動科論叢(12) : 77~87

Effects of Feeding Diets Containing Alfalfa, Nori or Pine-needle Meals on Cecal Size, Cecal Urease Activity and Serum Urea Concentration in Male and Female Rats

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알팔파, 김 또는 솔잎 분말을 함유한 사료의 급여가 쥐의 성별, 맹장 크기, 맹장 요소분해 효소 활성 및 혈청 요소수준에 미치는 영향

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요 약

이 연구는 알팔파, 김 또는 솔잎 분말을 함유한 사료의 급여가 쥐의 성별 맹장 크기, 맹장 요소분해 효소 활성 및 혈청 요소수준에 미치는 영향을 구명하기 위하여 실시되었다. 24마리의 암쥐와 24마리의 숫쥐(평균체중 92g)를 이용하여 사료 처리당 암쥐 6마리와 숫쥐 6마리를 2(성) × 4(사료) 요인 배치하였다.대조사료와 건조·분쇄한 알팔파, 김 또는 솔잎분말을 대조사료중의 옥수수 전분과 10%대치하여 만든 사료를 4주동안 급여하였다. 사양실험이 끝난 후 쥐들을 도살하여 혈청 요소함량과 맹장 내용물 중 요소 분해효소 활성을 각각 측정하였다. 일일 평균 사료섭취량(ADFI)과 일당 평균증체량(ADG)은 김을 섭취한 쥐들이 알팔파나 솔잎을 급여한 쥐들보다 높았다($P < .05$). 숫쥐가 암쥐에 비해 ADFI와 ADG는 많았고($P < .001$), 사료/증체(F/G)는 낮았다. 총 맹장무게 및 맹장내용물의 무게는 김이나 솔잎을 급여한 쥐가 대조구나 알팔파를 급여한 쥐보다 증가하였다.($P < .001$). 맹장내용물 g당 요소분해 효소 활성은 처리간에 유의차를 보이지 않았다. 숫쥐에서 암쥐에 비해 총 맹장내용물중의 요소분해 효소 활성이 높았다($P < .01$). 혈청 요소 수준은 암쥐가 숫쥐에 비해 현저히 높았으며($P < .001$), 대조구에 비해 솔잎 급여구에서 현저히 높았다($P < .05$). 이 실험결과는 김이나 솔잎에는 가용성 섬유질이 함유되어 있어 발효과정에서 맹장의 크기를 증가시키며 요소분해 효소 활성을 감소시키기 때문에 정장작용의 가능성이 있으나, 솔잎의 경우 다른 부작용이 우려되기 때문에 건강식품으로서의 이용성에 대해서는 더 검토가 필요하다. 또한 숫쥐가 암쥐보다 총 맹장내용물 중 요소분해 효소 활성이 높은 것은 흥미로우나 이에 대한 추가적인 연구가 필요하다고 하겠다. 암쥐에서 숫쥐에 비해 혈청 요소수준이 높은 이유는 암쥐가 숫쥐보다 단위 체중당 단백질을 더 많이 섭취했기 때문으로 사료된다.

(Key words : rats, urease activity, ammonia production, cecal size, alfalfa, nori, pine-needle)

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Ⅰ. INTRODUCTION

A variety of health-promoting food sources have been used in Korea as well as in other countries without knowing their specific effects. These sources include alfalfa, nori and pine-needles, which are used in forms such as food additives, pills or drinks, and contain significant amounts of different types of fibers. Dietary fiber, especially soluble fiber, is fermented, producing lactic acid and volatile fatty acids(VFA) in the large intestine of nonruminant animals including humans. Dietary cellulose increases bulk and the amount of water in intestinal contents, decreasing transit time and thus the concentration of toxic substances in contact with the intestinal mucosa(Kelsay, 1978).

One of the benefits of increasing soluble fiber in the diet is to reduce urease activity in the intestine(Wrong et al., 1981). The enzyme urease is produced by certain intestinal bacteria and serves an essential role in the process of urea recycling by catalyzing the conversion of urea to ammonia. Ammonia produced by the microbial urease may be used for microbial protein synthesis or be absorbed into the blood stream, and it is known to be toxic to animals(Visek, 1978). Pigs fed a high fiber diet have lower concentrations of ammonia-N in fecal, cecal and colonic samples(Varel, 1984), and rats fed a high cellulose diet had a lower cecal urease activity(Lee, 1992), compared to a control value. Lactulose and other fermentable substrates have been known to decrease ammonia production in a fecal incubation(Vince et al., 1978).

Urease-producing bacteria inhabiting the GI tract are considered to be important in both nutritional and pathological aspects because they are involved in nitrogen recycling(which wastes energy for urea resynthesis) and the resulting product ammonia can be harmful to animal and human health. The growth-promoting effects of subherapeutic-level antibiotics(Visek, 1978) or probiotics(Kim and Kim, 1992; Yeo and Kim, 1997) used in animal feeds have been ascribed to suppression of urea hydrolysis and subsequently reduced ammonia production in the GI tract. Urease immunization(Visek, 1978) or dietary urease inhibitors(Whitelaw et al., 1991) have also been suggested as a means of improving growth of farm animals.

Many ingredient used as health foods contain high levels of fiber which may influence microflora in the GI gract. Therefore, our studey was conducted to determine the effect of feeding diets containing alfalfa, nori or pine-needle meals on cecal size, cecal urease activity and serum urea concentration in male and female rats.

II. MATERIALS AND METHODS

1. Animals and diets

Twenty-four male and twenty-four female Sprague Dawley rats(mean initial weight, 92g) were housed individually in suspended wire cages in a room maintained at 20~25℃ with a 12-h light(07:00 to 19:00) and 12-h dark(19:00 to 07:00) cycle. Six male and six female rats were assigned to each of the following diets : a control diet or diets containing 10% alfalfa, 10% nori or 10% pine-needle meal(2 × 4 factorial design). The composition of the experimental diets is shown in Table 1. Diets and water were provided for ad libitum consumption.

After a 5-day adjustment period, during which a commerical chow was fed, rats were fed experimental diets for four weeks. Feed consumption and body weight were recorded every 2 d during the 4-week period.

Table 1. Composition of experimental diets(% on an air-dry basis)

Ingredient	Control	Alfalfa	Nori	Pine-needle
Casein[a]	20.0	18.0	18.0	20.0
L-methionine[a]	.3	.3	.3	.3
Corn oil[b]	5.0	5.0	5.0	5.0
Lard[c]	5.0	5.0	5.0	5.0
Choline chloride[a]	.2	.2	.2	.2
Vitamin mix[d]	1.0	1.0	1.0	1.0
Salt mix[d]	3.5	3.5	3.5	3.5
Cholic acid[a]	.2	.2	.2	.2
Sucrose[b]	20.0	20.0	20.0	20.0
Cholesterol[e]	.5	.5	.5	.5
Corn starch[f]	44.3	36.3	36.3	34.3
Alfalfa meal[g]	-	10.0	-	-
Nori meal[h]	-	-	10.0	-
Pine-needle meal[i]	-	-	-	10.0
Total	100.0	100.0	100.0	100.0

[a]United States Biochemical Co., Cleveland, Ohio.
[b]Jeil Jedang Co., Seoul, Korea.
[c]Samlip Yugi Co., Seoul, Korea.
[d]AIN-76A, Harlan, Madison, WI.
[e]Fluka Chemie, Switzerland.
[f]Sunil Pododang Co., Seoul, Korea.
[g]Alfalfa(Medicago sativa L.) containing 22.5% CP, 42.3% NDF and 29.4% ADF dried and ground.
[h]Nori(Porphyra spp., Dol-ghim) containing 22.1% CP, 45% NDF and 4.6% ADF dried and ground.
[i]Pine-needle(Pinus densiflora S.) containing 7.12% CP, 60.4% NDF and 43.5% ADF dried and ground.

2. Sample collection and incubation of cecal contents

At the end of the feeding experiment, rats were fasted for 12 hours and eight rats from different treatments were killed daily between 10:00 and 12:00 until all of the experimental rats were used.(The 12-hour fasting was carried out to increase the consistency in serum urea levels.) Blood samples were centrifuged at 3,000 × g for 15min and the serum stored at -20℃ for later analysis. Cecal contents were collected in 50-㎖ centrifuge tubes, weighed and diluted 1:4(w/v) with 0.2M phosphate buffer(pH 6.8). The cecal tissue was flushed clean with tap water, blotted on a paper towel and weighed(cecal wall weight). Duplicate 1-㎖ samples of diluted contents were transferred into 15-㎖ centrifuge tubes and 0.34㎖ of 0.44M urea solution was added to one sample, while 0.34㎖ distilled water was added to the other sample and this mixture was inactivated with 0.134㎖ of 6 N H_2SO_4 before incubation and this sample served as a blank. The former was incubated at 37℃ in a shaking water bath while being flushed with N_2 for the first 1min and then each unit was clamped sealed. The incubation was initiated within about 10min after rats were killed.

At the end of the 30-min incubation, an air stream was pulled through the reaction chamber, and 0.134㎖ of 6N H_2SO_4 was added to the inlet tube of the reaction chamber to stop the reaction. The inactivated samples were centrifuged at 3,000 × g for 15min and supernatants were collected into plastic vials and immediately stored at -20℃ for later analysis.

3. Determination of ammonia and urease activity

Ammonia concentrations in the 3,000 × g supernatants stored at -20℃ were determined using a colorimetric method(Chaney and Marbach, 1962). Net ammonia production during the 30-min incubation was calculated by the difference in ammonia concentrations between the blank and incubated sample, and was expressed as ㎛ol/30min per g collected contents or per total contents. Urese activity was calculated from net ammonia production, which was divided by a factor of two because one molecule of urea produces two molecules of ammonia upon hydrolysis.

Urea concentration in the plasma was determined using a colorimetric method(Chaney and Marbach, 1962). Two-hundred ㎕ of urease solution(urease 0.9g/L plus EDTA 0.4g/L) and 20㎕ of the plasma sample were mixed and

incubated at 37℃ for 15min, and the resulting ammonia concentration(㎛mol) was determined using the method described for determination of cecal ammonia concentration and urea concentration(㎛mol) was calculated by dividing ammonia concentration by two.

4. Statistical analysis of experimental data

Statistical analyses were carried out using the SAS package(SAS, 1988). Analyses of variance(ANOVA) were calculated in a factorial design(Table 2). Duncan's multiple range test was used to compare mean values of individual treatments, when the F-value was significant($P < .05$).

Table 2. Analysis of variance(ANOVA) of the data

Item	MS		
	Treat	Sex	Treat × Sex
Final Weight, g	1,370.93**	122,937.76**	570.02
ADFI, g	6.05*	264.09***	.64
ADG, g	1.66**	145.60***	.60
F/G	.34**	14.14**	.36**
Total Cecal Weight, g	3.47***	3.57**	.37
Cecal Wall Weight, g	.07**	.09*	.02
Cecal Contents, g	2.10**	3.46**	.47
Ammonia Production, ㎛mol			
Before incubation	.05**	.0001	.0005
After incubation	8.36***	.002	.215
Net production	7.66***	.005	.218
Urease Activity, ㎛mol			
per g contents	.174***	.0001	.004
per total contents	.037	.186**	.011
Plasma urea, mg/dl	2.05*	29.0***	.42

* $P < .05$.
** $P < .01$.
*** $P < .001$.

III. Result and Discussion

Rats fed the control or nori meal diets had higher($P < .01$) average daily feed intake and average daily gain, and lower feed/gain ratio(F/G) than rats fed diets

Table 3. Effects of feeding diets containing alfalfa, nori or pine-needle meal on body weight gain and feed efficiency

Item	Control			Alfalfa			Nori			Pine-needle			Sex class		SE
	male	female	mean	male	female	mean	male	female	mean	male	female	mean	male	female	
Initial weight, g	93.9	91.6	92.7	94.9	89.7	92.2	94.9	91.2	93.1	95.3	90.3	92.7	94.7	90.6	4.29
Final weight, g	307.1	213.8	260.4^{ab}	305.2	194.3	249.7^{b}	311.3	225.2	268.2^{a}	301.8	187.1	244.4^{b}	306.3^{A}	205.1^{B}	7.52
ADFI, g	18.9	14.9	16.9^{ab}	19.3	14.3	16.7^{b}	20.2	15.7	17.9^{a}	18.9	13.8	16.3^{b}	19.3^{A}	14.6^{B}	.53
ADG, g	7.6	4.4	6.0^{ab}	7.5	3.7	5.6^{bc}	7.7	4.8	6.2^{a}	7.4	3.5	5.4^{c}	7.5^{A}	4.0^{B}	.21
F/G[1]	2.5	3.4	2.9^{b}	2.6	3.8	3.2^{a}	2.6	3.3	3.0^{b}	2.6	4.0	3.3^{a}	2.5^{A}	3.6^{B}	.08

Values are means of 6 rats and pooled standard error(SE).
^{abc}Values in the same row sharing no common superscripts differ signficantly($P < .05$).
^{AB}Significant differences($P < .05$) between sexes.
[1]Significant($P < .01$) interactions between treatment and sex.

Table 4. Effects of feeding diets containing alfalfa, nori or pine-needle meal on cecal size and cecal contents

Item	Control			Alfalfa			Nori			Pine-needle			Sex class		SE
	male	female	mean	male	female	mean	male	female	mean	male	female	mean	male	female	
Total cecal weight,g	1.82	1.63	1.72^{bc}	1.47	1.10	1.28^{c}	2.50	1.87	2.18^{ab}	3.01	2.01	2.57^{a}	2.20^{A}	1.65^{B}	.20
Cecal wall weight,g	.57	.55	.56^{bc}	.60	.46	.53^{c}	.65	.65	.65^{ab}	.80	.60	.70^{a}	.65^{A}	.56^{B}	.04
Cecal contents, g	1.25	1.08	1.17^{bc}	.86	.63	.75^{c}	1.83	1.05	1.44^{ab}	2.21	1.25	1.73^{a}	1.54^{A}	1.09^{B}	.18

Values are means of 6 rats and pooled standard error(SE).
^{abc}Values in the same row sharing no common superscripts differ signficantly($P < .05$).
^{AB}Significant differences($P < .05$) between sexes.

containing alfalfa or pine-needle meal(Table 3). It is not surprising that alfalfa and pine-needle meals in the diet depressed growth because they contained 29.4% and 43.5% acid detergent fiber ADF, respectively, as shown in Table 1. Feeding diets supplemented with alfalfa or pine-needle meal reduced feed intake, indicating that these ingredients are not palatable.

Over the feeding period, male rats showed much higher($P < .01$) average daily gain and average feed intake, and lower($P < .01$) F/G than females, because the mature size of females is smaller than that of males, and the feeding experiment was extended over maturity.

Significant($P < .01$) differences were noted in the weight of the cecum(tissue + contents) and cecal contents between sexes as well as between dietary groups, showing that feeding nori or pine-needle meal increases the weight of the cecum and its contents compared to that found in the control or rats fed the alfalfa meal diet. Higher body weights of male rats were considered to be a major factor for the larger cecum sizes in males than in females. Younes et al.(1995) reported that when 7.5% fiber or oligosaccharide was substituted for the same amount of wheat starch in a semipurified diet, oat fiber did not cause an enlargement of the cecum, but gum arabic and oligosaccharides elicited a 35~60% enlargement of the cecum. Results of Younes et al.(1995) indicated that fermentable fiber enlarge the cecum because fermentation products, volatile fatty acids(especially bytyrate) stimulate cell proliferation. In the present study, rats fed diets containing nori or pine-needle meal had larger ceca and more contents, indicating that fiber in these sources are more fermentable than those in alfalfa meal.

Net ammonia production(or urease activity), serum urea and fecal nitrogen concentrations are presented in Table 5. Ammonia concentration(μmol of ammonia per g collected contents) before incubation was higher($P < .05$) in the control than in the other groups and no significant differences were found between sexes. Urease activity(1/2 × μmol of ammonia produced/30 min per g collected contents) in rats fed diets containing nori or pine-needle meal was much lower($P < .01$) than that in rats fed the control or alfalfa meal diet, but was not different between sexes. Total urease activity in the cecal contents was not significantly different between the dietary treatments, although it tended to be lower in rats fed nori or pine-needle meal than in the control or rats fed alfalfa meal. Interestingly, male rats showed higher($P < .01$) total urease activities in the contents but lower($P < .01$) plasma urea concentrations than did female rats. No significant differences in plasma urea concentrations were found between dietary

Table 5. Effects of feeding diets containing alfalfa, laver or pine-needle meal on ammonia production and urease activity in the cecal contents

Item	Control			Alfalfa			Nori			Pine-needle			Sex class		SE
	male	female	mean	male	female	mean	male	female	mean	male	female	mean	male	female	
Ammonia production[1]															
Before incubation	.44	.46	.45[a]	.35	.35	.35[b]	.29	.29	.29[b]	.34	.33	.33[b]	.35	.35	.03
After incubation	2.54	2.58	2.56[b]	3.34	2.94	3.13[a]	1.41	1.51	1.47[c]	1.35	1.56	1.46[c]	1.16	2.14	.25
Net production	2.10	2.12	2.11[b]	2.98	2.58	2.78[a]	1.12	1.21	1.17[c]	1.00	1.24	1.12[c]	1.80	1.79	.24
Urease activity[2]															
per g contents	.32	.32	.32[b]	.44	.38	.41[a]	.16	.17	.17[c]	.15	.18	.16[c]	.27	.26	.03
per total contents	.41	.34	.37[a]	.38	.23	.30[a]	.34	.14	.24[a]	.31	.23	.27[a]	.36[A]	.23[B]	.05
Plasma urea, mg/dl	8.8	13.5	11.1[b]	11.1	15.3	13.2[ab]	8.5	15.1	11.7[ab]	11.9	16.1	14.0[a]	10.0[A]	15.0[B]	1.20

Values are means of 6 rats and pooled standard error(SE).
[abc]Values in the same row sharing no common superscripts differ signficantly($P < .05$).
[AB]Significant differences($P < .05$) between sexes.
[1]μmol of ammonia per g contents.
[2]μmol of urea hydrolyzed/30 min at 37°C.

treaments. Feeding a diet containing fiber in rats(Lupton and Marchant, 1989) or a diet containing lactulose in humans(Weber and Veach, 1979) decreased urease activity or ammonia production in the GI tract. Bacterial urease activity could be inhibited by a high ammonia concentration in a medium, as shown with ureolytic ruminal bacteria(Cheng and Wallace, 1979). If oligosaccharides were present in the diet at a relatively low level together with other soluble and insoluble fiber, they could be useful in stimulating urea diffusion and ureolysis in the large intestine without inducing excessive nitrogen recycling(Younes et al, 1995). In the present study, feeding diets containing nori or pine-needle meal decreased urease activity(per g collected contents) compared to that found in the control or rats fed the alfalfa diet due primarily to dilution of cecal contents by soluble fiber.

A significant difference in blood urea concentration was found between male and female rats in the present study, perhaps because F/G was much lower($P < .01$) in males than in females and thus female rats consumed more protein per unit body weight than did male rats. Similarly, Tsuchiya et al.(1995) reported that blood urea nitrogen levels in female rats were higher than in male rats.

In conclusion, results of our study indicated that dietary nori or pine-needle meal increases cecal size and decreases cecal urease activity, perhaps due to their soluble fiber contents. The higher total urease activity in the cecal contents of male than that of female rats is interesting, but its mechanism is yet to be studied. The effect of sex on blood urea concentration is considered due to different amounts of protein intake per unit body weight.

IV. Summary

A study was conducted to investigate the effect of feeding diets containing alfalfa, nori or pine-needle meal on cecal size and cecal urease activity in male and female rats. Six male and six female Sprague Dawley rats(initial mean weight, 92g) were assigned to each of the following diets : a control diet or diets containing 10% alfalfa, nori or pine-needle meal. All the diets contained 5% corn oil and 5% lard. After a 4-week feeding period, rats were killed and cecal contents and blood samples were collected. Rats fed the control or nori meal diet had higher($P < .01$) average daily feed intake(ADFI) and average daily gain(ADG) and lower feed/gain ratio(F/G) than rats fed diets containing alfalfa and pine-needle meals. Male rats showed much higher($P < .01$) average daily gain, and lower($P < .01$)

F/G than females. Ammonia concentration(μmol ammonia per g collected contents) in the cecum(blank or before incubation) was higher($P < .05$) in the control than in the other groups. Urease activity in a g of cecal contents of rats fed diets containing nori or pine-needle meal was much lower($P < .01$) than that of rats fed the control or alfalfa meal diet, but was not different between sexes. However, total urease activity in the contents was not significantly different between the dietary treatments. Interestingly, male rats showed higher($P < .01$) total urease activity in the contents but lower($P < .01$) plasma urea concentration than did female rats. Results of our study indicated that dietary nori or pine-needle meal increases cecal size and decreases cecal urease activity perhaps due to soluble fiber present in the ingredients. The higher total urease activity in the cecal contents of male than that of female rats is interesting, but its mechanism is yet to be studied. The effect of sex on blood urea concentration is considered due to different amounts of protein intake per unit body weight.

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