

Impact of Osmotic Dehydration and Lactic Acid Fermentation of Onion on the Physiological Effects in Rats

by

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Supplementary Materials

Table S1. The composition of control and experimental diets (g/100 g diet). **Table S2.** Hematological parameters in rats fed diets with onion preparations and control diet. **Table S3.** Microbial enzymatic activity in the cecal digesta in rats fed diets with onion preparations and control diet. **Table S4.** Plasma relative protein levels (normalized protein expression, NPX) related to immunological status in rats fed diets with onion preparations and control diet. **Table S5.** Plasma relative protein levels (normalized protein expression, NPX) related to redox status, glucose/insulin, lipids metabolism and intestinal tissue integrity in rats fed diets with onion preparations and control diet.

Table S1. The composition of control and experimental diets (g/100 g diet).

Ingredient	Control diet	Experimental diet
Casein ¹	14.8	14.8
DL-Methionine	0.2	0.2
Cellulose ²	8	8
Choline chloride	0.2	0.2
Cholesterol	0.3	0.3
Rapeseed oil	8	8
Vitamin mix ³	1	1
Mineral mix ⁴	3.5	3.5
Saccharose	10	10
Onion preparation ⁵	0	2
Maize starch ⁶	54	52

¹Casein preparation: crude protein 89.7%, crude fat 0.3%, ash 2.0%, and water 8.0%.

² α -Cellulose (Sigma-Aldrich, Saint Louis, MO, USA), main source of dietary fibre.

³AIN-93G-VM, g/kg mix: 3.0 nicotinic acid, 1.6 Ca pantothenate, 0.7 pyridoxine-HCl, 0.6 thiamin-HCl, 0.6 riboflavin, 0.2 folic acid, 0.02 biotin, 2.5 vitamin B₁₂ (cyanocobalamin, 0.1% in mannitol), 15.0 vitamin E (all-*rac*- α -tocopheryl acetate, 500 IU/g), 0.8 vitamin A (all-*trans*-retinyl palmitate, 500000 IU/g), 0.25 vitamin D₃ (cholecalciferol, 400000 IU/g), 0.075 vitamin K₁ (phyloquinone), 974.655 powdered sucrose.

⁴Mineral mix, g/kg mix: 357 calcium carbonate anhydrous CaCO₃, 196 dipotassium phosphate K₂HPO₄, 70.78 potassium citrate C₆H₅K₃O₇, 74 sodium chloride NaCl, 46.6 potassium sulfate K₂SO₄, 24 magnesium oxide MgO, 18 microelement mixture, starch to 1 kg =213.62. Microelement mixture, g/kg mix: 31 iron (III) citrate (16.7% Fe), 4.5 zinc carbonate ZnCO₃ (56% Zn), 23.4 manganese (II) carbonate MnCO₃ (44.4% Mn), copper carbonate CuCO₃ (55.5% Cu), 0.04 potassium iodide KI, citric acid C₆H₈O₇ to 100 g.

⁵In the experimental diets, lyophilized onion preparations were used including untreated onion (v. Allurion), onion osmotically dehydrated in a glucose-fructose syrup, and onion osmotically dehydrated in a glucose-fructose syrup and then fermented with *Levilactobacillus brevis* LOCK 944 or *Lactiplantibacillus plantarum* P27.

⁶Maize starch preparation: crude protein 0.6%, crude fat 0.9%, ash 0.2%, total dietary fiber 0%, and water 8.8%.

Table S2. Hematological parameters in rats fed diets with onion preparations and control diet.

Parameter	C	G	GD	GDFa	GDFb	SEM	<i>p</i> -Value	Effect size η^2
WBC ($10^3/\mu\text{L}$)	6.532	6.299	5.766	5.917	5.779	0.127	0.220	0.117
LYM ($10^3/\mu\text{L}$)	5.490	4.998	4.819	4.988	4.902	0.115	0.393	0.085
MID ($10^3/\mu\text{L}$)	0.242	0.279	0.272	0.266	0.292	0.032	0.967	0.012
NEU ($10^3/\mu\text{L}$)	0.802 ^{ab}	1.019 ^a	0.663 ^b	0.662 ^b	0.582 ^b	0.040	0.002	0.304
LYM (%)	83.82	79.74	83.73	83.84	84.82	0.685	0.150	0.136
MID (%)	3.740	4.500	4.740	4.780	5.070	0.379	0.852	0.028
NEU (%)	12.45 ^{ab}	15.76 ^a	11.52 ^{ab}	11.41 ^{ab}	10.12 ^b	0.541	0.009	0.252
RBC ($10^6/\mu\text{L}$)	8.621	8.173	8.112	8.440	8.243	0.081	0.259	0.108
HGB (g/dL)	13.74	13.08	12.96	13.50	13.26	0.117	0.216	0.118
HCT (%)	42.80	40.66	40.76	42.08	41.54	0.364	0.302	0.100
MCV (fL)	49.70	49.80	50.30	50.00	50.60	0.255	0.807	0.034
MCH (pg)	15.97	16.01	15.96	16.00	16.13	0.065	0.934	0.017
MCHC (g/dL)	32.13	32.26	31.77	32.08	31.93	0.110	0.683	0.048
RDWc (%)	18.67	18.60	18.24	18.23	18.41	0.069	0.138	0.140
PLT ($10^3/\mu\text{L}$)	689.4	759.7	720.1	724.7	734.7	12.671	0.536	0.065
PCT (%)	0.554	0.598	0.574	0.582	0.576	0.010	0.718	0.044
MPV (fL)	7.980	7.890	7.978	8.030	7.830	0.035	0.385	0.086
PDWc (%)	34.93	34.62	34.97	35.30	34.38	0.107	0.063	0.175

Mean values within a row with unlike superscript letters are shown to be significantly different ($p < 0.05$; post-hoc Tukey test); η^2 , partial eta squared; SEM, pooled standard error of the mean (standard deviation for all rats divided by the square root of rat number, $n=50$); WBC, total white blood cells; LYM, lymphocytes; MID, mid-sized cells; NEU, neutrophils; RBC, red blood cells; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDWc, red cell distribution width; PLT, platelet count; PCT, platelet percentage; MPV, mean platelet volume; PDWc, platelet distribution width. Experimental groups: C, control diet; G, diet with 2% (w/w) of untreated onion (v. Allurion) preparation; GD, diet with 2% (w/w) of onion preparation obtained by osmotic dehydration in a glucose-fructose syrup; GDFa, diet with 2% (w/w) of onion preparation obtained by osmotic dehydration in a glucose-fructose syrup and fermentation with *Levilactobacillus brevis* LOCK 944; GDFb, diet with 2% (w/w) of onion preparation obtained by osmotic dehydration in a glucose-fructose syrup and fermentation with *Lactiplantibacillus plantarum* P27.

Table S3. Microbial enzymatic activity in the cecal digesta in rats fed diets with onion preparations and control diet.

Enzyme	C	G	GD	GDFa	GDFb	SEM	<i>p</i> -Value	Effect size η^2
α-Glucosidase								
Extracellular ($\mu\text{mol/h/g}$ digesta)	21.18	21.38	24.01	24.94	26.07	1.083	0.539	0.065
Intracellular ($\mu\text{mol/h/g}$ digesta)	3.543	2.217	1.760	1.385	2.172	0.276	0.131	0.143
Total ($\mu\text{mol/h/g}$ digesta)	24.73	23.60	25.77	26.33	28.24	1.138	0.771	0.038
Release rate (%)	86.82	90.61	93.23	93.67	92.24	0.897	0.098	0.156
β-Glucosidase								
Extracellular ($\mu\text{mol/h/g}$ digesta)	5.755	6.276	5.472	6.420	6.108	0.249	0.761	0.039
Intracellular ($\mu\text{mol/h/g}$ digesta)	1.792	1.750	1.250	1.199	0.959	0.110	0.057	0.181
Total ($\mu\text{mol/h/g}$ digesta)	7.547	8.026	6.722	7.619	7.066	0.287	0.658	0.051
Release rate (%)	76.79	77.55	81.72	83.34	85.97	1.219	0.080	0.165
α-Galactosidase								
Extracellular ($\mu\text{mol/h/g}$ digesta)	9.397	10.86	10.75	9.210	8.338	0.381	0.170	0.130
Intracellular ($\mu\text{mol/h/g}$ digesta)	4.528	4.642	4.185	3.443	3.531	0.254	0.440	0.078
Total ($\mu\text{mol/h/g}$ digesta)	13.93	15.50	14.93	12.65	11.87	0.555	0.198	0.122
Release rate (%)	67.56	71.61	72.75	73.68	69.50	1.141	0.447	0.077
β-Galactosidase								
Extracellular ($\mu\text{mol/h/g}$ digesta)	27.21	30.90	30.72	25.13	26.80	1.127	0.404	0.083
Intracellular ($\mu\text{mol/h/g}$ digesta)	2.516	3.571	2.436	1.922	2.590	0.284	0.482	0.072
Total ($\mu\text{mol/h/g}$ digesta)	29.73	34.47	33.15	27.05	29.39	1.146	0.241	0.112
Release rate (%)	91.35	89.39	92.54	92.93	89.81	0.874	0.640	0.053

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Table S4. Plasma relative protein levels (normalized protein expression, NPX) related to immunological status in rats fed diets with onion preparations and control diet.

Protein	C	G	GD	GDFa	GDFb	SEM	<i>p</i> -Value	Effect size η^2
Ahr	0.363	0.433	0.363	0.323	0.263	0.026	0.329	0.095
Casp3	6.421	6.178	6.581	6.440	6.538	0.105	0.795	0.035
Ccl2	10.21	10.20	10.26	10.17	10.27	0.022	0.542	0.065
Ccl3	3.975	3.787	3.743	3.530	3.938	0.097	0.626	0.055
Ccl5	8.157	8.229	8.132	7.905	8.497	0.139	0.775	0.038
Ccl20	0.286	0.354	0.202	0.225	0.242	0.059	0.940	0.016
Cxcl1	5.897	5.621	5.719	5.446	5.580	0.108	0.763	0.039
Cxcl9	3.961	3.544	3.951	3.469	3.700	0.079	0.156	0.134
Cyr61	5.233	5.025	4.979	4.906	5.160	0.100	0.849	0.029
Fst	7.296	7.721	7.571	7.706	7.401	0.084	0.427	0.080
Igsf3	3.891	3.849	3.902	3.885	3.840	0.024	0.916	0.020
Il1a	1.073	0.933	1.244	1.208	1.111	0.047	0.255	0.109
Il5	0.114	0.104	0.253	0.199	0.276	0.043	0.621	0.055
Il6	0.011	0.011	0.011	0.013	0.021	0.002	0.516	0.068
Il17a	0.984	1.300	1.13	1.110	1.363	0.053	0.156	0.134
Il23r	1.425	1.378	1.543	1.291	1.38	0.054	0.595	0.058
Itgb6	1.956	1.970	1.926	1.940	1.904	0.042	0.990	0.006
Map2k6	2.946	3.058	2.844	2.970	2.993	0.065	0.895	0.023
Plxna4	4.700	4.688	4.958	4.929	4.394	0.097	0.363	0.089
S100a4	7.976	8.034	7.995	8.073	7.976	0.032	0.854	0.028
Tgfb1	1.954	1.924	1.901	1.662	1.964	0.122	0.918	0.020
Tgfb3	10.84	10.78	10.86	10.76	10.85	0.015	0.121	0.146
Tnfrsf11b	0.198	0.211	0.168	0.144	0.065	0.025	0.396	0.084
Tnfrsf12a	6.321	6.161	6.295	6.359	6.122	0.062	0.704	0.046
Tnfsf12	2.967	2.925	2.790	2.870	2.987	0.052	0.775	0.038
Vsig2	1.035	1.255	0.834	0.916	0.828	0.075	0.349	0.092

Mean values within a row with unlike superscript letters are shown to be significantly different ($p < 0.05$; post-hoc Tukey test); η^2 , partial eta squared; SEM, pooled standard error of mean (standard deviation for all rats divided by the square root of rat number, $n=50$); Ahr, aryl hydrocarbon receptor; Casp3, caspase-3; Ccl2, C-C motif chemokine 3; Ccl2, C-C motif chemokine 3; Ccl5, C-C motif chemokine 5; Ccl20, C-C motif chemokine 20; Cxcl1, growth-regulated alpha protein; Cxcl9, C-X-C motif chemokine 9; Cyr61, cysteine-rich angiogenic inducer 61; Fst, follistatin; Igsf, immunoglobulin superfamily member 3; Il1a, interleukin-1 alpha; Il5, interleukin-5; Il6, interleukin-6; Il17a, interleukin-17A; Il23r, interleukin-23 receptor; Itgb6, integrin beta-6; Map2k6, mitogen-activated protein kinase kinase 6; Plxna4, plexin-A4; S100a4, S100 calcium-binding protein A4; Tgfb1, transforming growth factor beta-1; Tgfb3, transforming growth factor beta receptor type 3; Tnfrsf11b, tumor necrosis factor receptor superfamily member 11B; Tnfrsf12a, tumor necrosis factor receptor superfamily member 12A; Tnfsf12, tumor necrosis factor ligand superfamily member 12; Vsig2, V-set and immunoglobulin

domain-containing protein 2. Experimental groups: C, control diet; G, diet with 2% (w/w) of untreated onion (v. Allurion) preparation; GD, diet with 2% (w/w) of onion preparation obtained by osmotic dehydration in a glucose-fructose syrup; GDFa, diet with 2% (w/w) of onion preparation obtained by osmotic dehydration in a glucose-fructose syrup and fermentation with *Levilactobacillus brevis* LOCK 944; GDFb, diet with 2% (w/w) of onion preparation obtained by osmotic dehydration in glucose-fructose syrup and fermentation with *Lactiplantibacillus plantarum* P27.

Table S5. Plasma relative protein levels (normalized protein expression, NPX) related to redox status, glucose/insulin, lipid metabolism, and intestinal tissue integrity in rats fed diets with onion preparations and control diet.

Protein	C	G	GD	GDFa	GDFb	SEM	<i>p</i> -Value	Effect size η^2
Redox status								
Foxo1	0.498	0.419	0.599	0.676	0.438	0.045	0.321	0.096
Nadk	5.804	6.007	5.567	5.771	5.573	0.061	0.122	0.146
Prdx5	2.240	2.358	2.394	2.551	2.150	0.102	0.786	0.036
Riox2	1.675	1.935	1.867	1.898	1.870	0.057	0.665	0.050
Glucose/insulin								
Cpe	3.538	3.531	3.377	3.380	3.410	0.040	0.523	0.067
Dlk1	5.327	5.340	5.380	5.210	5.251	0.034	0.527	0.066
Eno2	0.259	0.125	0.199	0.192	0.089	0.029	0.396	0.084
Fstl3	5.136	5.193	5.023	5.018	5.089	0.034	0.424	0.080
Gcg	1.008	1.145	0.846	1.023	0.893	0.081	0.807	0.034
Ghr	2.672	2.838	2.707	2.652	2.704	0.048	0.782	0.037
Lipids								
Lpl	5.749	5.396	5.501	5.476	5.267	0.079	0.413	0.082
Plin1	0.042	0.020	0.084	0.011	0.027	0.014	0.498	0.070
Intestine integrity								
Clmp	6.670	6.726	6.573	6.688	6.638	0.034	0.685	0.048
Epcam	2.374	1.813	2.005	1.956	1.947	0.081	0.246	0.111
Matn2	2.038	2.105	1.999	2.152	1.914	0.050	0.625	0.055

Mean values within a row with unlike superscript letters are shown to be significantly different ($p < 0.05$; post-hoc Tukey test); η^2 , partial eta squared; SEM, pooled standard error of mean (standard deviation for all rats divided by the square root of rat number, $n=50$); Clmp, CXADR-like membrane protein; Cpe, carboxypeptidase E; Dlk1, delta-like 1 homolog; Eno2, enolase 2; Epcam, epithelial cell adhesion molecule; Foxo1, forkhead box protein O1; Fstl3, follistatin-related protein 3; Gcg, pro-glucagon; Ghr, ghrelin; Lpl, lipoprotein lipase; Matn2, matrilin-2; Nadk, NAD kinase; Plin1, perilipin-1; Prdx5, peroxiredoxin-5; Riox2, ribosomal oxygenase 2. Experimental groups: C, control diet; G, diet with 2% (w/w) of untreated onion (v. Allurion) preparation; GD, diet with 2% (w/w) of onion preparation obtained by osmotic dehydration in a glucose-fructose syrup; GDFa, diet with 2% (w/w) of onion preparation obtained by osmotic dehydration in a glucose-fructose syrup and fermentation with *Levilactobacillus brevis* LOCK 944; GDFb, diet with 2% (w/w) of onion preparation obtained by osmotic dehydration in a glucose-fructose syrup and fermentation with *Lactiplantibacillus plantarum* P27.