen and duodenum cannulae, at the National Institute of Animal Science (NIAS, RDA), Korea. Experimental steers in metabolic cages were preliminarily adapted 2 weeks before collection period. Steers were fed a basal diet of 8 kg/head/day with concentrate (60% FM) and rice straw (40% FM) during the experiment. The basal diets were offered twice a day equally. The components of the concentrate and the chemical composition of the basal diets are given in Tables 1 and 2.

The *in situ* nylon bag digestive experiment was conducted by using the method of Ørskovet al. (1980). Samples of rice straw, SCR and peppermint were ground with a Thomas Willey Mill (Thomas Scientific model 4, New Jersey, USA) and sieved with a standard test sieve (mesh no. 70, 212 µm; mesh no. 20, 850 µm). Nylon bags (15 × 8 cm, 45 µm pore size; Dacron polyester) were prepared as described by Shaver et al. (1986). Bags containing 5 g of the respective test samples (sample size: bag surface area = 16.67 mg/cm^2) were inserted in duplicate at each time point in each steer at 0, 3, 6, 9, 12, 24, 48, and 72 h. Bags were placed inside a mesh nylon bag that was tied to a weight chain and placed in the ventral sac. Blank bags were also placed in the mesh nylon bag to estimate influx of dry matter (DM) into the bag.

After incubation, all bags were removed at the same time, washed with cold water for 30 min to stop bacterial activity, and dried for 48 h. Zero-hour bags were washed and dried by the same method. Residual DM of each dried bag was corrected for DM by their corresponding blank bags. Dried residues from nylon bags for each steer at each time point were stored for further analysis. Dried samples from each time point were weighed to measure the percentage of DM that had disappeared and analysed for crude protein (CP) and neutral detergent fibre (NDF) (AOAC, 1990) to determine the proportion that had disappeared. Dry matter degradability of test samples at each time point was calculated by

Table 1. Formula of the experimental concentrate[A].

Item	DM%
Ground corn	47.8
Wheat bran	41.0
Soy bean residue	5.0
Rapeseed residue	2.0
Molasses	2.0
Calcium phosphate	1.5
Salt	0.4
Vitamin-mineral additive[B]	0.2
Lasalocid	0.1
Total	100

[A] Throughout the experiment, each steer had free access to water and commercial block mineral (Rincal block, DaehanNer Pham, Seoul, Korea) which provided the following nutrients per kilogram: I, 150 mg; Mn, 200 mg; S, 4000 mg; Co, 100 mg; Fe, 2000 mg; Zn, 100 mg; Ni, 50 mg; Cu, 100 mg; Mg, 3000 mg; Ca, 2000 mg; Se, 40 µg; NaCl, 380 g. [B] A kilogram of the additive (Grobic-DC, Bayer HealthCare, Leverkusen, Germany) provided the following nutrients: vitamin A, 2,650,000 IU; vitamin D3, 530,000 IU; vitamin E, 1050 IU; niacin, 10,000 mg; Mn, 4,400 mg; Fe, 13,200 mg; I,440 mg; Co, 440 mg.

Table 2. Chemical composition of the basal diets.

Item	Rice straw	Concentrate
Dry matter (%)	85.8±1.2	83.8±0.7
	--------------- % DM-----------------	
Crude protein	4.6±0.6	13.3±0.6
Ether extract	1.5±0.3	3.0±0.5
Crude fibre	27.9±2.1	5.9±1.7
Crude ash	11.3±1.1	5.1±1.0

the method of Ha and Kennelly (1984). Dry matter degradation parameters and effective degradabilities of test samples were also calculated with an exponential equation proposed by Ørskov and McDonald (1979), and applied rumen fractional outflow rate (*k*) was 0.02 to 0.06/h:

$$P = a + b\,(1 - e^{-ct})$$

$$ED = a + bc / (c + k)$$

Where, P = Degradation of nutrients at time t, a = rapidly degradable fraction, b = fraction degraded over time, c = constant for b fraction, t = rumen suspension time, k = rumen outflow rate, ED = effective degradability

The data from the experiment were analysed by using the SAS (1999) general linear model (GLM) procedure. Results are presented as mean values with the standard error of the means. Differences between treatments were determined by Duncan's multiple range test (1955) with $p < 0.05$ level of significance.

RESULTS AND DISCUSSION

Table 3 shows the chemical composition of rice straw, SCR and peppermint. SCR had lower DM, crude fibre (CF) and neutral detergent fiber (NDF) contents and

Table 3. Chemical composition of rice straw, soybean curd residue and peppermint.

Item	Rice straw	Soybean curd residue	Peppermint
Dry Matter (%)	85.8±1.2	21.0±1.5	86.2±1.1
	--------------------------------% DM------------------------------------		
Crude protein	4.6±0.6	20.7±1.3	8.9±1.3
Ether extract	1.5±0.3	5.0±0.7	4.0±0.2
Crude ash	11.3±1.1	3.4±0.5	10.4±1.4
Crude fibre	27.9±2.1	26.7±1.9	29.4±2.3
Neutral detergent fibre	68.3±3.4	50.6±2.4	43.7±2.6
Isoflavone (μg/g)	-	5.4±0.2	-
Menthol (ppm)	-	-	9,774.41±27.5

Table 4. Dry matter degradation parameters (%) and effective degradability (%) of rice straw,soybean curd residue and peppermint.

Time	Rice straw	Soybean curd residue	Peppermint
Parameters[A]			
a	14.37±1.20[b]	5.73±2.12[c]	37.75±2.75[a]
b	44.97±2.81[c]	94.7±3.15[a]	55.37±2.46[b]
C (per hour)	0.0089±0.001	0.034±0.002	0.025±0.005
EDD[B]			
0.02	28.22±1.78[b]	69.56±3.12[a]	72.81±5.18[a]
0.03	24.66±1.56[c]	57.98±2.15[b]	67.39±4.95[a]
0.04	22.56±2.10[c]	49.95±3.16[b]	63.41±3.85[a]
0.05	21.17±3.18[c]	44.06±3.35[b]	60.38±3.62[a]
0.06	20.18±1.75[c]	39.56±5.01[b]	57.99±5.47[a]

[a, b, c]Means with a different superscript in the same row are significantly different at p<0.05. [A]rapidly degradable fraction; b, slowly degradable fraction; c, rate of degradation, respectively.[B]EDD; effective dry matter degradability; calculated according to Ørskov and McDonald (1979), using a rumen outflow rate of 0.02 to 0.06 per hour.

higher crude protein (CP) and ether extract (EE) contents than rice straw and peppermint. The chemical composition of SCR was similar to results from the report by Kwak and Yoon (2003). Lee et al. (1992) also reported similar results in a study which analysed chemical composition of SCR extracted from domestic and imported soybean. In spite of the extraction process for tofu manufacture, highly valuable feed components remain in SCR which is thought to be a good feed source for ruminants.

Ryoo et al. (2004) reported that the isoflavone content of imported soybean was 504 μg/g, which is sufficient to have an effect on prevention and treatment of diseases caused by hormonal changes. The isoflavone content of SCR in this study was 5.35 μg/g, far less than in imported soybean. But, if SCR was supplemented with a total mixed ration to provide a fixed quantity of isoflavone every day, it could serve as a functional feed. Peppermint was 86.2% DM, which was 8.9% CP, 29.4% CF, 4.0% EE, 10.4% ash and 43.7% NDF, showing that the roughage and feed value of peppermint were higher than those of rice straw. Peppermint also contained 9774.4 ppm of menthol, which has antibacterial function, showing a similar level compared with an earlier study (Shin and Park, 1994).

Table 4 shows the degradability of DM calculated by the amount of DM disappearance per incubation time. The feed disappearance rate in ruminants is closely related to the passage rate in the rumen, so the disappearance rate of feeds has to be calculated with the passage rate. In Table 5, the calculated DM degradability was defined by the *a* fraction of fusible and resolvable materials (that is, materials that were smiley disappeared from nylon bag by washing), the *b* fraction that slowly degraded within a given time, and slope *c* which reflected the retention time within the rumen. The sum of the *a* and *b* fractions was expressed as a potential degradation rate or potential disappearance rate of each assessed sample (Ørskov et al., 1980). At this moment, fractional outflow rate in the rumen (*k*) was estimated by the resolvable DM degradation rate within the rumen by applying 0.02 to 0.06% per hour.

The *a* fraction was 14.3% for rice straw, 5.7% for SCR and 37.7% for peppermint ($p < 0.05$). The *b* fraction was

Table 5. *In situ* ruminal dry matter, crude protein and NDF disappearance rates (%) of rice straw, soybean curd residue and peppermint.

Time	Items	Rice straw	Soybean curd residue	Peppermint
0[A]	DM	14.98±1.8[b]	13.84±0.13[b]	41.33±0.03[a]
	CP	28.8±1.87[a]	21.04±2.84[b]	21.45±0.90[b]
	NDF	14.97±1.80[b]	7.15±0.96[c]	20.87±1.57[a]
3	DM	15.34±0.59[b]	15.23±0.43[b]	42.13±2.9[a]
	CP	29.35±1.46[a]	25.82±0.91[b]	21.60±2.13[c]
	NDF	15.47±0.76[b]	6.23±2.56[c]	20.78±1.76[a]
6	DM	16.96±0.47[b]	16.69±1.18[b]	44.61±3.02[a]
	CP	31.84±2.36[ab]	25.87±0.13[b]	35.75±2.08[a]
	NDF	16.96±0.46[b]	6.88±0.82[c]	20.85±2.35[a]
9	DM	18.29±0.37[b]	22.24±3.04[b]	51.43±0.6[a]
	CP	32.98±1.41[b]	26.05±0.47[c]	47.64±2.18[a]
	NDF	17.38±0.24[b]	5.76±1.37[c]	30.25±4.00[a]
12	DM	20.37±1.03[c]	28.96±3.77[b]	56.62±0.17[a]
	CP	34.19±1.74[b]	25.18±0.55[c]	50.89±3.83[a]
	NDF	18.29±0.37[a]	11.06±1.08[c]	41.81±0.51[b]
24	DM	22.49±1.31[b]	66.07±5.71[a]	70.92±1.04[a]
	CP	37.8±2.91[c]	47.61±0.29[b]	64.29±2.12[a]
	NDF	22.49±1.31[c]	43.85±0.30[b]	56.5±4.87[a]
48	DM	28.49±2.06[c]	90.47±6.02[a]	84.49±1.43[b]
	CP	41.6±2.74[c]	76.22±2.70[b]	79.86±2.34[a]
	NDF	28.85±2.06[c]	72.32±3.42[a]	68.78±4.93[b]
72	DM	38.36±2.90[c]	97.67±0.14[a]	86.81±0.11[b]
	CP	46.9±0.57[c]	96.42±0.09[b]	84.53±0.31[a]
	NDF	38.36±0.95[c]	95.78±0.19[a]	74.13±0.98[b]

[a, b, c]Means with a different superscript in the same row are significantly different at $p < 0.05$. [A]0 time means soluble fraction of feed dry matter as measured by washing loss from nylon bag.

44.9% for rice straw, 94.7% for SCR and 55.3% for peppermint ($p < 0.05$), and the slope *c* was 0.0089% per hour for rice straw, 0.043% per hour for SCR and 0.025% per hour for peppermint.

The DM degradation rate was estimated by applying 0.05% per hour for the rumen pass efficiency (Collucci et al., 1982; Hartnell and Satter, 1979) generally applied for beef cattle, was 21.1% for rice straw, 44.0% for SCR and 60.3% for peppermint ($p < 0.05$). This means that the highly effective DM degradability of peppermint between 0.02 to 0.06% per hour was attributable to the high rate of the *a* and *b* fractions. On the other hand, DM disappearance rate of SCR after 48 h was higher ($p < 0.05$) than that of peppermint. A higher rate of the *b* fraction, which was fraction of degradable at time infinity, in SCR than in peppermint contributed to this result. Thus, these are important data to consider for the SCR amount in the total mixed ration formulation.

The ruminal disappearance of DM, CP and NDF for rice straw, SCR and peppermint at 0 to 72 h are given in Table 5. The DM disappearance of SCR was 13.84% at the 0 time point, which was similar to rice straw (14.9%). The DM disappearance of SCR between 0 and 12 h increased gradually from 13.84 to 28.96% and increased dramatically between 12 and 48 h (28.96 to 90.47%), showing a disappearance rate of 1.7% per hour. The disappearance of SCR at 72 h was 97.6%, meaning it was almost completely digested, and the average disappearance rate was 1.16% per hour. The early disappearance of peppermint was much higher ($p < 0.05$) than rice straw or SCR, prompting consideration for its use in feeds.

The CP disappearance rate of SCR was 21.04% at the 0 h point and 25.18% at 12 h, for a disappearance rate of 0.34% per hour. In contrast, from the 12 to 24 h time points, the disappearance increased greatly to 47.61%, at a rate of 1.8% per hour.

The CP disappearance at 72 h was 96.42% for a rate of 1.19% per hour. In general, acid detergent (AD), neutral detergent (ND) and borate buffer are used to classify feed protein in five steps by digestive pattern (A: NPN; B1: soluble true protein; B2: ND soluble protein; B3: ND insoluble protein; C: AD insoluble protein) (Chalupa and Sniffen, 1994).

The protein digestion of SCR in the rumen showed a pattern of ND insoluble protein like the B3 type. Neutral detergent insoluble protein showed a rumen disappearance rate of 0.1 to 1.5% per hour and this exists at the form of protamine, cell wall protein, and denatured protein. When soybean meal was classified according to the five-step protein degradation model; more than 71% of the CP disappearance was ND soluble protein (Maeng, 1998). But, the protein degradation pattern of SCR in this study showed ND insoluble protein like B3 type.

The soluble protein of soybean is considered to be mostly dissolved through the tofu production process. The rumen CP disappearance of peppermint was 21.45% at the early stage, similar to SCR. It showed 21.60 and 50.89% at 3 and 12 h, respectively, showing a fast 2.43% per hour disappearance rate.

Maeng (1998) reported that alfalfa hay containing 20% CP had 80% of ND soluble protein, non-protein nitrogen (NPN) and soluble true protein and had about 10% of AD and unusable protein. In this study, the degradation pattern of protein for peppermint at an early stage was similar to alfalfa hay which has contents that are easily soluble in ND and borate buffer. After 24 h, ND insoluble protein had disappeared, showing the general roughage disappearance process.

The NDF disappearance rate of rice straw was 14.97% at the beginning and 38.36% at the end of trial, showing significantly low ($p < 0.05$) disappearance. Soybean curd residue also had a low disappearance rate to start with 7.15% at 0 h and 11.06% at 12 h. But after 12 h, it was faster, reaching 72.32% at 48 h, for a rate of 1.70% per hour.

The early NDF disappearance rate of peppermint was unchanged from 0 to 6 h, after which it consistently increased to 72 h. The fermentation rate of structural carbohydrate in rumen is 3 to 4% per hour, 5 times slower than starch, and has 12 h of interphase in the rumen degradation process.

This results from the adherence time of microorganisms to fibre in the rumen, the physical damage of fibre caused by rumination and the fermentation effect of microorganisms (Maeng, 1998). In this study, this is the reason why SCR and peppermint had low NDF disappearance until 6 h.

Conclusion

SCR and peppermint have greater nutrient composition as a feed for ruminants than rice straw. Soybean curd residue has a stable DM, CP and NDF degradation rate for 72 h, and the highest feed value for ruminants, with the highest disappearance rate. Peppermint has high DM, CP and NDF disappearance at an early stage, so it requires some consideration for use as a feed for ruminants. Using peppermint with materials like menthol has additional possibilities for producing functional livestock products.

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