

1 **Supplementation of Alhagi honey**
2 **polysaccharides contributes to the**
3 **improvement of the intestinal**
4 **immunity and regulating the**
5 **structure of intestinal flora in**
6 **mice**

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12 **SUPPLEMENTAL INFORMATION**

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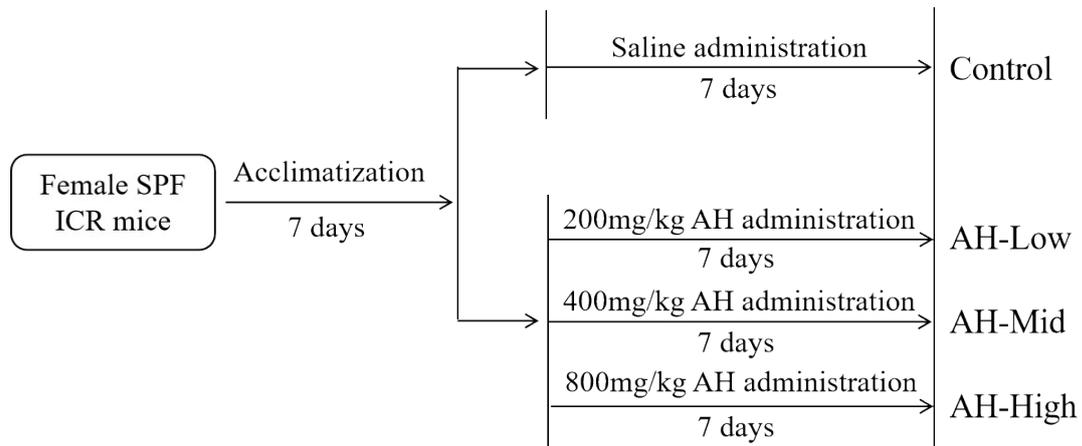
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20 **Supplementary Figure S1**



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22 **Supplementary Figure S1.** Experimental design (n = 5 in each group). Twenty ICR

23 mice were assigned to four groups randomly: Control, AH-Low group with 200

24 mg/kg AH; AH-Mid group with 400 mg/kg AH; AH-High group with 800 mg/kg AH.

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38 **Supplementary Table S1**

39 Compositions of experimental diets

Ingredient (g/kg)	
Protein	192
Fat	66
Fiber	33
Ash	58
Carbohydrate	610
Amino acid ^a	217.2
Minerals ^b	18.72
Vitamins ^c	2.32
Nutrient level	
Total energy of diets (kcal/g)	3.6
Protein energy supply ratio (%)	20.6
Fat energy supply ratio (%)	12
Carbohydrate energy supply ratio (%)	67.4
Total energy of AAP (kcal/kg)	2050
AAP-L energy supply ratio (‰)	0.56
AAP-M energy supply ratio (‰)	0.28
AAP-H energy supply ratio (‰)	0.14

40 ^a Provided per kilogram of Amino acid in diet: Alanine (12.9 g), Arginine (13 g),
 41 Aspartic acid (17.4 g), Cysteine (3.6 g), Glutamic acid (34.4 g), Glycine (13.2 g),
 42 Histidine (5.6 g), Isoleucine (11.8 g), Leucine (17.5 g), Lysine (14.2 g), Methionine (7
 43 g), Phenylalanine (9.2 g), Proline (16.7 g), Serine (10.6 g), Threonine (10.5 g),
 44 Tryptophan (2.6 g), Tyrosine (7.6 g), Valine (9.4 g); ^b Provided per kilogram of
 45 Minerals in diet: Arsenic (0.00032 g), Cadmium (0.000042 g), Calcium (11.1 g),
 46 Copper (0.016 g), Iodine (0.0011 g), Iron (0.32 g), Lead (0.00014 g), Magnesium

47 (2.16 g), Manganese (0.162 g), Mercury (Not detected), Phosphorus (8.5 g),
48 Potassium (7.84 g), Selenium (0.00037 g), Sodium (2.24 g), Zinc (0.14 g); ^c Provided
49 per kilogram of Vitamins in diet: Biotin (388 µg), Choline (1.84 g), Folic acid (0.0055
50 g), Niacin (0.088 g), Pantothenic acid (0.037 g), Pyridoxine (0.017 g), Riboflavin
51 (0.019 g), Thiamin (0.085 g), Vitamin A (25700 IU), Vitamin B12 (28.6 µg), Vitamin
52 D3 (4360 IU), Vitamin E (195 IU), Vitamin K3 (0.021 g).

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69 **Supplementary Table S2**

70 Primer sequences used for RT-qPCR

Gene	Direction	Primer sequence (5'–3')
IL-2	Forward primer	5'-TGAGCAGGATGGAGAATTACAGG-3'
	Reverse primer	5'-GTCCAAGTTCATCTTCTAGGCAC-3'
IL-4	Forward primer	5'-GGTCTCAACCCCCAGCTAGT-3'
	Reverse primer	5'-GCCGATGATCTCTCTCAAGTGAT-3'
IL-6	Forward primer	5'-CCAAGAGGTGAGTGCTTCCC-3'
	Reverse primer	5'-CTGTTGTTTCAGACTCTCTCCCT-3'
IL-10	Forward primer	5'-CTTACTGACTGGCATGAGGATCA-3'
	Reverse primer	5'-GCAGCTCTAGGAGCATGTGG-3'
IL-17	Forward primer	5'-TTTAACTCCCTTGGCGCAAAA-3'
	Reverse primer	5'-CTTCCCTCCGCATTGACAC-3'
TNF- α	Forward primer	5'-CAGGCGGTGCCTATGTCTC-3'
	Reverse primer	5'-CGATCACCCCGAAGTTCAGTAG-3'
INF- γ	Forward primer	5'-ATGAACGCTACACACTGCATC-3'
	Reverse primer	5'-CCATCCTTTTGCCAGTTCCTC-3'

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76 **Supplementary Table S3**

77 The body weight change of mice orally administrated with AH (AH-low, 200
78 mg/kg/day; AH-Mid, 400 mg/kg/day; AH-High, 800 mg/kg/day).

Days	Body weight (g/mouse)			
	Control	AH-Low	AH-Mid	AH-High
0	19.98±0.56	20.05±0.31	19.95±0.48	20.03±0.71
1	20.21±0.52	20.28±0.40	20.18±0.40	20.27±0.72
2	20.42±0.55	20.70±0.31	20.92±0.49	21.03±0.70
3	20.64±0.55	21.12±0.33	21.23±0.38	21.24±0.71
4	20.88±0.53	21.38±0.30	21.49±0.40	21.53±0.59
5	21.19±0.35	21.69±0.55	21.70±0.50	21.39±0.62
6	21.31±0.54	21.81±0.31	21.83±0.44	22.00±0.70
7	21.52±0.55	22.04±0.36	22.06±0.55	22.74±0.77

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88 **Supplementary Table S4**

89 The daily food intake of mice orally administrated with AH (AH-low, 200 mg/kg/day;
 90 AH-Mid, 400 mg/kg/day; AH-High, 800 mg/kg/day).

Days	Food intake (g/mouse)			
	Control	AH-Low	AH-Mid	AH-High
0	3.281±0.123	3.242±0.014	3.269±0.009	3.265±0.325
1	3.199±0.223	3.252±0.034	3.370±0.029	3.315±0.305
2	3.231±0.423	3.302±0.124	3.314±0.209	3.295±0.215
3	3.331±0.443	3.248±0.064	3.319±0.109	3.284±0.205
4	3.131±0.323	3.192±0.234	3.309±0.219	3.309±0.315
5	3.261±0.422	3.342±0.314	3.359±0.119	3.315±0.335
6	3.351±0.523	3.322±0.144	3.354±0.309	3.345±0.125
7	3.401±0.026	3.392±0.094	3.384±0.249	3.365±0.319

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100 **Supplementary Table S5**

101 Relative abundance of top four gut microbiota at the phylum level.

Groups	<i>Firmicutes</i> (%)	<i>Bacteroidetes</i> (%)	<i>F/B</i> (%)	<i>Proteobacteria</i> (%)	<i>Tenericutes</i> (%)
Control	52.56±1.82 ^a	41.75±1.20 ^b	1.26±0.05 ^a	4.94±0.32 ^a	0.42±0.01 ^b
AH-Low	48.96±0.64 ^a	46.56±1.08 ^{ab}	1.05±0.04 ^{ab}	3.41±0.18 ^b	0.41±0.04 ^b
AH-Mid	49.04±2.41 ^a	46.04±2.14 ^{ab}	1.07±0.10 ^{ab}	3.64±0.45 ^b	0.72±0.01 ^a
AH-High	45.74±3.02 ^a	49.95±3.78 ^a	0.94±0.13 ^b	2.88±0.05 ^b	0.70±0.10 ^a

102 Data are expressed as the mean \pm SD (n = 5). Values without the same letter (a-b) are
 103 significantly different ($p < 0.05$).

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117 **Supplementary Table S6**

118 Relative abundance of top seven classified gut microbiota at the genus level.

Groups	Oscillospir a(%)	Bacteroides s(%)	Prevotella (%)	Ruminococcus ccus(%)	Parabacteroides oides(%)	Coprococcus us(%)	Lactobacillus lus(%)	Staphylococcus occus(%)	Butyrivibrio onas(%)
Control	6.3±1.1 ^b	3.9±0.3 ^c	9.8±1.44 ^a	2.5±0.35 ^b	1.8±0.01 ^c	1.6±0.06 ^b	0.3±0.02 ^b	1.9±0.50 ^a	0.5±0.15 ^a
AH-Low	6.0±0.7 ^b	6.1±0.3 ^b	0.5±0.04 ^b	2.7±0.28 ^{ab}	2.6±0.24 ^b	1.5±0.17 ^b	0.8±0.11 ^b	1.0±0.08 ^b	0.5±0.10 ^a
AH-Mid	8.9±0.4 ^a	6.5±0.2 ^b	1.5±0.68 ^b	3.4±0.02 ^a	4.6±0.19 ^a	2.2±0.08 ^a	2.2±0.48 ^a	0.2±0.04 ^c	0.7±0.16 ^a
AH-High	6.9±0.2 ^{ab}	8.8±0.2 ^a	0.4±0.04 ^b	3.2±0.03 ^{ab}	2.8±0.30 ^b	2.3±0.16 ^a	0.9±0.02 ^b	0.2±0.02 ^c	0.4±0.02 ^a

119 Data are expressed as the mean \pm SD (n = 5). Values without the same letter (a-c) are

120 significantly different ($p < 0.05$).

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