SHIMADZU

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Measurement of Metabolites in Feces of Japanese Rock Ptarmigans by LC-MS/MS

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1. Overview

Metabolomics using LC-MS/MS was applied to increase the number of Japanese rock ptarmigans that is listed as an endangered species.

2. Introduction

Japanese rock ptarmigans, designated as a special natural monument are listed as an endangered species by Ministry of the Environment in Japan. However, estimated population size dropped from 3,000 birds in 1990 to less than 2,000 in 2009. In situ and ex-situ protection programs to increase the number of Japanese rock ptarmigans were endorsed. Metabolites produced by intestinal microbiota are absorbed constantly from the intestinal lumen and carried to systemic circulation; they play a direct role in health and disease. To increase the number of Japanese rock ptarmigans, it is important to analyze metabolites. In this study, metabolites in feces of Japanese rock ptarmigans under artificial raising and in situ protection at Mt. Kitadake (wild Japanese rock ptarmigans) were analyzed by LC-MS/MS.

3. Methods

3-1. Sample Preparation

Fresh cecal feces of Japanese rock ptarmigans were collected from four adult males and two chicks under artificial raising at Toyama Family park zoo and four adult hens under in situ protection at Mt. Kitadake. The feces were diluted with phosphate-buffered saline and extracted by intense mixing. The upper portion was centrifuged and the supernatant was centrifugally filtered. The filtrates were diluted 10-fold with water and analyzed by LC-MS/MS. Figure 1 shows the detailed procedures.

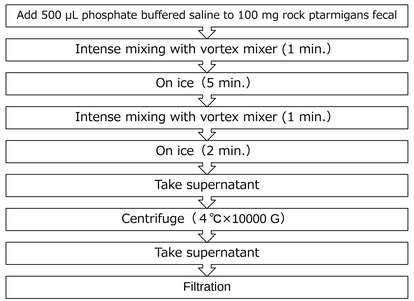

Figure 1 Process flow of sample preparation

3-2. Analytical Conditions

UHPLC (Nexera X2™ system)

Column: Discovery HS F5 (150 mmL. × 2.1 mmI.D., 3.0 µm)
Mobile phase A: 0.1% Formate/water
B: 0.1% Formate/acetonitrile
Flow rate: 0.25 mL/min
Injection vol.: 3 µL
Column temp.: 40°C

MS (LCMS-8050)

Ionization: ESI (Positive/Negative, MRM mode)
DL temp.: 250°C HB temp.: 400°C
Interface temp.: 400°C Nebulizing gas: 2.0 L/min
Drying gas: 10 L/min
Heating gas: 10 L/min

3. Result

3-1. Analysis of Metabolites in Feces of Japanese Rock Ptarmigans by LC-MS/MS

We analyzed 97 metabolites in feces of Japanese rock ptarmigans using LC-MS/MS. Main compounds of the metabolites were amino acids, organic acids, and nucleic acid-related substances. To investigate the repeatability of this analytical method, the feces of Japanese rock ptarmigans under artificial raising were analyzed. Fifty-five metabolites were detected from the feces and good repeatability (relative standard deviation of the peak area ratio of metabolites to internal standard <20%) was shown in 54 metabolites. The list of detected compounds and their repeatability is shown in Table 1.

Table 1 Detected compounds and their repeatability

Compounds	%RSD of peak area (n=6)	Compounds	%RSD of peak area (n=6)	Compounds	%RSD of peak area (n=6)
2-Aminobutyrate	6.41	Cystathionine	4.34	Met	3.84
Acetylcarnitine	3.75	Cytidine	1.55	Nicotinic acid	3.09
Adenine	14.1	Cytosine	11.8	Pantothenate	3.71
Adenosine	1.48	Dopamine	11.7	Phe	4.52
ADMA	4.09	FMN	8.18	Pro	2.57
Ala	3.95	GABA	6.69	Ser	3.99
AMP	4.03	Gln	2.89	Succinate	2.07
Arg	2.79	Glu	2.87	Taurocholate	9.27
Argininosuccinate	4.74	Gly	3.34	Thr/Homoserine	4.73
Asn	5.82	Guanosine	4.00	Thymidine	5.41
Asp	2.02	His	2.54	Thymine	9.20
cAMP	11.1	Hydroxyproline	2.88	Trp	3.37
Carnitine	5.72	Hypoxanthine	4.63	Tyr	3.82
Cholate	3.24	Ile	2.40	Uracil	6.27
Choline	2.34	Inosine	1.61	Uric acid	2.67
Citrulline	6.84	Leu	3.29	Uridine	1.44
CMP	3.08	Lys	2.29	Val	1.80
Creatine	4.17	Malate	20.8	Xanthine	3.60
Creatinine	5.85	MES (I.S.)	2.77		

3-2. Comparison between Artificial Raising and Wild

As a result of the analyses of the feces of Japanese rock ptarmigans under artificial raising and wild Japanese rock ptarmigans, 60 and 56 metabolites were detected, respectively. Main compounds of the metabolites were amino acids, nucleotides, nucleosides, and organic acids. Figure 2 shows the MRM chromatograms of extracts of feces of Japanese rock ptarmigans under artificial raising and wild Japanese rock ptarmigans obtained using LC-MS/MS.

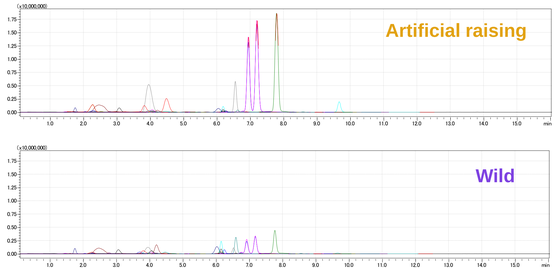

Figure 2 MRM chromatograms of extract of feces of Japanese rock ptarmigans

By Traverse MS™ software (Reifycs Inc., Japan), principal component analyses (PCA) was performed. As shown in Figure 3, two feces were successfully classified. Figure 4 shows the comparison of peak area rates of metabolites in the feces of Japanese rock ptarmigans under artificial raising and wild Japanese rock ptarmigans. The concentrations of 5 metabolites (adenine, adenosine, allantoin, cytidine and uridine) in the feces of wild Japanese rock ptarmigans were obviously higher ($p < 0.01$) than those in the feces of Japanese rock ptarmigans under artificial raising. In contrast, the concentrations of 31 metabolites (e.g. alanine, arginine, histidine, leucine, methionine and tryptophan) in the feces under artificial raising were obviously higher ($p < 0.01$) than those in the feces of wild Japanese rock ptarmigans.

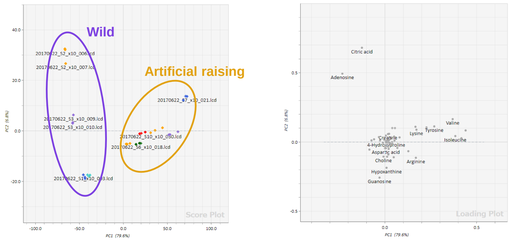

Figure 3 Score plot and loading plot

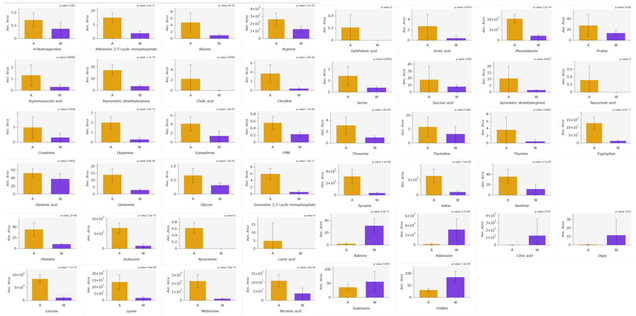

Figure 4 Comparison of peak area rate of metabolites between wild and artificial raising

5. Conclusions

- We analyzed metabolites in the feces of Japanese rock ptarmigans under artificial raising and wild Japanese rock ptarmigans using LC-MS/MS and 60 and 56 metabolites were detected, respectively.
- The concentrations of nucleoside in the feces of wild Japanese rock ptarmigans were obviously higher than those in the feces under artificial raising. In contrast, the concentrations of amino acids in the feces under artificial raising were obviously higher than those in the feces of wild Japanese rock ptarmigans.