Differential Effect of Non-Purified and Semi-Purified Standard Diets on Kynurenine and Peripheral Metabolites in Male C57BL/6J Mice

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ABSTRACT: The kynurenine (Kyn) pathway plays crucial roles in several inflammation-induced disorders such as depression. In this study, we measured Kyn and other related molecules in the blood plasma, brain, and urine of male C57BL/6J mice (B6) fed non-purified (MF) and semi-purified (AIN-93G and AIN-93M) standard rodent diets. Mice fed MF had increased plasma Kyn levels compared with those on AIN93-based diets, as well as decreased hippocampal Kyn levels compared with those fed AIN-93G. Previous studies showed that branched chain amino acids (BCAAs) suppress peripheral blood Kyn transportation to the brain, but plasma BCAA levels were not significantly different between the diet groups in our study. Urine metabolome analysis revealed that feed ingredients affected the excretion of many metabolites, and MF-fed mice had elevated excretion of kynurenic and quinolinic acids, pivotal metabolites in the Kyn pathway. Collectively, the level of critical metabolites in the Kyn pathway in the central and peripheral tissues was strongly affected by feed ingredients. Therefore, feed selection is a critical factor to ensure the reproducibility of experimental data in studies involving rodent models.

KEYWORDS: Feed, kynurenine, hippocampus, urine metabolome, peripheral metabolites

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Introduction

Diet affects animal physiology, behavior, metabolism, and other aspects in experimental animals, which might impact the results in animal studies. In a previous study, we revealed that a diet based on non-purified naturally derived materials increases stress resilience in a subchronic and mild social defeat (sCSDS) model of mice compared with that in animals on a semi-purified diet.[1,2] We also reported that dietary intake of a citrus flavonoid, hesperidin, could suppress elevated brain kynurenine (Kyn) levels and reduce stress vulnerability in the sCSDS model of mice.[3] The study was conducted using AIN-93G, a standard rodent diet formulated by the American Institute of Nutrition, which contains semi-purified feed ingredients.[4] Some critical molecules associated with depressive psychological disorders have been identified. In particular, the shift in the balance between Kyn and serotonin pathways of tryptophan (Trp) metabolism plays significant roles in the pathogenesis of depression.[5] Moreover, dysregulation of the Kyn pathway (KP) was observed in chronic diseases, including cancer and neurodegenerative and psychological disorders.[6] KP can also potentially alter the energy homeostasis in fat and muscle,[7,8] and can, therefore, play an important role in the pathogenesis of various diseases and physiological functions.

Based on the studies described above and our findings, we hypothesized that diet fed would have a significant effect on KP in rodent-based disease models. To ensure reproducibility of experimental data for research involving rodent-based disease models, it is critical to evaluate the effect of feed on KP in rodents. In this study, the relative levels of pivotal metabolites associated with Kyn metabolism were determined using brain tissue, blood plasma, and urine samples from mice fed non-purified and semi-purified standard feeds.

Methods

Animals

C57BL/6JJcl mice (7-weeks-old, male, B6) were purchased from Clea Japan (Tokyo, Japan) and reared in an animal facility at Ibaraki University under a 12/12 hours light/dark cycle (light from 07:00 to 19:00) at 23°C ± 2°C with a humidity of 50% to 60%. The B6 mice were divided into groups consisting of a non-purified diet (MF, Oriental Yeast, Tokyo Japan), semi-purified diet for the growth period (AIN-93G, Oriental Yeast), and semi-purified diet after the growth period (AIN-93M, Oriental Yeast). Dietary ingredients and feed composition of each diet are shown in Table 1. The amino acid composition of each feed is shown in Supplemental Table 1. This study protocol was approved by the Animal Care and Use Committee of Ibaraki University (Authorization Nos. 19230 and 20170).

Table 1. Composition of feeds used in the study.

	MF	AIN-93G	AIN-93M
Water (%)	8.1	9.0	9.0
Crude Protein (%)	23.2	17.9	12.5
Crude Fat (%)	4.9	7.0	3.9
Crude Ash (%)	6.0	2.5	2.4
Crude Fiber (%)	3.1	3.0	3.1
Nitrogen free extract (%)	54.7	60.6	69.1
Calorie (kcal)	356	377	362

Data from ORIENTAL YEAST CO., LTD, Tokyo, Japan.

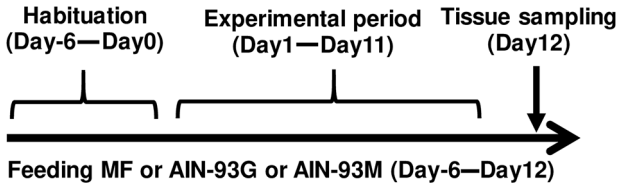

Figure 1. Study design: habituation, experimental period, and tissue sampling. Mice were fed indicated feeds throughout the habituation and experimental period.

Experimental design

The design of this study is illustrated in Figure 1. Mouse groups were fed the indicated diet from habituation (day-6) to tissue sampling (day 12).

Body weight gain, feed intake, and water intake

Throughout the experimental period (day 1-11), changes in the body weight gain, total feed intake, and total water intake were calculated as previously described.[10] Specifically, body weight gain was calculated by determining the difference between the body weight on day 0 and that on day 11. Food and water intake were calculated by determining the difference between the weight of feed and water bottle on the previous day and the weight of feed and water bottle on the measurement day, respectively.

Tissue sampling

Brain, liver, spleen, cecum, blood, and urine specimens were collected between 10:00 and 13:00 hours on day 12. After decapitation, brains were removed and stored at −80°C until analysis. The weights of other tissues were measured and recorded. Blood was collected in a tube and maintained at a final concentration of 0.1% 0.5 M ethylenediaminetetraacetic acid. Blood tubes were subsequently centrifuged (5 minutes, 1000×*g*, 4°C) and the supernatant was collected and stored at −80°C until analysis. Urine samples were collected by puncturing the bladder and were stored at −80°C until analysis.

Sample preparation for the determination of Trp and Kyn levels

The previously published sample preparation procedure for high-performance liquid chromatography (HPLC) analysis was performed with some modifications.[9] To determine Kyn and Trp levels in the brain region, the corresponding brain tissues, namely hippocampus, striatum, cerebellum, and prefrontal cortex, were used. These tissues were weighed and supplemented with 5 volumes of 0.1 N HCl for homogenization on ice. To remove the proteins, 24 µL of 30% trichloroacetic acid was added to 120 µL of the homogenate followed by vortexing. After centrifugation (20 minutes, 16 000×*g*, 4°C), 50 µL of the supernatant was used for HPLC analysis.

HPLC settings for the simultaneous determination of Trp and Kyn

A Shimadzu (Kyoto, Japan) chromatograph consisting of the following was used in this study: system controller (CBM-20A), degassing device (DGU-20A3), pump (LC-20AB), auto injector (SIL-20AC), photo diode array detector (SPD-M20A), and column oven (CTO-20AC). We used a column (4.6 mm internal diameter × 150 mm length; TSKgel ODS—100Z, TOSOH, Tokyo, Japan) with a particle diameter of 5 µm. A mobile phase composed of 10 mM ammonium acetate and 10% MeOH was used at a flow rate of 1.0 mL/minute at 35°C. The detection of Trp and Kyn at 280 and 360 nm, respectively, was performed using the photo diode array detector.

Analysis of plasma amino acids

Plasma amino acid analysis was performed using HPLC-electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS), employing a modification of the procedure described previously.[10,11] Briefly, for extraction, 5 µL of the plasma specimen was mixed with 15 µL of acetonitrile. After centrifugation (10 minutes, 20 000×*g*, 10°C) of the plasma specimen, 14 µL borate buffer and 4 µL APDSTAG® Wako Amino Acids Internal Standard mixture solution (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) were added to 4 µL of the supernatant and vortexed. After incubation (10 minutes, 55°C), 80 µL of 0.1% formic acid was added to the sample prior to HPLC-ESI-MS/MS analysis.

Sample preparation for urine metabolomic analysis

For this, previously reported methods were performed with some modifications.[12,13] Briefly, 20 µL of margaric acid and a tetracosane mixture (0.5 mg/mL), as well as 20 µL tropic acid (1 mg/mL), were added to 100 µL of the urine sample as the internal standard. Milli-Q water was added to a final volume of 2 mL, and thereafter, 0.5 mL of 5% hydroxylamine hydrochloride and 400 µL of 2.5 N NaOH aqueous solutions were added and mixed; samples were kept for 60 minutes at room temperature

(22°C). Subsequently, 350 µL of 6 N HCl was added to the mixture; the latter was stirred to prepare an extraction solvent. The extraction was performed twice using 6 mL ethyl acetate. Following the addition of ethyl acetate, the mixture was stirred and centrifuged (5 min, 14 000×*g*, 4°C) to obtain the component containing organic acid. Anhydrous sodium sulfate was then added, and the mixture was centrifuged (5 minutes, 14 000×*g*). The solution was then heated to 60°C under N_2 gas flow and dried. To the residue, 100 µL of *N,O*-Bis (trimethylsilyl) trifluoroacetamide + 1% trimethylchlorosilane solution was added, and the mixture was derivatized for 30 minutes at 80°C prior to gas chromatography-tandem mass spectrometry analysis.

Qualitative analysis of urinary metabolites

Gas chromatography-mass spectrometry solution (Shimadzu) was used to identify the urinary metabolites. Results were subsequently analyzed using a Microsoft Windows 7 Professional workstation and the Smart Metabolites Database (Shimadzu). Qualitative analysis was performed using the internal standard method, and the area ratio was determined as follows:

Area ratio = area value of compound Q.ION/area value of internal standard Q.ION, where Q.ION represents the peak area of a specific ion.

The relationships of detected metabolites were described on the metabolic pathway maps generated using the VANTED software (http://vanted.sourceforge.net/). Metabolite relationships were established using the KEGG pathway database (https://www.genome.jp/kegg/pathway.html).

Statistical analysis

Total feed intake, total water intake, body weight gain, tissue weight, Kyn and Trp levels in plasma and brain, and amino acid levels in plasma were analyzed using the Tukey–Kramer method. A P value $<.05$ was considered statistically significant. A P value $<.1$ was considered marginally significant. Analysis of the urine metabolome was performed on data that did not have zero values. Principal component analysis (PCA), one-way analysis of variance (ANOVA), and the Tukey–Kramer method was performed for the identified urinary metabolites. To control the P value for ANOVA, the false discovery rate was determined based on the methods of Benjamini and Hochberg.[14] The significance threshold was set at $q<.2$. Data are reported as the mean ± SEM. All data were analyzed using the R software (https://www.r-project.org/).

Results

Feed intake, water intake, and body weight gain

Data on total feed intake (A), total water intake (B), and body weight gain (C) are shown in Figure 2. Although there was no significant difference between groups in body weight gain (C), mice in the MF group consumed more feed than those in the AIN-93G and AIN-93M groups (MF vs AIN-93G, $P<.001$; MF vs AIN-93M, $P=.0040$; Figure 2A). Furthermore, mice in the MF group consumed more water than those in the AIN-93G and AIN-93M groups (MF vs AIN-93G, $P<.001$; MF vs AIN-93M, $P<.001$; Figure 2B).

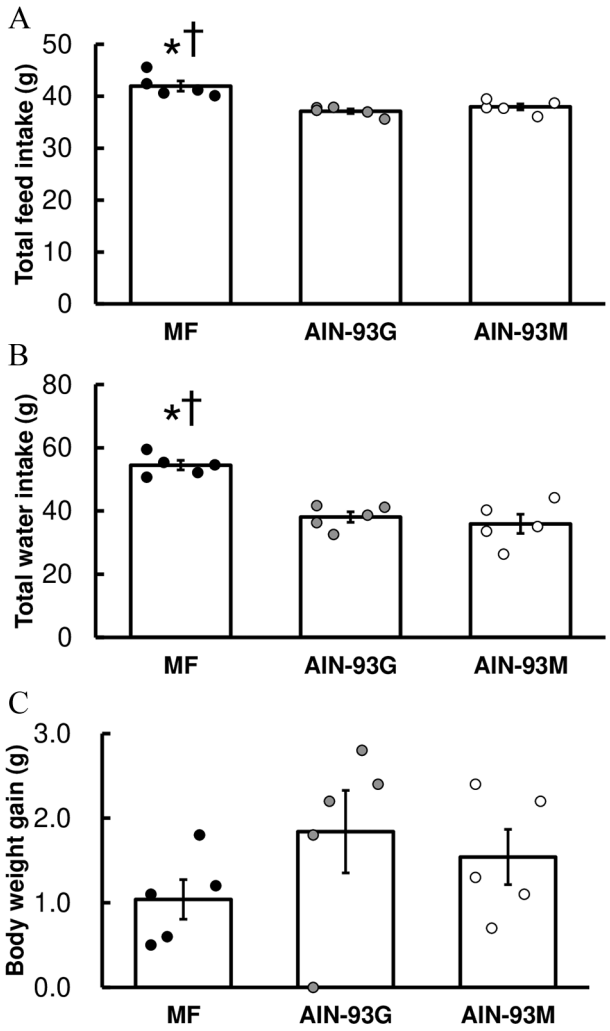

Figure 2. Total feed intake (A), total water intake (B), and body weight gain (C) in mice during the experimental period (n = 5 in each group). MF, data on MF-fed mice; AIN-93G, data on AIN-93G-fed mice; AIN-93M, data on AIN-93M-fed mice.
Data are expressed as means ± SEMs.
*$P<.05$ (MF vs AIN-93G).
†$P<.05$ (MF vs AIN-93M).

Tissue weight

Tissue weights were measured as a body weight ratio on day 12 (Supplemental Table 2). The liver weight of mice in the MF group was significantly higher than that of mice in the AIN-93M group (MF vs AIN-93M, $P=.0457$), which exhibited a feed-dependent trend compared with that in the AIN-93G group (MF vs AIN-93G, $P<.1$). The cecum weight of mice in the MF group was higher than that of mice in the AIN-93G and AIN-93M groups (MF vs AIN-93G, $P<.001$; MF vs AIN-93M, $P<.001$). Types of feed or diet had no effect on spleen weights for all groups.

Table 2. Effect of feeds composition on plasma kynurenine and tryptophan levels of mice.

		KYN (μM)	TRP (μM)	KYN/TRP RATIO × 10^{-2}
Plasma	MF	0.60 ± 0.07*†	48.2 ± 3.61	1.24 ± 0.10
	AIN-93G	0.36 ± 0.04	41.4 ± 4.66	0.88 ± 0.06
	AIN-93M	0.31 ± 0.07	30.7 ± 10.1	1.20 ± 0.17

Data were shown mean ± SEM. n = 5 per each group.
Tukey-Kramer method (MF vs AIN-93G: *$P < .05$, MF vs AIN-93M:†$P < .05$).

Table 3. Effect of feed composition on brain kynurenine and tryptophan levels of mice.

		KYN (NMOL/G)	TRP (NMOL/G)	KYN/TRP × 10^{-2}
Hippocampus	MF	0.32 ± 0.03**	220 ± 22.2††	0.15 ± 0.01
	AIN-93G	0.46 ± 0.03	303 ± 26.0	0.15 ± 0.01§
	AIN-93M	0.38 ± 0.03	396 ± 65.8	0.11 ± 0.02
Striatum	MF	0.36 ± 0.04	220 ± 28.7†	0.17 ± 0.02*†
	AIN-93G	0.35 ± 0.06	296 ± 30.1§	0.12 ± 0.01
	AIN-93M	0.36 ± 0.02	317 ± 16.2	0.12 ± 0.01
Cerebellum	MF	0.22 ± 0.03	277 ± 10.0	0.08 ± 0.01
	AIN-93G	0.28 ± 0.02	318 ± 26.4	0.09 ± 0.01
	AIN-93M	0.27 ± 0.01	314 ± 21.9	0.09 ± 0.01
Prefrontal cortex	MF	0.27 ± 0.03	192 ± 7.56	0.14 ± 0.01
	AIN-93G	0.32 ± 0.04	221 ± 16.4	0.15 ± 0.01
	AIN-93M	0.35 ± 0.02	200 ± 17.2	0.18 ± 0.02

Data were shown mean ± SEM. n = 5 per each group.
Tukey-Kramer method (MF vs AIN-93G: **$P < .05$, *$P < .1$. MF vs AIN-93M: ††$P < .05$, †$P < .1$. AIN-93G vs AIN-93M: §$P < .1$).

Trp and Kyn levels in plasma and brain tissues

Plasma samples were used to evaluate the effects of feed composition on peripheral Trp and Kyn levels. The plasma Kyn levels in the MF group were significantly higher than those in the AIN-93G and AIN-93M groups (MF vs AIN-93G, $P = .0343$; MF vs AIN-93M, $p = .0128$; Table 2). Additionally, we measured Trp and Kyn levels in brain tissues (Table 3) and found that hippocampal Kyn levels in the MF group were significantly lower than those in the AIN-93G group ($P = .0306$). Hippocampal Trp levels in the MF group were significantly lower than those in the AIN-93M group ($P = .0330$). Moreover, striatal Trp levels in the MF and AIN-93G groups were marginally lower than those in the AIN-93M group ($P < .1$). Collectively, plasma Kyn levels in mice fed MF were higher compared with those fed AIN93-based diets, whereas the hippocampal Kyn level was significantly lower than that in the AIN-93G group.

Plasma amino acid profile

To understand the effect of feed composition on plasma and brain Kyn levels in B6, the profiles of free amino acids in the plasma of these mice were evaluated (Table 4). As shown in Table 4, concentrations of 5 amino acids were significantly different among the diet groups. The asparagine level in the AIN-93M group was higher than that in MF and AIN-93G groups (MF vs AIN-93M, $P = .0390$; AIN-93G vs AIN-93M, $P = .0191$). The lysine level in the MF group was lower than that in AIN-93G and AIN-93M groups (MF vs AIN-93G, $P = .0353$; MF vs AIN-93M, $P = .0093$). The glutamine level in the MF group was lower than that in the AIN-93M group ($P = .0200$). The serine level in the MF group was lower than that in the AIN-93M group ($P = .0097$). Moreover, the ornithine level in the MF group was higher than that in the AIN-93G and AIN-93M groups (MF vs AIN-93G, $P = .0230$; MF vs AIN-93M, $P = .0261$).

Table 4. Effect of feed composition on plasma amino acid levels of mice.

AMINO ACID (μM)	MF	AIN-93G	AIN-93M
Alanine	580 ± 65	686 ± 81	763 ± 130
Arginine	116 ± 15	102 ± 14	106 ± 16
Asparagine	27 ± 1†	30 ± 5§	47 ± 6
Cysteine	11 ± 1	15 ± 2	*nd*
Glutamine	371 ± 27†	436 ± 25	592 ± 77
Glutamic acid	3 ± 1	3 ± 0.3	*nd*
Glycine	281 ± 30	197 ± 14	*nd*
Isoleucine	120 ± 9	108 ± 10	83 ± 17
Leucine	157 ± 14	118 ± 11	119 ± 14
Lysine	227 ± 9*†	382 ± 32	423 ± 57
Methionine	75 ± 6	85 ± 10	82 ± 17
Ornithine	85 ± 12*†	48 ± 5	49 ± 6
Phenylalanine	65 ± 13	51 ± 4	53 ± 7
Proline	594 ± 97	643 ± 143	804 ± 225
Serine	115 ± 10†	170 ± 25	207 ± 16
Taurine	234 ± 29	232 ± 14	235 ± 37
Threonine	177 ± 16	219 ± 27	*nd*
Tryptophan	38 ± 12	48 ± 3	45 ± 6
Tyrosine	72 ± 15	73 ± 10	82 ± 8
Valine	309 ± 31	252 ± 21	207 ± 50

Data were shown mean ± SEM. n = 3-5 per each group.
Tukey-Kramer method (MF vs AIN-93G, *$P < .05$; MF vs AIN-93M, †$P < .05$; §$P < .05$; AIN-93G vs AIN-93M).
Abbreviation: *nd*, not determined.

Data were shown mean ± SEM. n = 3-5 per each group. *Tukey-Kramer* method (MF vs AIN-93G, *$P < .05$; MF vs AIN-93M, †$P < .05$; *nd*, not determined).

Urinary metabolite profile

Previous studies showed that large neutral amino acids (LNAAs), namely Trp and tyrosine, and BCAAs suppress Kyn transport into the brain tissue via L-type amino acid transporter 1 (LAT1) competition,[15-17] and thus, we determined the effect of feed composition on plasma LNAA levels in B6 mice. However, plasma LNAA levels were not affected by types of feed, and plasma LNAAs did not interfere with the transport of Kyn into the brain tissues of mice fed MF. Therefore, we attempted to further elucidate whether feed composition could affect the profile of urinary metabolites involving Kyn and its associated pathways. From the results of metabolome analysis, 319 metabolites were detected in mouse urine (Supplemental Table 3), and the MF group was subsequently separated from AIN groups based on the results of PCA (Figure 3). As shown in Table 5, one way ANOVA revealed that the relative levels of 16 urinary metabolites were significantly different between groups ($P < .05$ and $q < .2$), and 3 metabolites were substantially different ($P < .05$, $q > .2$). A multiple comparison test was performed for the 19 metabolites. Relative levels of 15 metabolites in the urine of MF-fed mice were higher than those in AIN-93G-fed mice. Relative levels of 16 metabolites were higher in the urine of MF-fed mice than in AIN-93M-fed mice. Furthermore, the levels of 2 metabolites were relatively lower in the urine of MF-fed mice compared with the levels in AIN-93G-fed mice, and the level of one metabolite was relatively lower in the urine of MF-fed mice compared with the levels in AIN-93M-fed mice. We generated a metabolic map to evaluate the effect of diet composition on the Trp–Kyn pathway and related pathways in mouse urine. We observed changes in many metabolites between groups with respect to these pathways (Figure 4).

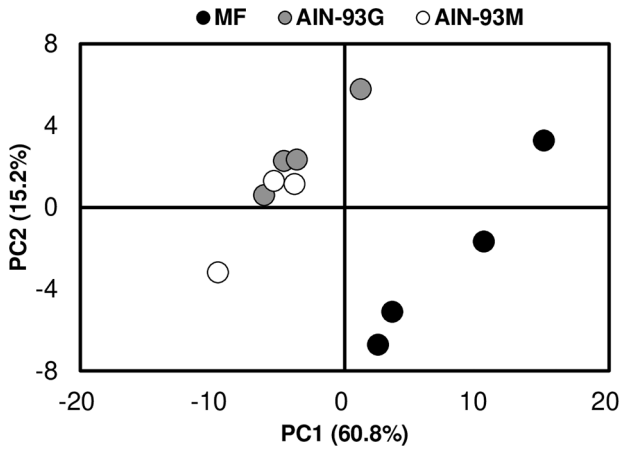

Figure 3. PCA of urinary metabolites for each group (n = 3-4). PCA, principal component analysis; PC1, principal component 1; PC2, principal component 2; MF, data on MF-fed mice; AIN-93G, data on AIN-93G-fed mice; AIN-93M, data on AIN-93M-fed mice.

Discussion

In this study, we demonstrated that diet composition, namely non-purified (MF) and semi-purified (AIN-93G, and AIN-93M) feeds, differentially affects the peripheral and central metabolite profiles in mice. Mice fed AIN-93G or AIN-93M consumed less feed and water than those fed MF; however, there was no significant difference in body weight gain among the 3 groups (Figure 2). This result is consistent with our previous finding that cumulative feed intake in mice fed MF is higher than that in mice fed AIN-93G, but the cumulative calorie intake and body weight gain were similar between the groups.[18] There could be several possible reasons for this phenomenon. Whereas AIN-93 based diets only have cellulose as

Table 5. Effect of feed composition on urinary metabolites of mice.

METABOLITES	MF	AIN-93G	AIN-93M	*ANOVA*	
				P-VALUE	*Q*-VALUE
Citric acid-4TMS	2.1953 ± 0.1584*†	0.0132 ± 0.0041	0.0612 ± 0.0271	.0016	.1517
2-Hydroxyglutaric acid-3TMS	1.2738 ± 0.1190*†	0.0483 ± 0.0147	0.0966 ± 0.0416	.0027	.1260
Anthranilic acid-TMS	0.0020 ± 0.0004*	0.0062 ± 0.0004	0.0042 ± 0.0011	.0052	.1600
Ornithine-4TMS	0.0152 ± 0.0017*†	0.0035 ± 0.0010	0.0028 ± 0.0001	.0061	.1426
Aconitic acid-3TMS	1.3541 ± 0.1509*†	0.2052 ± 0.0455	0.3375 ± 0.1104	.0068	.1264
Fumaric acid-2TMS	0.2946 ± 0.0422*†	0.0043 ± 0.0006	0.0074 ± 0.0018	.0106	.1641
HydroquinoneTMS	0.0206 ± 0.0031*†	0.0054 ± 0.0018	0.0042 ± 0.0007	.0149	.1984
Azelaic acid-2TMS	0.0112 ± 0.0024*†	0.0025 ± 0.0002	0.0015 ± 0.0002	.0167	.1936
Kynurenic acid-2TMS	0.0592 ± 0.0193	0.0382 ± 0.0046	0.0153 ± 0.0024	.0182	.1877
Hippuric acid-2TMS	0.8759 ± 0.1882*†	0.0177 ± 0.0047	0.0109 ± 0.0047	.0197	.1829
Uracil-2TMS	0.0856 ± 0.0273†	0.0368 ± 0.0039	0.0190 ± 0.0009	.0208	.1757
Glutaric acid-2TMS	0.3449 ± 0.0733*†	0.0062 ± 0.0013	0.0071 ± 0.0017	.0240	.1862
Hippuric acid-TMS	0.0007 ± 0.0003*†	0.1665 ± 0.0558	0.3386 ± 0.0676	.0262	.1873
2-Ketoadipic acid-oxime-3TMS(2)	0.0195 ± 0.0040*†	0.0014 ± 0.0007	0.0008 ± 0.0002	.0267	.1774
4-Hydroxybenzoic acid-2TMS	0.8185 ± 0.1563*†	0.1221 ± 0.0379	0.1112 ± 0.0141	.0278	.1721
Ornithine-d7-4TMS	0.3667 ± 0.0933*†	0.0219 ± 0.0062	0.0059 ± 0.0010	.0302	.1755
Glyceric acid-3TMS	0.0712 ± 0.0141*†	0.0165 ± 0.0045	0.0146 ± 0.0038	.0377	.2060
Quinolinic acid-2TMS	0.0259 ± 0.0058*†	0.0037 ± 0.0014	0.0037 ± 0.0012	.0415	.2145
3-Hydroxyisobutyric acid-2TMS	0.0529 ± 0.0124*†	0.0145 ± 0.0034	0.0083 ± 0.0004	.0492	.2406

Data (relative area) were shown mean ± SEM. n = 3-4 per each group.
Tukey-Kramer method (MF vs AIN-93G, *$P < .05$. MF vs AIN-93M, †$P < .05$).

ingestible plant fibers, MF contains both insoluble and soluble fibers from natural feed materials, which may affect the intestinal ecosystem (microbiome and metabolites) and morphology, such as gut volume and villus.[18-20] Cecum was larger in mice fed MF than in those fed AIN-93s (Supplemental Table 2). The different gastrointestinal environment may affect the expression of critical molecules that regulate the feeding behavior. Furthermore, plasma levels of amino acids were different among the 3 groups (Table 4). Collectively, these peripheral differences might globally influence the feeding behavior. Also, mice fed MF consumed more drinking water compared with those fed AIN-93s (Figure 2B). Although it may be a reflection of the amount of feed intake, there would be several possibilities for this; possibly, water absorption in the gastrointestinal tract was influenced by dietary fibers in feed.

In this study, we observed that liver weight (% of BW) of mice fed MF was higher than that of mice fed AIN-93 based diets. This result is consistent with our previous finding.[18] However, body and liver weights were not significantly different among the 3 groups in this study (data not shown). Thus, physiological problems might not have occurred in the liver. In general, fat deposition is one of the causes for enlargement of the liver. Although AIN-93 based diets induce fat deposition in the liver, short term MF feeding in this study did not induce fatty liver and abnormal hypertrophy in mice.[20]

Hippocampal inflammation is implicated in mental disorders such as depression,[21] and activated microglia play a pivotal role in brain inflammation by upregulating the production of kynurenic acid (KA) and quinolinic acid (QUIN).[22] In a previous study, we showed that the hippocampal Kyn level in sCSDS mice is negatively correlated with stress resilience.[3] Additionally, peripheral Kyn moves to the neural tissues in the brain via the blood brain barrier (BBB),[23] and therefore, the inhibition of Kyn transport into the brain could prevent the onset of depression. Several studies have reported that exercise activates the expression of Kyn aminotransferase in the periphery and the

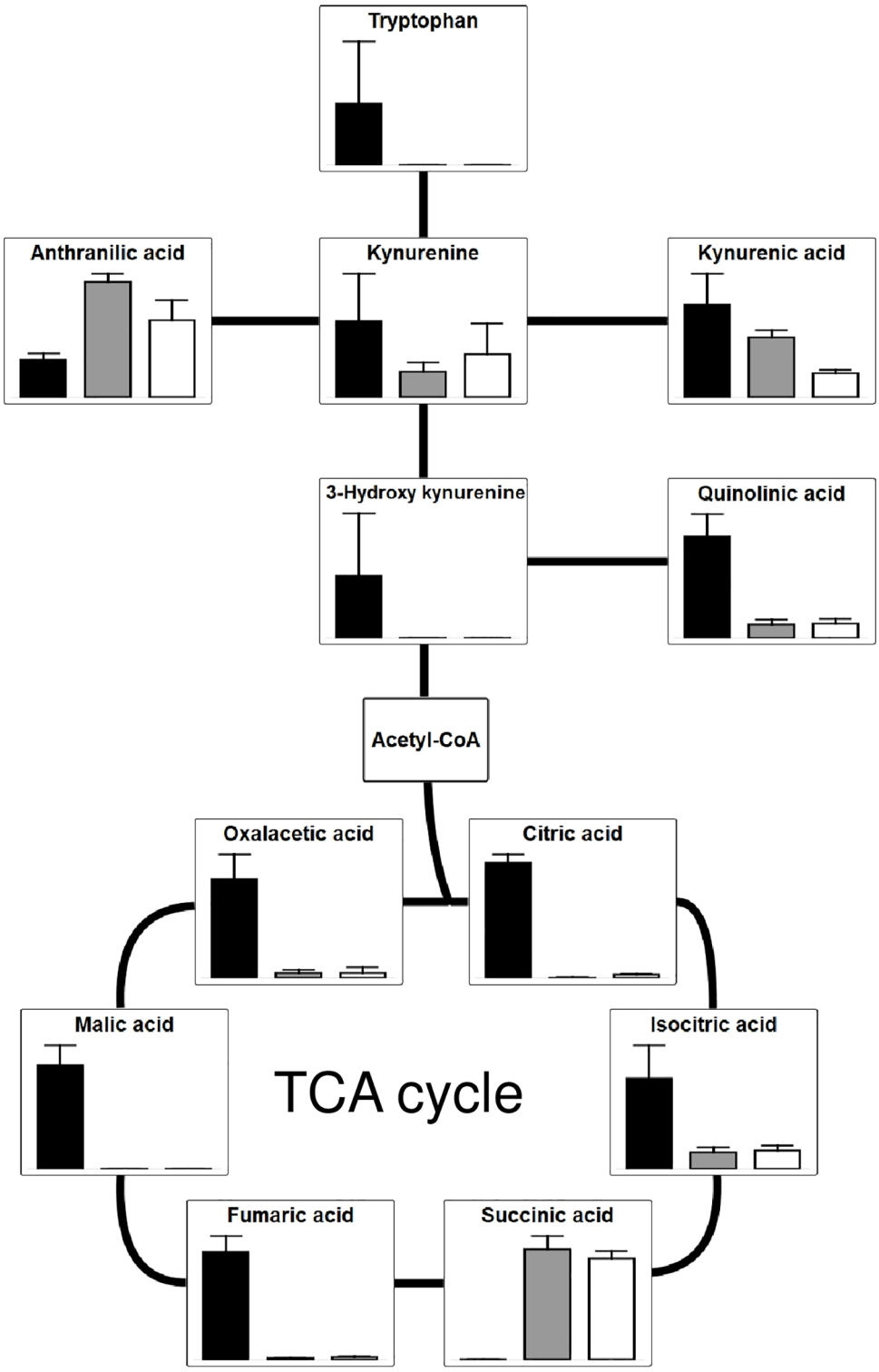

Figure 4. Differential effect of feed ingredients on urinary metabolites in male mice. Black bars indicate values for mice fed MF. Gray bars indicate values for mice fed AIN-93G. White bars indicate values for mice fed AIN-93M.

conversion of Kyn to KA, which cannot pass through the BBB, thus contributing to the prevention of mental disorders.[24,25] In this study, we found that the diet of non-purified feed increased blood plasma Kyn levels but reduced hippocampal Kyn levels compared with the respective levels observed with the semi-purified feed (Tables 2 and 3). There are various possibilities for these phenomena, and we inferred that peripheral blood metabolites in mice fed MF suppress Kyn transport into the neural tissues in the brain. In this study, there was no significant difference in the blood LNAA levels between groups (Table 4), whereas the leucine (Leu) level in mice fed MF was slightly higher than that in mice fed AIN-93G, indicating the possible role of peripheral Leu in suppressing blood Kyn transport into the brain.[16] Trp levels in the hippocampus and

striatum of the mice fed MF were lower than those in the mice fed AIN-93 based diets; however, no differences were observed in the cerebellum and prefrontal cortex (Table 3). These results indicate that feed composition affects Trp levels in the brain. In addition, it indicates that the uptake ability of Trp might differ among brain regions.

Urine metabolome analysis revealed that B6 mice fed MF excrete higher levels of metabolites than those fed AIN-93 based diets; however, anthranilic acid (AA) levels in mice fed MF were lower than those in mice fed AIN-93G (Table 5). Conversely, urine KA and QUIN levels in mice fed MF were higher than those in mice fed AIN-93G and AIN-93M. Moreover, QUIN is used for nicotinamide adenine dinucleotide synthesis downstream of the KP; however, feed composition did not affect the urinary nicotinic acid and nicotinamide levels (Supplemental Table 3). Our results showed that MF and AIN-93 based diets differentially affect the KP and/or urinary excretion profiles of AA, KA, and QUIN in mice. Urinary excretion of KA and QUIN indicates the degradation of Trp.[26] There are several possible causes for this, such as the activation of Trp-degrading enzymes and differences in the composition of amino acids in the feed.[27,28] Peripheral QUIN is mainly synthesized in the liver[29] and is regulated by α-amino-β-carboxy muconate-ε-semialdehyde decarboxylase (ACMSD). In this study, dietary conditions might have differentially affected the ACMSD activity in the liver. The ACMSD activity is regulated by Trp and protein intake besides other factors, such as fatty acid composition.[30] MF and AIN-93 based diets differentially affected metabolites of the tricarboxylic acid (TCA) cycle in the urine (Figure 4). Specifically, citric acid plays a central role in energy production in mitochondria and coordinates glucose, fatty acid, and amino acid metabolism.[31,32] The different compositions of amino acids, carbohydrates, and fatty acids in the MF and AIN-93 based diets are likely to have contributed to the different urinary excretion profiles of organic acids in the present study.[33] Given that the TCA cycle follows the KP via acetyl-CoA, the feed composition might affect energy metabolism in mice. Amino acid metabolism associated with the KP is linked to both carbohydrate and fat metabolism through the TCA cycle. However, differences between metabolome results of AIN and MF groups might be attributable to the varying composition of proteins, carbohydrates, and fatty acids for each type of feed, and therefore, further studies are needed.

The study has several limitations. We used only male mice. Dysregulation of the KP has been reported not only in males but also in females.[34] Therefore, it is necessary to elucidate the effects of diets on the KP in female mice. Moreover, we did not analyze critical metabolites, such as QUIN and KA, in the brain KP. Thus, the complete picture of brain KP should be elucidated in future studies.

In this study, we demonstrated that non-purified and semi-purified feeds differentially affect the levels of Kyn and associated metabolites in the brain, blood plasma, and urine. The metabolites associated with the KP have been identified in many research studies. This indicates the critical roles of feed ingredient metabolism in modulating inflammatory pathway mechanisms involved in various chronic diseases, including cancer, and neurodegenerative and psychological disorders. Based on our findings, we reiterate the importance of researchers choosing the appropriate feed ingredients for laboratory animals used as disease models. Therefore, the design of a standard diet used in animal-based research should be further examined and validated to ensure the reproducibility of experimental data.[20]

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Author Contributions

Y.Y., A.O., and A.T. designed the experiment. Y.Y. and A.T. wrote the manuscript. A.O., I.N., T.M., and A.H. helped draft the manuscript. Y.Y. and A.O. analyzed kynurenine and tryptophan. Y.Y. and I.N. performed metabolome analysis. Y.Y. and T.M. performed amino acid analysis. A.T. supervised the present study.

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Supplemental Material

Supplemental material for this article is available online.

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