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Effect of Dietary Supplementation of Allium Hookeri Root and Processed Sulfur on the Growth Performance of Guinea Pigs

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***Abstract*—**This study investigated the effects of the dietary supplementation of the *Allium hookeri* root, and processed sulfur, on the growth performance of guinea pigs. The guinea pigs were fed a control diet (CON), as well as the control diet including 1% freeze-dried *Allium hookeri* root (AH), or 0.1% processed sulfur (S), or including both the freeze-dried *Allium hookeri* root and the processed sulfur (AHS). The weight of perirenal adipose tissue (PAT) and the epididymal adipose tissue (EAT) in the AH were significantly lower than CON ($p < 0.05$). The serum cholesterols levels of the AH and the AHS were significantly lower than the S ($p < 0.05$). While the total saturated fatty acid content in the serum of the AH and AHS groups showed a tendency to decrease, the total monounsaturated fatty acid increased. The results of this study suggested that dietary consumption of *Allium hookeri* root may help to decrease fat accumulation, lower serum cholesterol levels, and control serum free fatty acid contents in the guinea pigs.

Keywords*—Allium hookeri*, processed sulfur, dietary supplementation, growth performance, guinea pig.

I. Introduction

THERE are many diseases caused or exacerbated by obesity, so many studies have investigated foods for the prevention of obesity [1]. Some functional foods have been approved for obesity prevention [2]. Functional foods such as plants rich in phenolic compounds and organic sulfur compounds help to decrease metabolic syndrome by reducing cholesterol, body weight, and triglycerides [3]. Sulfur is a mineral that has been associated with improvement of cardiovascular functions, as well as being an anti-oxidant; it also has anti-obesity abilities, has been used in oriental medicine for neural paralysis and cold extremity treatment, and in western medicine to treat constipation, hemorrhoids, and to support homeostasis [4], [5]. However, sulfur is highly toxic, which means that purification is necessary for it to be used as a dietary supplement [6]. There are several methods of purification [7], such processing the sulfur by heating and melting it over a material or light mineral, and then separating the liquid sulfur from the cooled material. Recently, studies were done on animals that were fed processed sulfur, to reveal the positive effect of sulfur on the quality of meat [8], [9]. *Allium hookeri*, belonging to the Allium genus (which includes plants such as garlic, onions, and chives), has been shown to improve the nutritional quality of food [10]. It is used for medical purposes, because of its anticancer and anti-inflammatory properties, and is listed in the medical dictionary of Myanmar [11]. Plants rich in organic sulfur compounds have antioxidant, anticancer, anti-inflammatory and cholesterol-lowering effects; *Allium hookeri* has six times higher than the organic sulfur compound content than garlic, and two times higher than the content of onions cultivated in Korea [12]. Recently, *Allium hookeri* was successfully cultivated in Korea [13]. There are many studies which document the effects of the dietary supplementation of Allium species (such as garlic and onions) but few studies on *Allium hookeri* have been done. Therefore, the aim of this study was to investigate the effect of the dietary supplementation of the *Allium hookeri* root, and processed sulfur, on the growth performance of Guinea pigs.

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II. Materials and Methods

A. Composition of Diet

Allium hookeri root was purchased from Samchaenara Co. (Gyeongsangnamdo, Korea) and freeze-dried at Dongil Cold Storage & Foods Co. (Gyungkido, Korea). Processed sulfur was obtained from Ebada Eco Technology (Seoul, Korea). The freeze-dried *Allium hookeri* root used in this study contained 12.72±0.79% crude proteins, 0.46±0.06% crude fat, 12.24±2.24% crude ash, 1.60±0.16% moisture, and 31.67±0.12% crude fiber. The sulfur used was 99% processed sulfur.

B. Animal Experimental Design

20 6-week old conventional Hartley male guinea pigs weighing between 250 g and 300 g (RaonBio Co., Gyunggido, Korea) were used. During the acclimation period, the guinea pigs were fed cabbage and control diets at the ratio of 5:5, which was gradually reduced by removing the cabbage and introducing the control diets [14]. After a week of acclimation, they were randomly divided into four groups and placed in individual cages. The control group (CON) was fed the control diet. The AH group was fed the control diet, plus 1% *Allium hookeri* root. The S group was fed the control diet, plus 0.1% processed sulfur. The AHS group was fed the control diet, with the addition of both 1% *Allium hookeri* root and 0.1% processed sulfur. The composition of the diets is presented in

Table I. The animals received free access to clean water containing ascorbic acid (500mg/L) [15]. All guinea pigs were maintained under a 12 hour light and dark cycle at a controlled room temperature of 22±2°C, with moisture levels of 50±5%. The experiment was conducted over an 8-week period. The animal experiment was approved by the Konkuk University Institutional Animal Care and Use Committee (IACUC, Approval No. KU14025).

TABLE I
COMPOSITION OF CONTROL DIET (G/KG DIET)

Ingredients	Groups[1)] CON[2)]	AH	S	AHS
Casein, 30 Mesh	200	198	200	198
L-Cystine	3	3	3	3
Corn Starch	397	393	397	393
Maltodextrin 10	132	131	132	131
Sucrose	100	99	100	99
Cellulose	50	50	50	50
Soybean Oil	70	69	70	69
Mineral Mix	35	5	35	5
Vitamin Mix	10	10	10	10
Choline Bitartrate	3	3	3	3
Processed sulfur			1	1
Allium hookeri root		10		10
Total (g)	1,000	1,000	1,000	1,000
Total (kcal)	4,000	4,000	4,000	4,000

1) Con, guinea pigs fed the control diet; AH, guinea pigs fed control diet including 1% *Allium hookeri* root; S, guinea pigs fed control diet including 0.1% sulfur; AHS, guinea pigs fed control diet including both 1% *Allium hookeri* root and 0.1% sulfur.
2) AIN 93 G (Research Diets, INC., New Brunswick, NJ, USA).

C. Initial and Final Weight, Weight Gain, Total Food Intake and Feed Efficiency Ratio

The guinea pigs were weighed before they were divided into random groups, and the final body weight of the guinea pigs was checked before fasting for 24 hours, prior to dissection. Food intake and changes in body weight were measured at the same time every day. The guinea pigs were weighed using an electronic balance (AR2140, Ohaus, Pine Brook, NJ). The feed efficiency ratio (FER) was calculated using:

$$\text{Total food intake (g)} = \text{ration of food (g)} - \text{remaining food (g)}$$

$$\text{FER} = \text{Total body weight gain (g)} / \text{Total food intake (g)}$$

D. Organ Weight

The liver, spleen, kidney, perirenal adipose tissue (PAT), epididymal adipose tissue (EAT) and heart were removed immediately after sacrificing, and weighed after being cleaned with 95% saline solution. The organ weight was presented as the organ to body weight ratio (g/kg body weight) [16].

E. Blood Analysis

The blood was collected with a sterilized-syringe after dissection. The blood was left at room temperature for 3 hours and then centrifuged at 5,000 rpm for 20 min at 4°C to separate the serum. Before analysis, the blood was frozen in a refrigerator set below -20°C. Triglyceride, total cholesterol, HDL-cholesterol and LDL-cholesterol were determined by a chemistry analyzer at GC Lab (Gyunggido, Korea). Measurement of amino acids and fatty acids was performed by the Korean Standards Codex method [17]. After the preconditioning procedure, blood samples were analyzed by an automatic amino acids analyzer (S2100, S4300, S5200, SYKAM, Germany) to measure amino acid content. The free fatty acids were measured using a gas chromatograph (5890, Agilent Technologies, USA) with an SP-2560 column (100 m×0.25 mm×0.2 μm), after preconditioning.

F. Statistical Analysis

A statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA). All data were analyzed using the General Linear Models (GLM). All data are represented as the mean ± standard deviation. Significant differences were determined by the Duncan's multiple range test ($p<0.05$).

III. RESULTS AND DISCUSSION

A. Growth Performances

The growth performances are provided in Table II. The final weight, body weight gain, total food intake, and the FER of the AH and AHS groups were not significantly different from the CON ($p<0.05$). However, the final weight, body weight gain, total food intake, and the FER of the S group was significantly lower than the CON ($p<0.05$). Previous studies reported the decreased body weight of processed sulfur-fed rats [7], [8]. This experiment suggested that processed sulfur decreased the body weight, but *Allium hookeri* and *Allium hookeri* root together with processed sulfur did not affect the body weights of the guinea pigs.

TABLE II
BODY WEIGHT STATISTICS, TOTAL FOOD INTAKE, AND FER

Body weight	Groups[1)] CON	AH	S	AHS
Initial	269.4±22.8[2)]	261.6±15.0	284.2±9.3	273.2±18.1
Final	447.6±36.3[a]	423.0±34.0[ab]	377.0±42.0[b]	469.4±50.4[a]
Body weight gain	178.2±26.5[a]	161.4±24.8[a]	92.8±41.9[b]	196.2±41.2[a]
Total food intake	716.5±67.4[a]	729.5±33.0[a]	613.6±76.2[b]	745.9±84.3[a]
FER (%)	0.23±0.04[a]	0.20±0.02[a]	0.12±0.06[b]	0.26±0.04[a]

1) Con, guinea pigs fed the control diet; AH, guinea pigs fed control diet including 1% *Allium hookeri* root; S, guinea pigs fed control diet including 0.1% sulfur; AHS, guinea pigs fed control diet including both 1% *Allium hookeri* root and 0.1% sulfur.
2) Values in each row with different letters are statistically different ($p<0.05$). All values are expressed as mean ± standard deviation (n=5).

B. Organs and Adipose Tissue

The organ weight and adipose tissue weights were presented in Table III. The weight of the livers in the AH group were significantly higher than that of the S group ($p<0.05$). The weight of the spleens in the AH were significantly lower than that of the CON group ($p<0.05$). Although the weight of the kidneys in all groups was not significantly different from the CON group, the S was significantly higher than AHS ($p<0.05$). The weight of the liver, spleen and kidneys between each group showed some significant differences ($p<0.05$). However, a previous study reported that differences in sulfur concentration did not affect the weight of organs in rats [18]. Therefore, these results also suggested that diets supplemented by *Allium hookeri* root processed sulfur may not directly affect the weight of the organs in guinea pigs. The EAT weight of the AH was significantly lower compared to that of the CON ($p<0.05$). The PAT of the AH was significantly lower than that of the CON ($p<0.05$). This result may suggest that the decreased final weight, body weight gain, total food intake, and FER resulted in reduced adipose tissue weights in the group with the diet supplemented by processed sulfur; however, while the final body weight, body weight gain, total food intake and the FER in the AH and AHS were no different compared to the CON, the weight of the adipose tissue decreased. Previous research reported that processed sulfur-fed chickens had an increase of abdominal fat, but a decrease in the total body fat, because supplementing with sulfur accelerated the lipid metabolism in the chickens [19]. This result may suggest that *Allium hookeri* root may decrease the adipose tissue, although it did not affect the body weight.

TABLE III
WEIGHTS OF LIVER, SPLEEN, KIDNEY, EAT AND PAT

Groups[1)]	CON	AH	S	AHS
Liver	38.8±7.91[ab,2)]	40.6±9.81[a]	29.6±2.46[b]	35.3±4.82[ab]
Spleen	1.7±0.48[a]	1.3±0.12[b]	1.3±0.13[b]	1.2±0.27[b]
Kidney	8.0±1.19[ab]	7.8±0.76[ab]	9.2±1.09[a]	7.4±0.61[b]
Heart	3.4±0.52	3.5±0.59	3.0±0.15	3.3±0.21
Epididiymal fat	4.4±0.27[a]	3.8±0.47[b]	3.0±0.39[c]	4.4±0.50[ab]
Retroperitioneal fat	6.1±1.84[a]	3.0±1.30[b]	4.2±1.89[ab]	5.5±0.91[ab]

1) Con, guinea pigs fed the control diet; AH, guinea pigs fed control diet including 1% *Allium hookeri* root; S, guinea pigs fed control diet including 0.1% sulfur; AHS, guinea pigs fed control diet including both 1% *Allium hookeri* root and 0.1% sulfur.
2) Values in each row with different letters are statistically different ($p<0.05$). All values are expressed as mean ± standard deviation (n=5).

C. Triglyceride and Cholesterol Levels Serum

TABLE IV
TRIGLYCERIDE, TOTAL CHOLESTEROL, HDL-CHOLESTEROL, LDL-CHOLESTEROL ANALYSIS IN SERUM

Groups[1)]	CON	AH	S	AHS
Triglyceride	75.4±18.66[2)]	81.2±33.67	86.00±9.72	72.0±21.39
Total cholesterol	62.2±6.50[b]	61.0±18.11[b]	84.6±24.30[a]	57.6±10.90[b]
HDL-choleste rol	6.8±0.84[b]	5.8±1.64[b]	8.6±2.07[a]	6.4±1.52[b]
LDL-choleste rol	57.2±6.53[b]	52.2±14.86[b]	79.4±24.86[a]	52.8±8.23[b]

1) Con, guinea pigs fed the control diet; AH, guinea pigs fed control diet including 1% *Allium hookeri* root; S, guinea pigs fed control diet including 0.1% sulfur; AHS, guinea pigs fed control diet including both 1% *Allium hookeri* root and 0.1% sulfur.
2) Values in each row with different letters are statistically different ($p<0.05$). All values are expressed as mean ± standard deviation (n=5).

The triglyceride, total cholesterol, HDL-cholesterol, and LDL-cholesterol results are shown Table IV. The serum triglyceride levels in the *Allium hookeri* root treated groups were lower than in the CON. The levels of total cholesterol, HDL-cholesterol, and LDL-cholesterol in the S group were all significantly higher than the other groups ($p<0.05$). However, the levels of total cholesterol, HDL-cholesterol, and LDL-cholesterol in the AH and AHS groups were lower than the CON group (though the difference was not significant). Previous studies on the consumption of foods rich in organic sulfur compounds and polyphenols also reported a cholesterol-lowering effect [20], [21]. This study also indicated that a dietary supplement of *Allium hookeri* root can lower serum cholesterol levels, which is in agreement with previous reports. However, the consumption of processed sulfur increased both the triglyceride and cholesterol levels in blood plasma, which was also reported previously [3], [5].

D. Free Fatty Acids in Serum

Analysis of the free fatty acids analysis in the serum is shown in Table V. Total SFA (saturated fatty acid) in AH showed tendency to be lower than CON. However, the total SFA in S and AHS was less than the detection limit of 30 ppm. Total MUFA (monounsaturated fatty acid) in AH showed a tendency to be higher than CON, while it was significantly higher in S and AHS compared to CON ($p<0.05$). A previous study reported higher levels of SFA and lower levels of MUFA in obese rats [20]. This result suggests that consuming *Allium hookeri* root and/or processed sulfur decreased the total SFA, while increasing MUFA in the serum.

TABLE V
CONTENTS OF FATTY ACIDS IN SERUM (%)

Groups[1)]	CON	AH	S	AHS
Total SFA	3.5±1.37[2)]	2.3±0.57	ND	ND
Total MUFA	51.8±7.57[a]	57.2±3.78[ab]	61.4±1.53[b]	62.6±1.52[b]
Total PUFA	7.3±2.96	5.2±4.49	2.7±2.38	2.7±2.37
Total PUFA/SFA	1.2±1.99	2.3±2.07	-	-

1) Con, guinea pigs fed the control diet; AH, guinea pigs fed control diet including 1% *Allium hookeri* root; S, guinea pigs fed control diet including 0.1% sulfur; AHS, guinea pigs fed control diet including both 1% *Allium hookeri* root and 0.1% sulfur; SFA, saturated fatty acid; ND, not detected (limit of detection 30ppm); MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.
2) Values in each row with different letters are statistically different ($p<0.05$). All values are expressed as mean ± standard deviation (n=5).

IV. CONCLUSIONS

Dietary processed sulfur consumption significantly decreased the final body weight, body weight gain, FER and EAT ($p<0.05$). However, it significantly increased cholesterol levels ($p<0.05$), and increased the triglyceride in the serum. Consuming *Allium hookeri* root significantly decreased EAT and PAT ($p<0.05$). Moreover, while dietary processed sulfur increased various serum amino acids contents, a combination of both *Allium hookeri* root and processed sulfur had an increase in more amino acids than CON and *Allium hookeri* root alone. This study suggested that dietary *Allium hookeri* root may have significant effects on decreasing fat accumulation in guinea pigs.

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