

Table S1. Composition of the control and HGB diets.

	(g/kg diet)	
	Control	HGB
Casein	200	157.4
Corn starch	197.5	-
α -Corn starch	132	27.3
Sucrose	100	100
Soybean oil	70	70
Lard	200	192.4
Cellulose	50.0	-
Barley flour ("White Fiber")	-	402.4
AIN-93G mineral mixture	35	35
AIN-93 vitamin mixture	10	10
L-cystine	3	3
Choline bitartrate	2.5	2.5
<i>t</i> -butylhydroquinone	0.014	0.014

Barley flour ("White Fiber") from the Hakubaku Co. Ltd (Yamanashi, Japan), White fiber is contained β -glucan, 8.0%; total dietary fiber, 12.4%; protein, 8.4%; fat, 1.9%.
HGB: High β -glucan barley group.

Table S2. List of standard bacterial strain and primer sequence for real time PCR.

Phylum	Forward (Fw) / Reverse (Rv)	Primer sequence	Standard bacteria strain. Strain No.		Reference
<i>Bacteroidetes</i>	Fw	5'-CRAACAGGATTAGATACCCT-3'	<i>Bacteroides fragilis</i>	JCM11019	De Gregoris <i>et al</i> : J. Microbial Methods, Sep;86(3): 351-6, 2011.
	Rv	5'-GGTAAGGTTCTCGCGTAT-3'			
<i>Firmicutes</i>	Fw	5'-TGAAACTYAAAGGAATTGACG-3'	<i>Ruminococcus albus</i>	JCM14654	De Gregoris <i>et al</i> : J. Microbial Methods, Sep;86(3): 351-6, 2011.
	Rv	5'-ACCATGCACCACCTGTC-3'			
<i>Actinobacteria</i>	Fw	5'-TACGGCCGCAAGGCTA-3'	<i>Bifidobacterium longum</i>	JCM1217	De Gregoris <i>et al</i> : J. Microbial Methods, Sep;86(3): 351-6, 2011.
	Rv	5'-TCRTCCCCACCTTCTCCG-3'			
<i>Total bacteria</i>	Fw	5'-AAACTCAAAGKAATTGACGG	<i>Ruminococcus albus</i>	JCM14654	De Gregoris <i>et al</i> : J. Microbial Methods, Sep;86(3): 351-6, 2011.
	Rv	5'-CTCACRRCACGAGCTGAC-3'			
Genus	Fw / Rv	Primer sequence	Standard bacteria	Strain No.	Reference
<i>Bacteroides fragilis</i> group	Fw	5'-GTCAGTTGTGAAAGTTTGC-3'	<i>Bacteroides fragilis</i>	JCM11019	Bernard AE <i>et al</i> : Appl Environ Microbiol, 66: 1587-94, 2000.
	Rv	5'-CAATCGGAGTTCTTCGTG-3'			
<i>Lactobacillus</i>	Fw	5'-TGGAAACAGGTGCTAATAGGC-3'	<i>Lactobacillus ramosus</i>	ATCC8530	Roy Byun <i>et al</i> : J Clin Microbiol, 42(7): 3128-36, 2004.
	Rv	5'-GTCCATTGTGGAAGATTCCC-3'			
<i>Bifidobacterium</i>	Fw	5'-AGGGTTCGATTCTGGCACAG-3'	<i>Bifidobacterium longum</i>	JCM1217	Kok RG <i>et al</i> : Appl Environ Microbiol, 62: 3668-72, 1996.
	Rv	5'-CATCCGGCATTACCACCC-3'			
<i>Prevotella</i>	Fw	5'-CACCAAGGCGACGATGA-3'	<i>Prevotella malaninogenica</i>	JCM6325	Larsen <i>et al</i> : PLoS ONE, 5: e9085, 2010.
	Rv	5'-GGATAACGCCTGGACCT-3'			
<i>Clostridium leptum</i> subgroup	Fw	5'-GCACAAGCAGTGGAGT-3'	<i>Ruminococcus albus</i>	JCM14654	Matsuki <i>et al</i> : Appl Environ Microbiol, 70: 7220-8, 2004.
	Rv	5'-CTTCCTCCGTTTTGTCAA-3'			
<i>Clostridium coccoides</i> group	Fw	5'-AAATGACGGTACCTGACTAA-3'	<i>Clostridium coccoides</i>	JCM1395T	Matsuki <i>et al</i> : Appl Environ Microbiol, 70: 7220-8, 2004.

	Rv	5'-CTTTGAGTTTCATTCTTGCGAA-3'			
<i>Atopobium cluster</i>	Fw	5'-GGTTGAGAGACCGACC-3'	<i>Collinsella aerofaciens</i>	JCM10188	Matsuki <i>et al</i> : Appl Environ Microbiol, 70: 7220-8, 2004.
	Rv	5'-CGGRGCTTCTTCTGCAGG-3'			

R =A or G, K=T or G, Y=C or T.

Table S3. List of primers used for real-time PCR.

gene symbol	Forward	Reverse	reference
<i>IFN-γ</i>	5'-GCATCTTGGCTTTGCAGCT-3'	5'-CCTTTTTTCGCCTTGCTGTTG-3'	Takiguchi, H <i>et al</i> : J Oral Sci, 54: 23-32, 2012.
<i>IL-1β</i>	5'-CGCAGCAGCACATCAACAAGAGG-3'	5'-TGTCCTCATCCTGGAAGGTCCAC-3'	Aoe, S <i>et al</i> : Int J Hum Cult Stud, 26: 277-282, 2016.
<i>IL-4</i>	5'-CTTCCAAGGTGCTTCGCATA-3'	5'-AAGCCCGAAAGAGTCTCTGC-3'	Yoon <i>et al</i> : BMC Complement Altern Med, 15: 353, 2015.
<i>IL-5</i>	5'-CCCCACGGACAGTTTGATT-3'	5'-TGAGGCTTCCTGTCCCTACT-3'	Primer design was performed by NCBI Primer-Blast tool.
<i>IL-6</i>	5'-CGGCCTTCCCTACTTCACAA-3'	5'-TCTGCAAGTGCATCATCGTT-3'	Mio, K <i>et al</i> : nutrients, 12: 3546, 2020.
<i>IL-10</i>	5'-TGGACAACATACTGCTAACC-3'	5'-GGATCATTTCCGATAAAGGCT-3'	Paiva <i>et al</i> : BMC Microbiology, 13: 69, 2013.
<i>IL-12</i>	5'-AGCACCAGCTTCTTCATCAGG-3'	5'-GCGCTGGATTGAAACAAAG-3'	Paiva <i>et al</i> : BMC Microbiology, 13: 69, 2013.
<i>IL-17</i>	5'-GCTCCAGAAGGCCCTCAGA-3'	5'-CTTTCCCTCCGCATTGACA-3'	Paiva <i>et al</i> : BMC Microbiology, 13: 69, 2013.
<i>IL-33</i>	5'-GGGCTCACTGCAGGAAAGTA-3'	5'-TTTGCCGGGAAATCTTGGA-3'	Primer design was performed by NCBI Primer-Blast tool.
<i>TNF-α</i>	5'-ACCCTCACACACTCAGATCATCTTC-3'	5'-TGGTGGTTTGCTACGACGT-3'	Aoe, S <i>et al</i> : Int J Hum Cult Stud, 26: 277-282, 2016.
<i>TGFβ</i>	5'-AGGGCTACCATGCCAACTTC-3'	5'-CCACGTAGTAGACGATGGGC-3'	Lai D <i>et al</i> : PLoS One, 9(5): e98749, 2014.
<i>pIgR</i>	5'-TTGTTACGCTCTTGTAAGT-3'	5'-ACAGGCCTCGGTTACTGGTACC-3'	Blanch, VJ <i>et al</i> : J Immunol, 62: 1232-5, 1999.
Reference <i>36B4</i>	5'-GGCCCTGCACTCTCGCTTTC-3'	5'-TGCCAGGACGCGCTTGT-3'	Aoe, S <i>et al</i> : nutrients, 11: 1674, 2019.

R =A or G. IFN-γ, interferon gamma; IL-10, interleukin 10; IL-12, interleukin 12; IL-17, interleukin 17; IL-1β, interleukin 1 beta; IL-33, interleukin 33; IL-4, interleukin 4; IL-5, interleukin 5; IL-6, interleukin 6; pIgR, polymeric immunoglobulin receptor; TGFβ, transforming growth factor beta; TNF-α, tumor necrosis factor-α.

Table S4. KEGG enrichment analysis with upregulated DEGs in the ileum of barley group by using DAVID database.

ID	Pathway name	Count	P value	FDR
mmu00591	Linoleic acid metabolism	12	8.3e-6	2.0e-3
mmu00590	Arachidonic acid metabolism	15	3.0e-5	3.6e-3
mmu04640	Hematopoietic cell lineage	14	7.8e-5	6.2e-3
mmu00830	Retinol metabolism	14	1.3e-4	7.4e-3
mmu04060	Cytokine-cytokine receptor interaction	25	1.5e-4	7.4e-3
mmu05150	Staphylococcus aureus infection	10	2.9e-4	1.1e-2
mmu05340	Primary immunodeficiency	8	5.8e-4	2.0e-2
mmu05204	Chemical carcinogenesis	13	6.7e-4	2.0e-2
mmu04514	Cell adhesion molecules (CAMs)	18	7.5e-4	2.0e-2
mmu04662	B cell receptor signaling pathway	11	9.1e-4	2.2e-2
mmu00140	Steroid hormone biosynthesis	12	1.4e-3	3.1e-2
mmu04610	Complement and coagulation cascades	11	1.7e-3	3.4e-2

FDR were adjusted by using Benjamini-Hochberg algorithm.

Table S5. Final weight, body weight gain, food intake, and organ weight in mice fed the control and HGB diets.

	Control	HGB
Initial weight (g)	20.0 ± 0.3	20.0 ± 0.4
Final weight (g)	42.4 ± 1.5	38.4 ± 1.2*
Body weight gain (g/day)	0.25 ± 0.02	0.20 ± 0.01*
Food intake (g/day)	2.85 ± 0.06	2.82 ± 0.04
Food efficiency ratio (%)	8.7 ± 0.4	7.2 ± 0.3*
Liver (g)	1.5 ± 0.1	1.2 ± 0.1*
Cecum with digesta (g)	0.2 ± 0.02	0.4 ± 0.03*
Epididymal fat (g)	2.3 ± 0.1	2.2 ± 0.2
Retroperitoneal fat (g)	1.0 ± 0.1	0.7 ± 0.1*
Mesenteric fat (g)	1.1 ± 0.2	0.6 ± 0.1*

Values are mean ± SE (n=8). * $p < 0.05$ showed significantly different among each group. Food efficiency ratio (%) = Body weight gain/Food intake × 100. HGB: High β -glucan barley group.

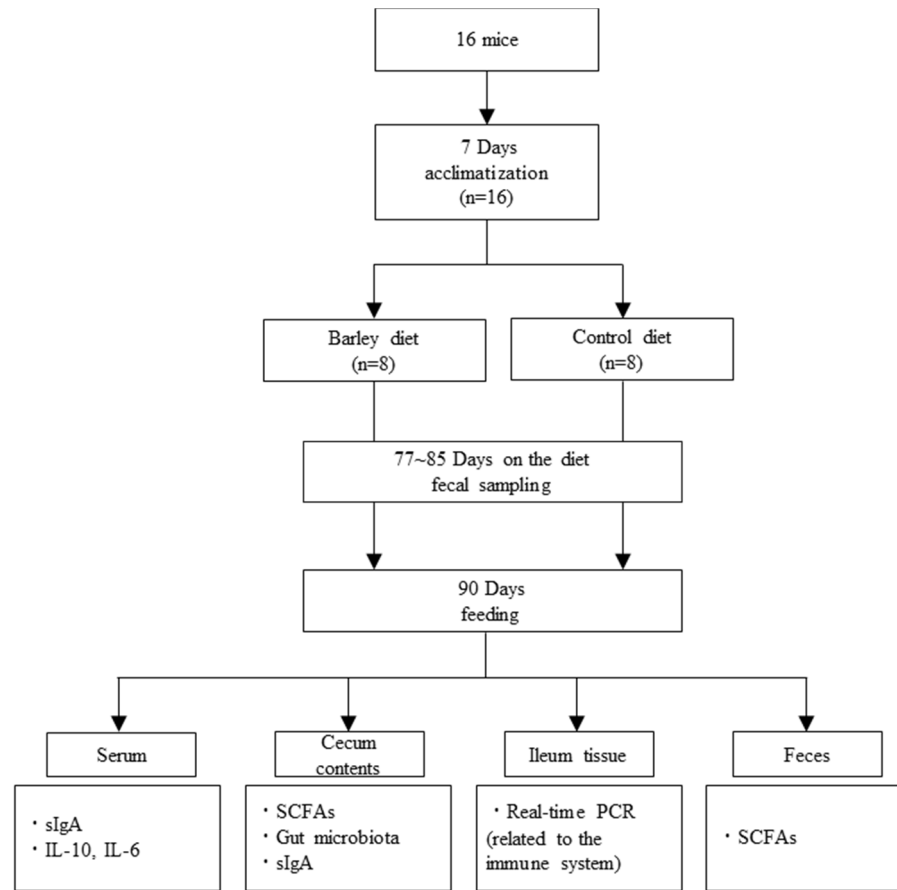


Figure S1. Flow chart showing the study design of the animal studies. sIgA, secretory immunoglobulin A; SCFAs, Short-chain fatty acids.

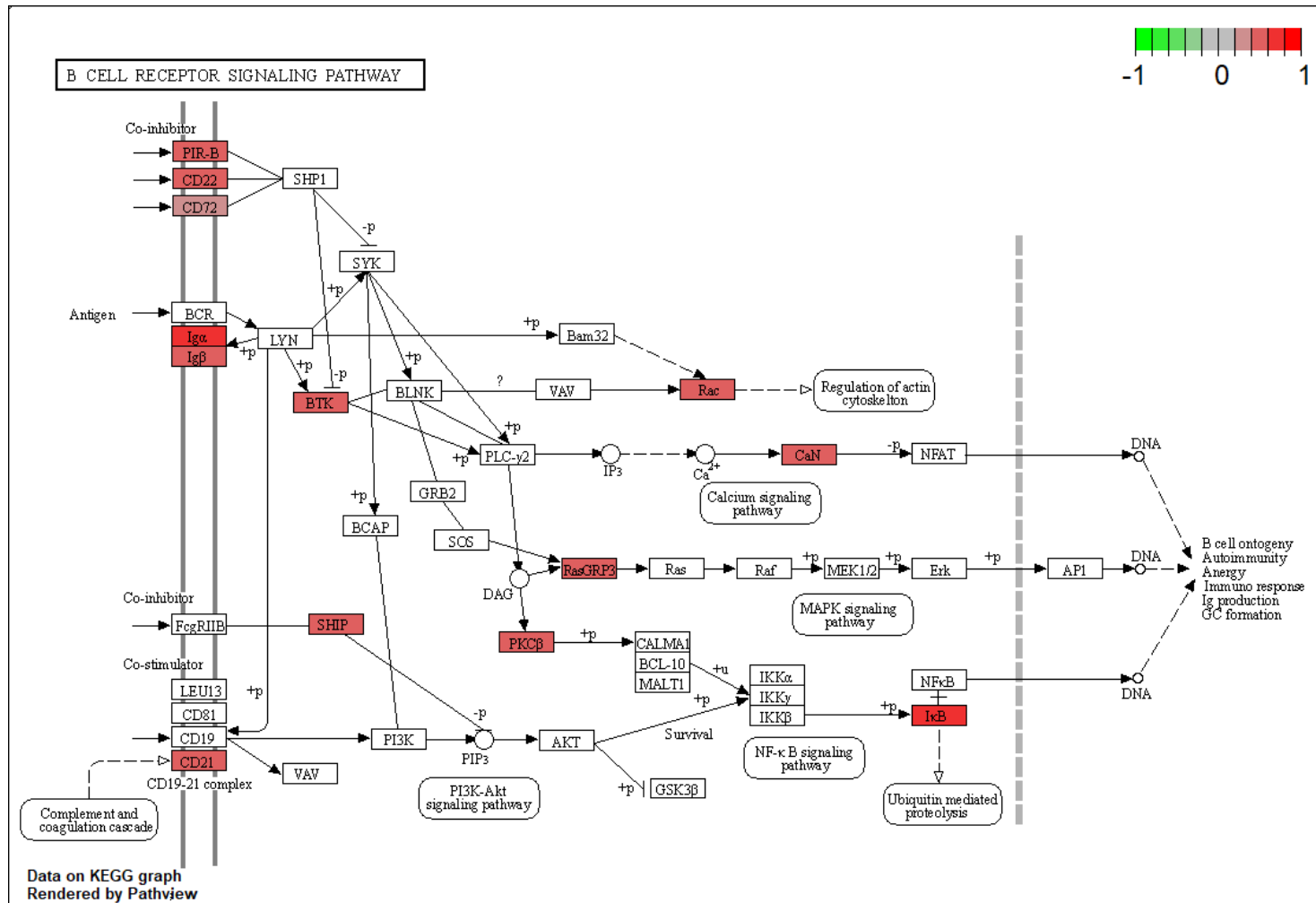
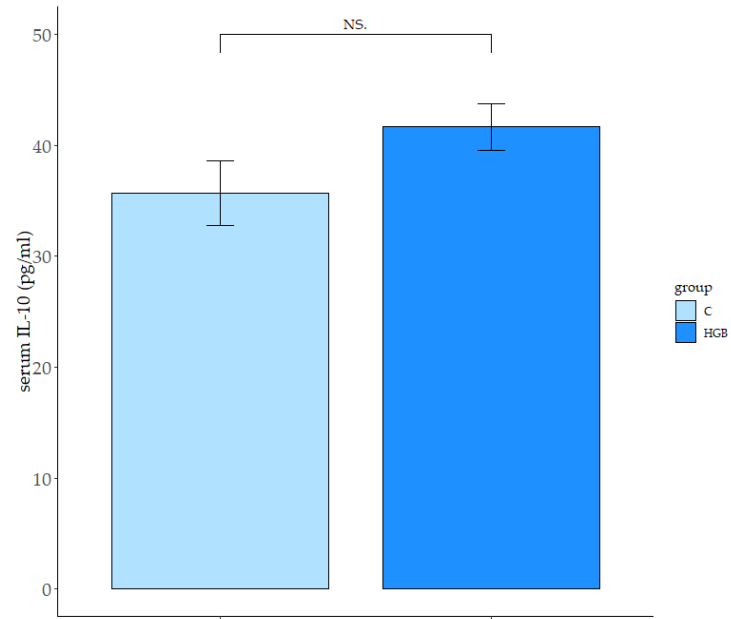


Figure S2. DEGs up-regulated by barley group involved in B cell receptor signaling pathway (extracted from Kyoto Encyclopedia of Genes and Genomes pathway). DEGs: Differential expressed genes had selected the cut-off criteria (Log-ratio > 1.3 fold in the barley group compared with the control group). The color depth in the figure indicates the Log-ratio values.

(a)



(b)

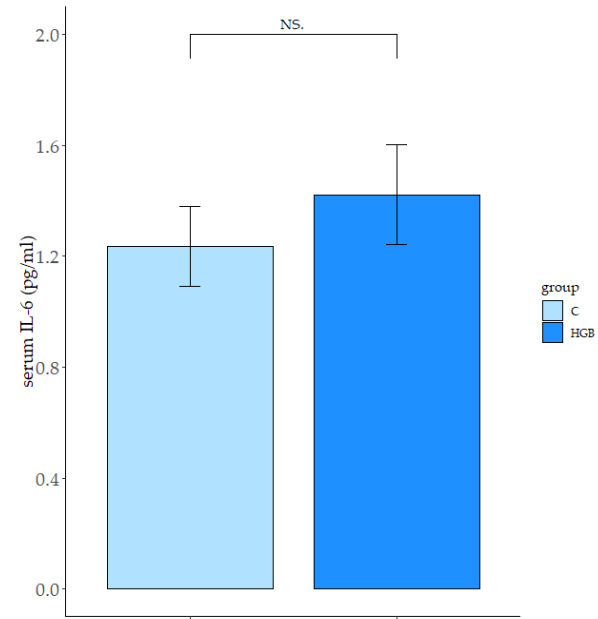


Figure S3. The concentration of IL-10 (a) and IL-6 (b) in serum. Values are means \pm SE, n=8. C: control group, HGB: high β -glucan barley group. "NS" is not significant difference between each group.

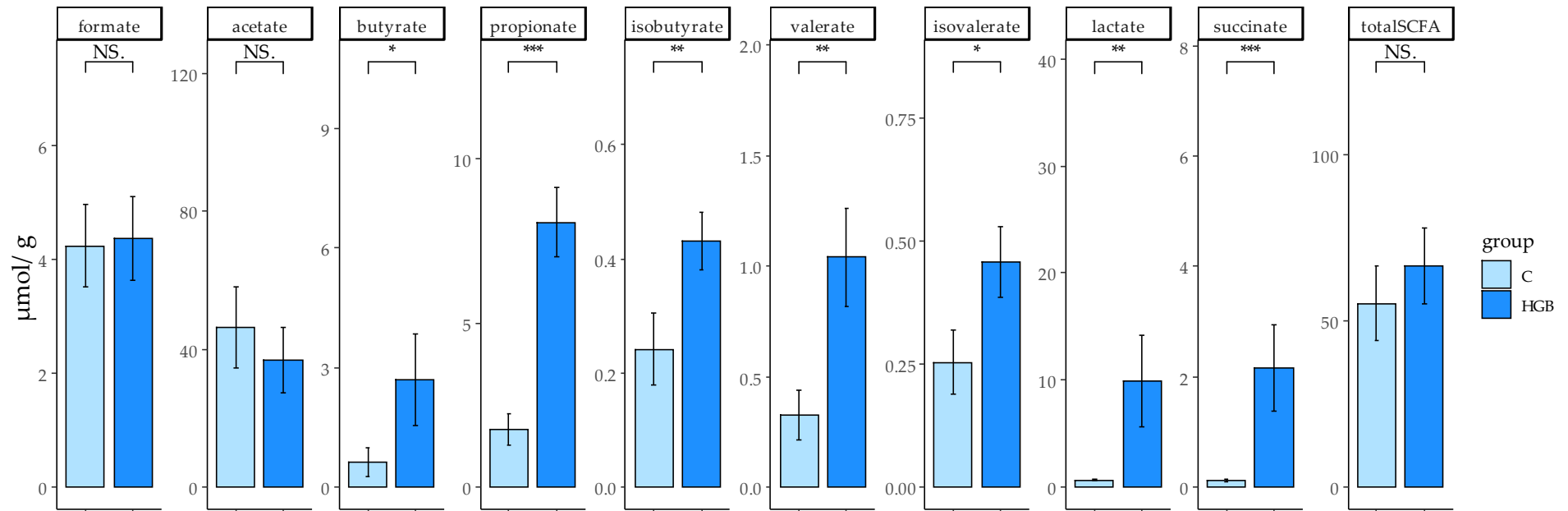


Figure S4. The concentration of SCFAs and organic acids in feces. Values are means \pm SE, n=8. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ showed a significant difference between each group, "NS" is not significant. C: control group, HGB: high β -glucan barley group. C: control group, HGB: high β -glucan barley group.