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Research Article

Relationship Linking Dietary Quercetin and Roughage to Concentrate Ratio in Feed Utilization, Ruminal Fermentation Traits and Immune Responses in Korean Indigenous Goats

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ABSTRACT

A total of nine Korean indigenous goats were used in a cross-over arrangement to give nine replicates per treatment, and they were housed individually assigned to 1 of 9 dietary treatments. Nine treatments were 0, 500, and 1000 ppm of quercetin supplementation in diets by mixing roughage and concentrate with different ratios (RC ratio) of 3:7 (RC 30), 5:5 (RC 50) and 7:3 (RC 70). Nutrient utilizations of dry matter, crude fat and NDF were not affected by neither RC ratio nor dietary quercetin (p>0.05), but the rate of crude protein and ADF increased in animals in RC 70 group regardless of quercetin supplementation (p<0.05). In addition, higher RC ratio increased (p<0.05) N retention and N retention rate. Total VFA, acetic acid, propionic acid, iso-butyric acid, butyric acid, iso-valeric acid and valeric acid contents were not affected (p>0.05) by dietary quercetin. Meanwhile, lower total cholesterol level exhibited in animals in RC 70 group compared to RC 30 or 50 groups, unrelated to dietary quercetin (p<0.05), however other plasma parameters were not influenced (p>0.05) by RC ratio and dietary quercetin. Our results indicated that both RC ratio and dietary quercetin may not directly affect the production indices and immune responses in Korean indigenous goat.

(**Key words** : Feed utilization, Korean indigenous goat, Roughage to concentrate ratio, Ruminal fermentation, Quercetin)

Ⅰ. INTRODUCTION

Livestock would be exposed to stress factors from poor environmental conditions like air pollution, noise, heat and oxidative stress (Rahal et al., 2014). These unfavorable conditions can lead to critical diseases such as foot and mouth disease and epizootic fever, which resulted in decline of animal production and economic loss in livestock farms (Rahal et al., 2014). Thus, antibiotics have been excessively utilized to animals to deal with such problems (Gillespie and Flanders, 2009). However, European Union began the restriction of antibiotic use from 2006, because antibiotic resistant bacteria and its residues in meat products could threaten human health (Cogliani et al., 2011). Consequently, alternatives as an antibiotic substitute are required for the food safety. Flavonoid, which is a polyphenol compound, is characterized by the thoughtful antioxidant, and is generally found in plants in the form of flavonol, flavones, iso-flavones, flavanones, anthocyanidins and flavonols (Dai and Mumper, 2010; Kumar and Pandey, 2013). In particular, quercetin, the type of flavonol is the most prominent dietary antioxidant contained in fruits, vegetables, flowerers, teas, and even in red wines (Leopoldini et al., 2006; Boots et al., 2008).

Quercetin was considered as an antioxidant owing to reducing peroxidation and scavenging free radicals (Farombi and Onyema, 2006; Boots et al., 2008). Previous studies demonstrated that it was involved in the inhibition of cancer, pulmonary or cardiovascular disease, and in anti-aging (Boots et al., 2008; Chen et al., 2010). In circulatory system, quercetin prevented cardiac muscle cell damage, hardening of the arteries, ischemic stroke, hyperpiesia and arrhythmia (Yang et al., 2015). Furthermore, it showed neuroprotective effects on cerebral ischemia by reducing lipid peroxidation (Yang et al., 2015). Therefore, quercetin has diverse functional properties associated with oxidant stress, and has a potential as a therapeutic agent in pathological problems (Lee et al., 2011;

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Yang et al., 2015).

Although many advantages of quercetin have been reported in medicine, few research have been reported its application in domestic animals. When pig fed with a diet containing 500 mg quercetin/kg body weight for 3 days, quercetin was highly accumulated in liver and kidney, while small amounts were detected in tissues of brain, heart, spleen and muscle, suggesting that its short-term exposure did not contribute to distribute quercetin metabolites in tissues (Boer et al., 2005). On the other hands, quercetin had a positive effect on pork quality by minimizing water loss and pH decrease, when pigs fed with quercetin prior to loading and transport (Kremer et al., 1998). Also, chicken fed a diet supplemented with quercetin indicated higher oxidation stability in leg meat than those had a diet with antibiotics (Jang et al., 2010). Recently, Korean indigenous goat had a diet containing quercetin showed increased digestibility, total volatile fatty acids (VFA) in rumen fluid and the content of propionate (Cho et al., 2010).

The purpose of this study is to investigate the effect of quercetin supplementation in diets by mixing roughage and concentrate with different ratios on feed utilization, blood profiles, properties of fermentation and immune system in Korean indigenous goat, to enhance the growth performance and the resistance to diseases. And finally we suggest appropriate usage of dietary quercetin to improve animal management and industry.

Ⅱ. MATERIALS AND METHODS

All management and experimental procedures were reviewed and approved properly by the Animal Ethics Committee of the Chungnam National University

1. Animals, housing, experimental design and diets

Nine male Korean indigenous goats (20 months old, weighing 22 ± 1.8 kg) were allotted in a cross-over arrangement to give nine replicates per treatment with three different combinations of diet, and three different levels of quercetin (0, 500 and 1000 mg per concentrated feed per kg; Quercetin hydrate, 95%; ACROS Co., NJ) along with different ratio of roughage and concentrate feeds, were 3:7 (RC 30), 5:5 (RC 50), and 7:3 (RC 70), respectively. The chemical compositions of experimental diets are presented in Table 1. Quercetin was top dressed to experimental diets for a daily ration basis.

The goats were housed in a metabolism cage (0.5×0.7×1.0 m^3) individually equipped with a nipple connected to a

Table 1. Composition and analyzed chemical composition of the experimental diets (g/kg, as-fed basis)

Item	Treatments[1]		
Ingredients, g/kg	RC 30	RC 50	RC 70
Timothy hay	-	250	700
Barley	300	250	-
Concentrate[2]	700	500	300
Analyzed chemical composition, DM[3] basis			
Crude protein, g/kg	117.0	102.1	115.1
Crude fat, g/kg	38.0	32.0	30.0
NDF[4],g/kg	550	601	653
ADF[5],g/kg	255	372	422
Crude ash, g/kg	80	77	78

[1] Treatments: RC 30 (300 g/kg roughage: 700 g/kg concentrate), RC 50 (500 g/kg roughage: 500 g/kg concentrate), RC 70 (700 g/kg roughage: 300 g/kg concentrate)
[2] Commercial formula feeds for fattening: crude protein 110 g/kg, crude fat 15 g/kg, calcium 5 g/kg, phosphorus 3 g/kg
[3] Dry matter
[4] Neutral detergent fiber
[5] Acid detergent fiber

calibrated bottle, a feeder and a metal trough for collection of feces and urine. All animals had access to feed on an ad libitum basis and water was available all the times. The experiment lasted for 126 days with 9 periods and each period lasted for 14 days with 7 days adaptation and 7 days sample collection. The respective diet was offered at 9:00 am and 4:00 pm every day.

2. Sampling

Feed intake was measured on a weekly basis as feed disappearance from the feeder, and water intake were recorded as disappearance from the plastic water bottle. Urine was obtained into a tube containing 15 mL of 50% (V/V) hydrochloric acid to inhibit nitrogen loss and frozen at -20ºC. The ambient temperature was maintained at 20-25ºC for the experimental period.

3. Dry matter digestibility and nutrients utilization

Dry matter digestibility and nutrients utilization were determined through comparison between feed intake and feces. The feeds and feces were analyzed for proximate composition using the procedures of AOAC (1995). The feces were oven-dried at 70ºC (to constant weight), then weighed to determine dry matter.

The proximate compositions of samples were determined by the methods of AOAC [18]. Briefly, crude protein content was measured by the Kjeldahl method (VAPO45, Gerhardt Ltd., Germany). The amount of nitrogen obtained was multiplied by 6.25 to calculate the crude protein content. Crude fat content was measured by the Soxhlet extraction system (TT 12/A, Gerhardt Ltd., Germany). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined following the procedures of Robertson and Van Soest (Robertson et al., 1981).

Nitrogen retention (NR) was calculated by subtracting fecal and urinary nitrogen from collected fecal and urinary nitrogen.

4. Ruminal pH and VFA concentrations

Approximately 100 mL of ruminal fluid was obtained as grab samples of digesta via a flexible PVC stomach tube (Cristallo Extra, FITT S.p.A., Sandrigo, Italy) which has been validated by Ramos-Morales et al. (2014). Collected fluid sample was strained through four layers of cheesecloth and then pH of the ruminal fluid was immediately determined using a pH meter (750P, Istek Co., Republic of Korea). A 10 mL sample of strained ruminal fluid was acidified with 0.5 mL of H_2SO_4 and was stored at -20ºC until analyzed for VFA. These samples were prepared as follows: 1) sample tubes were thawed and centrifuged at 2000 × g, 4°C for 15 minutes (Micro 12, Hanil Science Co. Ltd., Incheon, Korea), 2) supernatant (1 mL) was transferred into a microfuge tube, 0.2

Table 2. Effect of a diet supplemented with dietary quercetin and roughage and concentrates ratio (RC ratio) on feed intake, water intake,urine excretion and feces elimination in Korean indigenous goats

Item	Treatments[1]									SEM[2]	P-value[3]		
	RC 30			RC 50			RC 70				RC	QU	RC × QU
	QU 0	QU 500	QU 1000	QU 0	QU 500	QU 1000	QU 0	QU 500	QU 1000				
Feed intake, g/day	530.2	538.5	543.7	528.3	536.5	540.6	527.9	534.1	542.0	42.38	NS	NS	NS
Water intake, mg/g feed	1.71	1.68	1.66	1.61	1.62	1.62	1.58	1.62	1.59	0.112	NS	NS	NS
Urine excretion, mL/day	309.6	352.6	305.9	276.0	274.5	291.5	246.7	283.9	278.2	42.28	NS	NS	NS
Feces elimination, g/day	190.1	191.0	188.9	198.0	204.9	195.9	191.5	192.7	192.6	11.24	NS	NS	NS

[1] Treatments: RC 30 (300 g/kg roughage: 70 g/kg concentrate), RC 50 (500 g/kg roughage: 500 g/kg concentrate), RC 70 (700 g/kg roughage: 300 g/kg concentrate), QU 0 (dietary quercetin 0 mg/kg), QU 500 (dietary quercetin 500 mg/kg), QU 1000 (dietary quercetin 1000 mg/kg)
[2] Pooled standard error of mean
[3] Significance level: NS: Not significant

ml of 25% metaphosphoric acid was added, and the mixture was vortexed before incubating at room temperature (20-25ºC) for 30 minutes, and 3) supernatant was transferred into a gas chromatograph (GC-17A, Shimadzu, Japan). The analytical condition of organic acid by gas chromatograph is presented in Table 2.

5. Blood characteristics and immune responses

Blood samples (8-10 mL) were collected via jugular vein puncture into vacutainer tubes (EDTA-coated, Becton Dickinson Vacutainer systems, Franklin Lakes, NJ). All samples were immediately centrifuged (Micro 12, Hanil Science Co. Ltd., Incheon, Korea) at 2,000 × g for 10 minutes at 5°C to recover plasma and serum which was stored at -20°C until analyzed. No hemolysis was detected in any of the samples analyzed. Concentrations of creatinine (kinetic Jaffe method), glucose (glucose oxidase method), cholesterol (cholesterol oxidase method), total bilirubin (dichloroanylin method), blood urea nitrogen (BUN, urease/glutamate dehydrogenase method), and the activity of aspartate aminotransferase (AST, L-aspartate/2-oxoglutarate as substrate), alanine aminotransferase (ALT, L-alanine/2-oxoglutarate as substrate), alkaline phosphatase (P-nitrophenylphosphate as substrate) and gamma glutamyl transferase (γ-GT, L-gamma-glutamyl-3-carboxy-4-nitroanilide as substrate) glutamic oxaloacetatic transaminase (GOT), glutamic pyruvic transaminase (GPT), total protein (Biuret method), albumin (bromcresol green method), calcium (Arsenazo III method), inorganic phosphorus (phosphomolybdate method) were measured by commercial kits (Selectra 2, Merk Ltd. Co. Netherland) using an auto analyzer. Plasma IgA, IgM, and IgG concentrations were determined in appropriately diluted samples by a sandwich ELISA using micro-titer plates and goat IgA, IgM, and IgG ELISA quantitation kits (BlueGene Biotech). The ELISA procedure was conducted to according to the protocol of the manufacturer and absorbance was measured at 450 nm. The concentrations of IgA, IgM, and IgG were determined using standard curves constructed from respective Ig standards run on the assay micro-titer plate and were expressed as milligrams of IgA, IgM, or IgG per milliliter of plasma.

6. Statistical analyses

Data were analysed using the GLM procedure of SAS version 9.1 (2002-2003 by SAS Institute Inc., Cary, NC). The cross-over design was included in the statistic model with the main effects being (1) different combinations of diet, and (ii) different levels of quercetin. The individual goat was considered as the experimental unit for all measurements. The main effects were assessed by least-squares analysis and a 2-factor linear model with all interactions was used to evaluate responses. Statistical significance was accepted at $p<0.05$. Pair-wise comparisons between means were made using Fisher's-protected LSD test when appropriate.

Table 3. Effect of a diet supplemented with dietary quercetin and roughage and concentrates ratio (RC ratio) on nutrient utilization in Korean indigenous goats

Item	Treatments[1]									SEM[2]	P-value[3]		
	RC 30			RC 50			RC 70				RC	QU	RC×QU
	QU 0	QU 500	QU 1000	QU 0	QU 500	QU 1000	QU 0	QU 500	QU 1000				
Dry matter, %	64.10	63.96	64.53	61.95	61.46	62.39	63.32	62.96	63.41	0.98	NS	NS	NS
Crude protein, %	67.66^{b}	67.72^{b}	68.44^{b}	69.33^{b}	68.45^{b}	68.46^{b}	73.49^{a}	73.81^{a}	73.80^{a}	0.68	*	NS	NS
Crude fat, %	74.98	75.18	75.67	75.66	76.77	77.01	77.51	76.67	76.13	0.65	NS	NS	NS
NDF4,%	58.21	57.83	58.77	57.71	56.82	58.42	60.79	59.84	60.75	1.32	NS	NS	NS
ADF5,%	48.03^{b}	48.02^{b}	47.13^{c}	50.91^{b}	50.16^{b}	51.10^{b}	56.41^{a}	56.32^{a}	57.77^{a}	1.52	*	NS	NS

[ab] Means in the same row with different superscripts differ in RC ($P<0.05$)

[1] Treatments: RC 30 (300 g/kg roughage: 70 g/kg concentrate), RC 50 (500 g/kg roughage: 500 g/kg concentrate), RC 70 (700 g/kg roughage: 300 g/kg concentrate), QU 0 (dietary quercetin 0 mg/kg), QU 500 (dietary quercetin 500 mg/kg), QU 1000 (dietary quercetin 1000 mg/kg)

[2] Pooled standard error of mean

[3] Significance level: NS: Not significant, * $P<0.05$

[4] Neutral detergent fiber

[5] Acid detergent fiber

Ⅲ. RESULTS

All the animals were performed well and no symptoms of illness observed during the entire experiment period.

The feed samples of the three different diets (based on roughages to concentrate ratio; RC) were analyzed and the ingredient and chemical composition are presented in Table 1. Dietary treatments both RC ratio and quercetin had no effect on feed intake, water intake, urine excretion and feces elimination in Korean indigenous goats (Table 2). In addition, no interaction between RC ration and dietary quercetin were observed (Table 2).

Nutrient utilization in Korean indigenous goats fed with different level of dietary quercetin supplemented diets is presented in Table 3. Utilizations of dry matter, crude fat, NDF and were not affected (p>0.05) by neither RC ratio nor dietary quercetin. However, higher RC ratio increased N retention and N retention rate in Korean indigenous goats (p<0.05; Table 4). Nonetheless, roughages to concentration ratio was affect (p<0.05) on the crude protein and ADF utilization of Korean indigenous goats, although it was not affected other nutrients (i.e., DM, CF and NDF) utilization (Table 3). Total VFA, acetic acid, propionic acid, iso-butyric acid, butyric acid, iso-valeric acid and valeric acid contents were not affected by dietary quercetin (Table 5). Nevertheless, higher RC ratio increased (p<0.05) acetic acid molar proportion and acetic acid to propionic acid ratio (Table 5). Higher RC ratio decreased total cholesterol level (p<0.05), however neither RC ratio nor dietary quercetin had no effect on the other plasma parameters (Table 6). Furthermore, either RC or dietary quercetin or the interaction between those factors had no effect (p>0.05) on immune responses in Korean indigenous goats (Table 7).

Ⅳ. DISSCUSSION

The present study determined the effect of dietary quercetin supplementation, along with roughage to concentrate ratio on feed intake, nitrogen utilization, ruminal characteristics, plasma parameters and immune responses in Korean indigenous goats.

In this study, diets were formulated with three different roughages to concentrate ratios (i.e., 3:7, 5:5. 7:3), and barley and timothy hay were used as source of roughages in the current study. However, minor variations of chemical composition were observed (see Table 1). In this light, such differences might not affect our major outcomes observed in the study. Previous studies demonstrated that variations between chemical composition of experimental diets might be possible when analyzed the nutrition profiles in total mixed ration (Nienaber, 2008; Dung et al., 2014; Benavides et al., 2013). The crude fat level was numerically higher in RC 30 where it places second and third in RC 50 and RC 70, respectively. This crude fat level might influence with the

Table 4. Effect of a diet supplemented with dietary quercetin and roughage and concentrates ratio (RC ratio) on nitrogen utilization in Korean indigenous goats

Item	Treatments[1]									SEM[2]	P-value[3]		
	RC 30			RC 50			RC 70				RC	QU	RC×QU
	QU 0	QU 500	QU 1000	QU 0	QU 500	QU 1000	QU 0	QU 500	QU 1000				
Total N intake, g/day	10.11	10.11	10.11	9.64	9.83	9.64	10.58	10.58	10.58	0.61	NS	NS	NS
Feces N loss, g/day	3.25	3.24	3.20	2.96	3.09	3.03	2.81	2.75	2.79	0.18	NS	NS	NS
Urine N loss, g/day	5.80	5.01	5.17	4.18	4.22	4.62	3.96	3.94	4.49	0.44	NS	NS	NS
Retained N, g/day	1.78^{b}	1.85^{b}	1.74^{b}	2.51^{ab}	2.51^{ab}	2.00^{ab}	3.81^{a}	3.89^{a}	3.30^{a}	0.51	*	NS	NS
N retention rate, %	17.06^{b}	17.28^{b}	17.03^{b}	25.96^{ab}	25.44^{ab}	20.27^{ab}	35.56^{a}	35.63^{a}	31.31^{a}	4.11	*	NS	NS

^{ab} Means in the same row with different superscripts differ in RC (P<0.05)

[1] Treatments: RC 30 (300 g/kg roughage: 70 g/kg concentrate), RC 50 (500 g/kg roughage: 500 g/kg concentrate), RC 70 (700 g/kg roughage: 300 g/kg concentrate), QU 0 (dietary quercetin 0 mg/kg), QU 500 (dietary quercetin 500 mg/kg), QU 1000 (dietary quercetin 1000 mg/kg)

[2] Pooled standard error of mean

[3] Significance level: NS: Not significant, * P<0.05

Table 5. Effect of a diet supplemented with dietary quercetin and roughage and concentrates ratio (RC ratio) on ruminal characteristics in Korean indigenous goats

Item	Treatments[1]									SEM[2]	P-value[3]		
	RC 30			RC 50			RC 70				RC	QU	RC×QU
	QU 0	QU 500	QU 1000	QU 0	QU 500	QU 1000	QU 0	QU 500	QU 1000				
pH	6.14	6.17	6.18	5.91	6.03	5.97	5.84	5.88	5.83	0.07	NS	NS	NS
Total VFA mM/L	88.57	91.82	88.35	88.25	88.02	90.41	89.95	89.07	90.29	12.07	NS	NS	NS
Acetic acid molar %	38.61b	38.04^{b}	38.92^{b}	39.77^{ab}	40.35^{ab}	40.58^{a}	41.59^{a}	41.56^{a}	42.46^{a}	0.87	*	NS	NS
Propionic acid molar %	32.41	32.81	31.91	30.52	29.12	30.29	30.51	32.28	30.56	1.01	NS	NS	NS
Iso-butyric acid molar %	1.66	1.78	1.87	1.58	1.58	1.53	1.97	1.94	1.91	0.12	NS	NS	NS
Butyric acid molar %	21.94	22.08	22.12	22.38	23.88	22.75	20.92	18.98	20.11	1.11	NS	NS	NS
Iso-valeric acid molar %	2.20	2.07	2.16	2.03	2.09	2.05	2.14	2.25	2.08	0.15	NS	NS	NS
Valeric acid molar %	3.17	3.23	3.02	2.73	2.98	2.80	2.87	2.98	2.89	0.14	NS	NS	NS
Acetic acid/Propionic acid	1.20^{b}	1.17^{b}	1.25^{b}	1.31^{ab}	1.40^{a}	1.36^{ab}	1.38^{a}	1.30^{ab}	1.41^{a}	0.04	*	NS	NS

[ab] Means in the same row with different superscripts differ in RC (P<0.05)

[1] Treatments: RC 30 (300 g/kg roughage: 70 g/kg concentrate), RC 50 (500 g/kg roughage: 500 g/kg concentrate), RC 70 (700 g/kg roughage: 300 g/kg concentrate), QU 0 (dietary quercetin 0 mg/kg), QU 500 (dietary quercetin 500 mg/kg), QU 1000 (dietary quercetin 1000 mg/kg)

[2] Pooled standard error of mean

[3] Significance level: NS: Not significant, * P<0.05

Table 6. Effect of a diet supplemented with dietary quercetin and roughage and concentrates ratio (RC ratio) on plasma parameters in Korean indigenous goats

Item	Treatments[1]									SEM[2]	P-value[3]		
	RC 30			RC 50			RC 70				RC	QU	RC×QU
	QU 0	QU 500	QU 1000	QU 0	QU 500	QU 1000	QU 0	QU 500	QU 1000				
Creatinine, mg.dL	1.03	0.91	0.98	0.97	1.02	1.06	0.99	1.07	1.02	0.05	NS	NS	NS
Alkaline phosphate, U/L	531.2	446.4	538.4	456.4	494.4	466.2	478.4	481.6	456.4	60.96	NS	NS	NS
Glucose, mg/dL	73.33	71.78	77.33	73.67	70.56	72.44	69.56	72.00	73.00	3.28	NS	NS	NS
Total cholesterol, mg/dL	86.44^{a}	84.00^{a}	84.89^{a}	83.78^{a}	80.67^{a}	85.44^{a}	67.89^{b}	72.67^{b}	70.56^{b}	4.85	*	NS	NS
Blood urea nitrogen, mg/dL	15.33	14.89	15.78	14.22	14.11	14.89	13.78	13.56	13.67	0.91	NS	NS	NS
Total bilirubin, mg/dL	0.42	0.44	0.41	0.43	0.42	0.42	0.48	0.44	0.44	0.03	NS	NS	NS
GOT4,U/L	62.33	72.89	63.22	70.11	63.89	58.89	65.89	65.11	74.11	9.85	NS	NS	NS
GPT5,U/L	14.00	13.67	17.50	12.50	11.80	15.33	15.00	16.29	14.80	2.83	NS	NS	NS
Total protein, g/dL	6.33	6.40	6.12	6.27	6.38	6.41	6.48	6.58	6.29	0.18	NS	NS	NS
Albumin, g/dL	3.26	3.18	3.27	3.22	3.30	3.24	3.28	3.28	3.21	0.05	NS	NS	NS
Calcium, mg/dL	11.84	11.86	12.12	11.63	11.80	11.68	12.02	11.62	11.94	0.25	NS	NS	NS

[ab] Means in the same row with different superscripts differ in RC (P<0.05)

[1] Treatments: RC 30 (300 g/kg roughage: 70 g/kg concentrate), RC 50 (500 g/kg roughage: 500 g/kg concentrate), RC 70 (700 g/kg roughage: 300 g/kg concentrate), QU 0 (dietary quercetin 0 mg/kg), QU 500 (dietary quercetin 500 mg/kg), QU 1000 (dietary quercetin 1000 mg/kg)

[2] Pooled standard error of mean

[3] Significance level: NS: Not significant, * P<0.05

[4] Glutamate exaloacetate transaminase

[5] Glutamate pyruvate transaminase

concentrate level, as crude fat level reduced with reducing concentrate level in the experiment diets. Moreover, it was observed high crude fat level in the ration consisted with higher concentrate and lower level in low concentrate rations (Nienaber, 2008). The level of NDF and ADF was improved with the increase of roughage portion in roughage to concentrate mix rations (Nienaber, 2008; Dung et al., 2014). With agreement of this, it was observed positive relation between roughage level with NDF and ADF in mix ration formulated for this experiment.

The effect of diet supplemented with quercetin on feed intake, water intake, urine excretion and feces elimination in Korean indigenous goats were not significant differences among the dietary treatments. Further, no significant difference showed in RC×QU interaction and roughage to concentration ratio. Identically, Cho et al. (2010) reported that no significant effects on feed intake, water intake, urine excretion and feces elimination in Korean indigenous goats fed with diet supplemented with dietary quercetin. Also, different roughage to concentrate ratios did not statistically affect feed intake, water intake, urine excretion and feces elimination in Korean indigenous goats. These observations are in agreement with previous study with Holstein cows (Nienaber, 2008), which observed no significant difference in feed intake with deferent roughage to concentrate ratios.

It was reported (Cho et al., 2010) dietary inclusion of quercetin was not affected on crude fat, NDF and ADF digestibility in Korean indigenous goats. Moreover, it was reported that digestibility of crude protein was improved with dietary quercetin supplementation (Cho et al., 2010). Similarly, dietary quercetin was not affected on dry matter, crude fat, NDF and ADF utilization in this study. Dispute to previous reported reported (Cho et al., 2010), crude protein utilization was not affected in this experiment. The results in our study also showed that dietary quercetin supplementation had no significant difference in dry matter utilization in Korean indigenous goats. This observation is in an agreement with (Benavides et al., 2013) previous observed results with male Merino lambs. Interaction between RC×QU was not affected on nutrition utilization of Korean indigenous goats. It was reported that digestion coefficient of dry matter, crude protein, were improved except NDF and ADF when RC ratio increased in the ration in swamp buffaloes buffaloes (Wanapat and Wachirapakorn, 1990). Nonetheless, higher roughages to concentration ratio was significantly resulted in increased crude protein and ADF utilization of Korean indigenous goats, although it was not affected other measured nutrients (DM, CF and NDF) utilization in the current study.

In this experiment, either quercetin along or RC×QU interaction had no effect on total nitrogen intake, fecal nitrogen loss, urine nitrogen loss, N retention and N retention rate of Korean indigenous goats. Nevertheless, different ratio of roughage and concentrate in rations were significantly affected daily N retention and N retention rate while it was not significant on total N intake, feces N loss and urine N loss in Korean indigenous goats. Similarly, observed nitrogen intake, nitrogen in feces and nitrogen in urine was not significantly affect from forage to concentrate ratio in non-pregnant Granadina goats (Cantalapiedra-Hijar et al., 2009).

It was reported (Cho et al., 2010) rumen total VFA, propionate, acetate to propionate ratio and butyrate significantly increased in Korean indigenous goat fed a diet supplemented with dietary quercetin. Whilst, quercetin supplementation was not made any differences on rumen pH, total VFA, acetic acid-, propionic acid-, iso-butyric acid-, butyric acid-, iso-valeric acid-, valeric acid-molar proportions and acetic acid to propionic acid ratio in Korean indigenous goats. Further, RC ratio was not affected the aforementioned ruminal characteristics except acetic acid molar and acetic acid to propionic acid ratio. In addition, it was examined (Cantalapiedra-Hijar et al., 2009) that significant ruminal pH difference in the Granadina goats fed with different forage: concentration ratios. This could be due to higher lactic acid concentration yield with concentrated diets which consist with high amount of starch (Slyter, 1976). Especially, the evidence found in the current study that ruminal pH level was decreased with lower RC ratio (i.e., close to RC 30).

Addition of dietary quercetin to a diet did not affect the blood parameters except blood urea nitrogen and blood urea nitrogen to creatinine ratio in Korean indigenous goats (Cho et al., 2010). Similarly, in this experiment, it is postulated the plasma parameters were not affected by the dietary quercetin supplementation to a diet. Moreover, interactive effects between roughage and dietary quercetin on the plasma

parameters of Korean indigenous goats were non-significant in the current study. Similar to our results, Santra and Pathak (2000) reported that protein, albumin and globulin in blood were similar in the nine months old cross breed calves fed a diet with different RC ratios. Contrasting to our results, previous study with lactating dairy cows demonstrated that negative relationship between plasma cholesterol and amount of concentrate feed in a diet (Zebeli et al., 2011).

Immune responses are strongly relating with the nutrition status of the animal (Benavides et al., 2013; Carroll and Forsberg, 2007). Further, phenolic like substances which incorporate to animal diets are showed the immune-modulator properties (Benavides et al., 2013). However, it was documented that quercetin supplementation in a diet had no effect to antibody production in host animals (Exon et al., 1998; Sforcin et al., 2005; Benavides et al., 2013). Similarly, Ig G, Ig A, and Ig M levels were not affected with RC ratio, QU level to Korean indigenous goats in the current study. This is an agreement with previous calves' study (Santra and Pathak, 2000) that was demonstrated that no differences in immune responses of calves when those fed a diet with different ratio of roughages to concentrates. Previous studies reported (Berger et al., 2012; Benavides et al., 2013), incorporation of dietary quercetin in to ruminant diet had no significant effects due to its lower bio-availability compared to when it fed to monogastrics. Although clear explanations are not documented, it may be the reason for our observed results throughout the study that we found no effect on immune responses in Korean indigenous goats.

Ⅴ. CONCLUSIONS

In conclusion, Korean indigenous goats fed a diet supplemented with dietary quercetin commensurate with different RC ratio had no significant direct effect on the productivity and health status indices. However, further investigation is warranted to determine the effects of higher dietary quercetin on direct or indirect impact of antioxidant activity, an animal disease resistant and subsequent their economic influences in production animals, where the use of antimicrobial growth promoters is restricted as for a substitution of antibiotics.

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