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

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This study investigated the effects of four different dietary onion (*Allium cepa* L.) preparations from the new Allurion variety on body composition, metabolic parameters, hepatic gene expression, and gut microbial activity in healthy male Wistar rats. Fifty 7-week old rats were randomly assigned to five dietary groups and fed semi-purified diets for four weeks: control, untreated onion, osmotically dehydrated onion with a glucose-fructose syrup, and dehydrated onion further subjected to lactic acid fermentation with *Levilactobacillus brevis* ŁOCK 944 and *Lactiplantibacillus plantarum* P27. Body weight, feed intake, and body composition were monitored, and blood, liver, and cecal samples were collected for biochemical, enzymatic activity, bile acid, short-chain fatty acid, and gene expression analyses. Dietary onion preparations reduced hepatic expression of lipogenic genes *Hif1a*, *Scd1*, and *Acly*, indicating anti-lipogenic effects. Osmotic dehydration boosted these benefits by suppressing inflammatory and lipogenic genes (*Nfkb1*, *Srebf1*, *Tlr4*) and improving plasma markers of liver function and lipid metabolism. Fermentation had a preparation-specific impact on liver genes that regulate fatty acid oxidation, bile acid metabolism, and inflammation. The percentage of lean tissue decreased in dehydrated onion-fed rats, yet there were no changes in their overall body weight, and fermentation did not further affect their body composition. Cecal enzyme activities and bile acid profiles were modulated, reflecting favorable gut microbial activity. These findings demonstrate that dietary onion, particularly when processed *via* osmotic dehydration and selective fermentation, can beneficially modulate hepatic gene expression, plasma biochemistry, and gut microbial function, supporting its potential as a functional dietary component to promote metabolic health.

Keywords: intestine, liver, microbial acidification, onion, osmotic drying, rat

ABBREVIATIONS

AC, atherogenic coefficient; AIP, atherogenic index of plasma; BA, bile acid; C, control diet (group); G, diet (group) with 2% (*w/w*) of untreated onion preparation; GD, diet (group) with 2% (*w/w*) of onion preparation obtained by osmotic dehydration in a glucose-fructose syrup; GDFa, diet (group) with 2% (*w/w*) of onion preparation obtained by osmotic dehydration in a glucose-fructose syrup and fermentation with *Levilactobacillus brevis* ŁOCK 944; GDFb, diet (group) with 2% (*w/w*) of onion preparation obtained by osmotic dehydration in a glucose-fructose syrup

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and fermentation with *Lactiplantibacillus plantarum* P27; PSCFA, putrefactive short-chain fatty acids; SCFA, short-chain fatty acids.

INTRODUCTION

Onion (*Allium cepa* L.) is a widely consumed vegetable rich in vitamins and bioactive compounds, including flavonoids (such as quercetin), sulfur-containing compounds, and prebiotic fibers, which together exert diverse health-promoting effects. Regular intake of onion has been associated with improvements in cardiometabolic risk factors, including reductions in body weight, waist circumference, blood lipid levels, and markers of glucose metabolism in humans, particularly with longer supplementation periods or higher daily doses (>300 mg/day), as shown in randomized trials and meta-analyses [Hejazi *et al*., 2023]. This metabolic modulation likely reflects potent antioxidant and anti-inflammatory effects of onion, which support cardiovascular health through lipid and blood pressure reduction, endothelial function improvement, as well as potential antidiabetic effects *via* enhanced glycemic control [Li *et al*., 2020]. Onions also contain significant levels of prebiotic fructans that favorably influence gut microbiota composition and short-chain fatty acid production, contributing to digestive health and systemic metabolic benefits, and their flavonoid content has been linked to reduced oxidative stress [Benítez *et al*., 2011; Gupta *et al*., 2025].

Osmotic dehydration is a mild food processing technique in which plant tissues are partially dehydrated in a hypertonic solution, leading to water removal while limiting high-temperature exposure and preserving key quality attributes of fruits and vegetables. Compared with conventional high-heat drying methods, osmotic dehydration can help retain important bioactive compounds, including phenolic compounds [Yazidi *et al*., 2024]. Studies on onion have shown that significant amounts of flavonols, such as quercetin derivatives, can be preserved or stabilized during osmotic dehydration, suggesting that this process may enhance the functional value of dried onion preparations while improving their texture and shelf stability [Grzelak-Błaszczyk *et al*., 2021]. Despite several studies on osmotic dehydration and compositional changes in plant foods, in the current scientific literature there are no published *in vivo* animal or human trials testing health endpoints (*e.g.*, blood cholesterol and triglyceride content, liver enzyme activity) specifically after consumption of osmotically dehydrated vegetables or fruit.

Osmotic dehydration prior to further processing has been shown to affect the retention and stability of bioactive compounds, indicating that careful selection of dehydration conditions can help maintain nutritional quality of dried onion products. Subsequent lactic acid fermentation of osmodehydrated onion allows for the development of fermented products with potentially enhanced functional properties. It has been shown that fermentation does not adversely affect phenolic content and may even improve the profile of beneficial organic acids without compromising the availability of fructooligosaccharides [Grzelak-Błaszczyk *et al*., 2023]. Fermentation processes are also known to increase the bioavailability of phenolic compounds and modify carbohydrate profiles in plant foods, which can lead to increased antioxidant activity and greater potential for modulating gut health [Nowak *et al*., 2025].

The goal of this study was to thoroughly evaluate the efficacy of osmotic dehydration techniques utilizing a glucose-fructose syrup solution. This was done both as a standalone method and in combination with lactic acid fermentation processes. The aim was to develop innovative preparations from onion of the Allurion variety with enhanced functional properties. The research also focused on investigating the *in vivo* effects of these specially formulated preparations on large intestinal function and various metabolic parameters in rats, providing a comprehensive understanding of their potential health benefits.

MATERIALS AND METHODS

Onion preparation

The preparations were made from red-skinned onion of the Allurion variety grown at the Institute of Horticulture in Skierniewice, Poland. After harvest, the bulbs were stripped of inedible parts and cut lengthwise into eight pieces. Then, five times the amount of the hypertonic solution was added. Osmoconcentration was carried out using a 50% glucose-fructose syrup (Kemikals, Gdynia, Poland) and a 5% sodium chloride (Chempur, Piekary Śląskie, Poland) solution, in a water bath with a shaker (GFL 1086, Lauda GFL, Burgwedel, Germany) at 25°C and a shaking frequency of 150 cycles/min for 2 h. After osmotic dehydration, the onion samples were double-rinsed with water for 5 s, blotted dry, and then one-third of the samples were lyophilized using an Alpha 1-2 LDplus lyophilizer (Christ, Osterode am Harz, Germany) at a vacuum of 0.26 mbar for 48 h. The remaining samples were immersed in sterile water at a ratio of 50 g of the material *per* 100 mL and divided into two parts. Each experimental variant was inoculated separately with a monoculture prepared from an overnight culture of either *Lactiplantibacillus plantarum* P27 [Ścieszka *et al*., 2025] or *Levilactobacillus brevis* ŁOCK 944 [Klewicka *et al*., 2013]. In each case, the inoculum was added at 10% (*v/v*), providing approximately 10^7 cells/mL. Fermentation was conducted at 30°C for 96 h. After fermentation, the onion was separated from the broth, and the obtained fermented samples were lyophilized under the same conditions as described earlier.

Analysis of onion preparations

The proximate composition of the preparations was determined by AOAC International methods using the following procedures: protein content – 920.152; crude fat – 930.09; dry matter and ash content – 940.26, total dietary fiber (TDF) – 985.29 [Horwitz & Latimer, 2007].

Phenolic compound profile was determined according to the method described by Grzelak-Błaszczyk *et al*. [2020]. Onion preparations weighing 0.5 g (±0.0001 g) were extracted with 70% (*v/v*) methanol for 15 min at room temperature in an IS 4 ultrasonic cleaner (Intersonic S.C., Olsztyn, Poland). The process was repeated three times, and the filtrates from each step were combined. The extracts were analyzed by the high-performance liquid chromatography (HPLC) method using a Dionex Ultimate 3000 system with a photodiode array (PDA) detector and Chromeleon software (Thermo Fisher Scientific Inc., Waltham, MA, USA). A Kinetex C18 column (100 Å, 150 mm×2.1 mm, 2.6 µm; Phenomenex, Torrance, CA, USA) was used. The mobile phase consisted of solvent A (0.1% formic acid in H_2O, *v/v*) and solvent B (0.1% formic acid in 90% acetonitrile, *v/v*), which were applied in the following gradient program: 0–1 min 5% B; 1–15 min 5%–35% B; 15–17 min 35%–80% B; 17–21 min 80% B; 21–22 min 80–5% B; and 22–30 min 5% B. The injection volume was 5 µL, and the mobile phase flow rate was 0.5 mL/min. Separation was carried out at 35°C. Chromatograms were recorded at a wavelength of 360 nm for flavonols and 520 nm for anthocyanins. The quantification was based on the following standards: quercetin 3,4'-diglucoside, quercetin 4'-glucoside, quercetin 3-*O*-glucoside, quercetin, and cyanidin 3-glucoside chloride (Extrasynthese, Genay, France). The total content of identified phenolic compounds was calculated as the sum of flavonols and anthocyanins and expressed in g/100 g of preparation.

The composition of mono-, di-, and oligosaccharides was determined according to the method described by Grzelak *et al*. [2009] with modifications. An onion preparation of 0.5 g (±0.0001 g) was extracted three times for 15 min using: 2, 1.5, and 1.5 mL of 70% (*v/v*) methanol. This process was carried out in an IS 4 ultrasonic cleaner (Intersonic S.C.) at room temperature. The combined extracts were concentrated using a speed-vacuum centrifuge (ScanSpeed 40, Denmark). Finally, the concentrated solution was diluted with 1 mL of 50% (*v/v*) acetonitrile and analyzed using a Knauer HPLC Smartline system (Berlin, Germany) equipped with a ClarityChrome 5.0.5. data acquisition system and a Knauer refractive index detector (K-2301). Separation was carried out under isocratic conditions on a Shodex NH_2P column (250×4 mm; Shodex, Tokyo, Japan). The mobile phase consisted of acetonitrile and water (68:32, *v/v*) and was used at a flow rate of 0.7 mL/min. The column temperature was maintained at 25°C. The following standards were used: fructose, glucose, sucrose, kestose, and nystose (Merck, Darmstadt, Germany). The total content of identified fructooligosaccharides was calculated as the sum of kestose, nystose, and frutosyl nystose and expressed in g/100 g of preparation.

To enumerate lactic acid bacteria (LAB) in the onion preparation, 1 g of the sample was aseptically transferred into 9 mL of sterile physiological saline (0.9% NaCl) and homogenized thoroughly to obtain the initial 10^{-1} dilution. Serial tenfold dilutions were subsequently prepared using the same diluent. Then, 1-mL dilutions were surface-plated in duplicate onto de Man, Rogosa, and Sharpe (MRS) agar (Merck, Darmstadt, Germany), a selective medium for the cultivation of LAB. The inoculated plates were incubated at 30±1°C for 48 h under anaerobic conditions. After incubation, plates containing 30-300 colonies were selected for enumeration, and the bacterial counts were expressed as $\log_{10}$ colony-forming units *per* gram (log CFU g^{-1}) of onion preparation.

Animals and diet

The experiment included 50 healthy male Wistar (Cmdb: WI) rats, aged 49 days and weighing 183.5 g on average (standard error of the mean: 3.447). This study was conducted in accordance with the approval granted by the Local Institutional Animal Care and Use Committee (Approval No. 41/2022; Olsztyn, Poland, June 15, 2022). We took every precaution to minimize suffering and enhance the welfare of the experimental animals, following the principles outlined in the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, Directive 2010/63/EU. The four-week feeding experiment involved five distinct experimental groups, each with 10 animals (*n*=10). The group size was calculated to ensure reliable research findings suitable for rigorous statistical analysis, adhering to the 3R principle – replacement, reduction, refinement – for ethical animal research. The rats were randomly assigned and housed individually in stainless steel cages within a controlled environment. Temperature was maintained at 21–22°C with a relative humidity of 60±10%. The facility featured a 12-h day-night cycle and a ventilation system providing 15 air changes *per* hour.

For four weeks, the rats were given unrestricted access to tap water and semi-purified diets. These diets were prepared and subsequently stored at 4°C in airtight containers until the termination of the experiment (details are provided in **Table S1** in Supplementary Materials). The diets were adapted from a casein diet for laboratory rodents as recommended by the American Institute of Nutrition. Five experimental treatments were utilized to assess the effects of four different onion preparations, each added at a 2% (*w/w*) dietary level, replacing an equivalent amount of maize starch (**Table S1**). The control diet was devoid of any onion supplementation. In the experimental onion groups, four lyophilized onion preparations were evaluated: (i) untreated, (ii) osmotically dehydrated using a glucose-fructose syrup solution, (iii) osmotically dehydrated in a glucose-fructose syrup solution followed by lactic acid fermentation using *Lev. brevis* ŁOCK 944 (stain 944), and (iv) osmotically dehydrated with a glucose-fructose syrup solution followed by lactic acid fermentation with *Lb. plantarum* P27 (strain P27).

Sample collection and analyses

The individual feed consumption and body weight gains of the rats were assessed. At the termination of the experiment, the rats were fasted for 8 h before being subjected to time-domain nuclear magnetic resonance (NMR) analysis using a minispec LF 90II analyzer (Bruker, Karlsruhe, Germany) to determine fat and lean tissue mass. The minispec emitted various radio frequency pulse sequences into soft tissues to realign the nuclear magnetic spins of hydrogen and subsequently detected the radio frequency signals generated by these hydrogen spins from the tissues. The contrast in relaxation times of hydrogen spins between adipose tissue and water-rich tissues was utilized to estimate fat and lean body mass. Following the NMR procedure, the rats were anesthetized intraperitoneally using ketamine

(100 mg/kg body weight) and xylazine (10 mg/kg body weight), in accordance with established guidelines for the anesthesia and euthanasia of laboratory rodents. After performing a laparotomy, blood samples were collected from the caudal vena cava into heparinized and ethylenediaminetetraacetic acid tubes, after which the animals were euthanized by cervical dislocation. Blood plasma was obtained through centrifugation at 350×*g* for 10 min at 4°C and stored at −70°C until analysis. Selected internal organs and tissues were then dissected and weighed.

Cecal samples were promptly analyzed for contents of ammonia, dry matter, bile acids, and short-chain fatty acids (SCFA). The remaining digesta were stored at −70°C. Ammonia was extracted using boric acid in Conway dishes and measured through titration with sulfuric acid [Conway, 1957]. The dry matter content was determined by drying to constant weight at 105°C. SCFA profile was analyzed by gas chromatography, where 0.2-g samples were mixed with formic acid, diluted with deionized water, and then centrifuged [Mikulski *et al*., 2024]. The supernatant was injected into a capillary column (SGE BP21; Trajan Scientific and Medical, Melbourne, Australia) and separated according to a specified temperature program: the initial oven temperature was 85°C, it was raised to 180°C at 8°C/min and held for 3 min. The temperature of the flame ionization detector and the injector was 180°C and 85°C, respectively. Identification and quantification of SCFAs were based on standards sourced from Sigma-Aldrich (Saint Louis, MO, USA). Contents of individual compounds were expressed as µmol *per* g of cecal digesta. In addition, the cecal putrefactive SCFA (PSCFA) content was calculated as the sum of isobutyric, isovaleric, and valeric acids. Analysis was performed in duplicate. The bile acids (BA) in the cecum were determined by liquid chromatography-mass spectrometry (LC-MS) using a system consisting of an Agilent 1200 chromatograph (Stockport, United Kingdom) and an AB Sciex 4000 QTrap triplequadrupole mass spectrometer (Warrington, UK). A Supelco Ascentis Express C18 column (15×4.6 mm, 2.7µm; Sigma Aldrich) maintained at 40°C was used for compound separation. Elution was carried out in a gradient system of solvent A (5 mM ammonium acetate in water) and solvent B (0.012% (*v/v*) formic acid in methanol) according to the following program: 0–2 min, 50% (*v/v*) B; 2–20 min, 50–95% (*v/v*) B; 20–22 min, 95% (*v/v*) B; 22–23 min, 95–50% (*v/v*) B; and 23–40 min, 50% (*v/v*) B. The injection volume was 5 µL, and the mobile phase was pumped at the flow rate of 0.6 mL/min. Mass data acquisition was performed using Analyst 1.6.2 software (SCIEX, Marlborough, MA, USA). The internal standards: d4-cholic, d4-glycochenodeoxycholic, d4-glycocholic, d4-litocholic, d4-deoxycholic, and d4-chenodeoxycholic acids, and the relative response factor, were used to quantify the BA. Content of individual BA was expressed as mg *per* g of cecal digesta.

Bacterial extracellular enzyme activity in cecal digesta was evaluated by measuring the release rates of *p*- or *o*-nitrophenol from nitrophenylglucosides [Juśkiewicz *et al*., 2024]. This assessment focused on enzymes, such as α- and β-glucosidase, α- and β-galactosidase, β-glucuronidase, α-arabinopyranidase, and β-xylosidase. The reaction mixtures were incubated at 37°C with *p*-nitrophenol and *o*-nitrophenol being quantified by absorbance measurement at 400 and 420 nm, respectively, following the addition of sodium carbonate. The enzymatic activity was expressed as µmol of product released *per* hour *per* gram of digesta. To measure the total enzyme activity, the cecal digesta was vortexed with glass beads and centrifuged, and the resulting supernatant was analyzed in the same manner as for extracellular activity. Intracellular enzyme activity was derived by comparing total activity against secreted (extracellular) enzyme activities. Standard curves for *p*-nitrophenol and *o*-nitrophenol were used for calculations, and analyses were conducted in duplicate.

In whole blood, hematological parameters were measured, including total white blood cell count (WBC), lymphocytes (LYM), granulocytes (GRA), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), and platelet count (PLT), using the ABACUS Jr VET Analyzer (DIATRON MI PLC, Budapest, Hungary). Blood plasma analysis included triglycerides (TG), total cholesterol (TC), HDL cholesterol (HDL), urea, glucose, albumin, and the activity of aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT), and was performed with a biochemical analyzer (Pentra C200, Horiba, Tokyo, Japan). Based on blood plasma parameters, atherogenic coefficient (AC) and atherogenic index of plasma (AIP) were calculated according to Equations (1) and (2), respectively:

$$AC = (TC - HDL)/HDL \tag{1}$$

$$AIP = \lg(TG/HDL) \tag{2}$$

Liver lipids were extracted according to the method of Folch *et al*. [1957]. The cholesterol and triglyceride contents in the extract were determined with the spectrophotometric method using commercial kits (cholesterol DST, triglycerides DST, Alpha Diagnostics, Ltd., San Antonio, TX, USA). Total RNA was isolated from liver tissue fragments weighing approximately 30 mg using the MagnifiQ 16 Total RNA Plus instant kit (A&A Biotechnology, Gdańsk, Poland) on an NPA-32P automated nucleic acid extractor, following the manufacturer's instructions. Samples were first homogenized in 400 µL of a Phenozol Plus reagent using a TissueLyser II homogenizer (Qiagen, Hilden, Germany), and the resulting lysates were processed automatically according to the programmed isolation protocol. RNA concentration and purity were determined with a NanoDrop Lite spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). All RNA samples were normalized to a concentration of 10 ng/µL using a TE buffer (Thermo Fisher Scientific, Cat. No. 12090015) prior to reverse transcription. cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific) according to the manufacturer's user guide (Pub. No. MAN0017977). Reverse transcription reactions (20 µL) were performed under the following thermal profile: 25°C for 10 min, 37°C for 120 min, and 85°C for 5 min, followed by storage at 4°C. The resulting cDNA was pre-amplified using the Fluidigm

PreAmp Master Mix (PN 100-5744) with a custom pool of TaqMan Gene Expression Assays. Pre-amplification was conducted on a QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific) following the Fluidigm protocol (PN 100-5876), with the following cycling conditions: 95°C for 2 min; 14 cycles of 95°C for 15 s and 60°C for 4 min. The pre-amplified products were diluted (1:5, *w/v*) in a Dilution Reagent (Fluidigm PN 100-8726) before quantitative PCR (qPCR) analysis. Quantitative gene expression analysis was carried out using the Biomark HD System and Juno Controller (Fluidigm Corporation, South San Francisco, CA, USA) with the 192.24 Dynamic Array Integrated Fluidic Circuit (IFC), following the manufacturer's guidelines (PN 100-6170). Each reaction contained diluted, preamplified cDNA combined with TaqMan Universal PCR Master Mix (2X), TaqMan Assay, GE Sample Loading Reagent and GE Assay Loading Reagent. The IFC was loaded using the Juno Load Mix 192.24 GE script, and qPCR was performed on the Biomark HD using the GE 192×24 Standard v1 protocol. Data was acquired using the Fluidigm Real-Time PCR Analysis Software, with default baseline and threshold settings applied. Gene expression data were expressed relative to the reference gene (glyceraldehyde-3-phosphate dehydrogenase, GAPDH) and were presented as relative fold gene.

Plasma samples were analyzed utilizing the Olink Target 96 Mouse Exploratory kit (Olink Proteomics, Uppsala, Sweden) in accordance with the manufacturer's recommended protocol. Initially, serum samples were thawed on ice and centrifuged at 4°C to eliminate any particulate matter prior to processing. The samples then underwent an overnight incubation at 4°C with oligonucleotide-labeled antibody pairs specific to the target proteins, facilitating the formation of antigen-antibody complexes and enhancing assay sensitivity and specificity. Following the formation of these immune complexes, proximity extension assay (PEA) technology was employed to promote the hybridization of complementary oligonucleotides, followed by enzymatic extension to generate unique DNA reporter sequences for each target protein. The QuantStudio 5 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) was used for pre-amplification of these reporter sequences, ensuring they reached detectable levels to enhance signal reliability. Sample loading and integrated fluidic circuit (IFC) priming were performed using the Juno system (Standard BioTools, South San Francisco, CA, USA), strictly adhering to the manufacturer's instructions to maintain optimal fluidic integrity and assay performance. Protein expression signals were quantified using qPCR amplification with the Biomark HD system (Standard BioTools), enabling the simultaneous quantification of multiple targets in a highly multiplexed assay format. Data normalization was conducted with the help of internal and external assay controls provided by the manufacturer, ensuring the quality and consistency of the results. Protein expression values were reported as Normalized Protein eXpression (NPX) units, calculated using the Olink NPX Signature software (Olink Proteomics).

■ Statistical analysis

The results are presented as average values, complemented by the standard error of the mean (SEM) to provide insight into data variability. To assess the statistical significance of differences among the various experimental treatments, namely the control, untreated onion, dehydrated onion, and dehydrated/fermented onion groups, a one-way analysis of variance (ANOVA) was employed (STATISTICA software, version 14.0; StatSoft Corp., Krakow, Poland). Following this analysis, a Tukey post-hoc test was conducted to identify significant differences among the groups. Additionally, effect sizes were calculated using partial eta squared (ηp^2), which quantifies the proportion of total variance that can be attributed to the specific factor under consideration, while effectively isolating the influence of other factors. Data were tested for normality using the Shapiro-Wilk test prior to ANOVA.

RESULTS AND DISCUSSION

Results showing the effects of the applied technological processes on the chemical composition of onion preparations are presented in **Table 1**. The rate of loss of chemical compounds during osmotic dehydration is affected by process conditions, including the concentration and temperature of the hypertonic solution, immersion time, the sample-to-solution ratio, and the agitation or circulation of the osmotic solution [Revaskar *et al*., 2014; Yazidi *et al*., 2024]. The onion preparations subjected to osmotic concentration followed by lactic acid fermentation exhibited reduced contents of protein, dietary fiber, fructooligosaccharides (FOS), and phenolics. In contrast, the contents of their simple sugars, sucrose, and ash increased. Similar changes have been reported in previous studies on onion subjected to osmotic dehydration in sucrose and sodium chloride solutions [Grzelak-Błaszczyk *et al*., 2021], followed by lactic acid fermentation [Grzelak-Błaszczyk *et al*., 2023].

The effect size analysis revealed that the applied dietary treatments with onion preparations had a moderate to large effect on fat and lean tissue content in the rat's body as well as on relative mass of spleen, liver, kidneys and epididymal white adipose tissue (ηp^2=0.063–0.201; **Table 2**). The one-way ANOVA and Tukey post-hoc test showed a significant increase in the body's lean tissue percentage in rats fed a diet supplemented with osmotically dehydrated onion preparation (p<0.05; GD *vs*. control group). These moderate-to-large effect size values indicate that a substantial proportion of the variance in internal organ weights as well as fat and lean tissue contents can be attributed to the dietary onion intervention, suggesting it has practical significance. In a 12-week randomized, double-blind, placebo-controlled trial, supplementation with an onion peel extract significantly reduced body weight and percentage of body fat in overweight and obese adults compared with placebo [Lee *et al*., 2016]. In Zucker diabetic fatty rats, dietary supplementation with a whole onion extract reduced body weight gain and adipose tissue weight compared with controls [Yoshinari *et al*., 2012]. In our study, we observed that the lean tissue percentage in rats was significantly reduced when they consumed a diet containing dehydrated onion, without any accompanying changes in body weight. The fermentation processing applied to the dietary onion preparation did not yield any additional effects on body fat or lean tissue composition. Some researchers

Table 1. Chemical composition (g/100 g) and count of live lactic acid bacteria (LAB) (log CFU/g) of preparations from onion (v. Allurion) obtained by lyophilization (untreated onion), osmotic dehydration in a glucose-fructose syrup, and a combination of osmotic dehydration and fermentation with *Levilactobacillus brevis* ŁOCK 944 or *Lactiplantibacillus plantarum* P27.

Parameter	Lyophilization	Osmotic dehydration	Osmotic dehydration and fermentation with strain 944	Osmotic dehydration and fermentation with strain P27
Dry matter	84.50±0.03	88.69±0.11	84.23±0.08	84.06±0.20
Protein	11.34±0.28^{a}	4.83±0.07^{b}	5.67±0.32^{b}	4.71±0.19^{b}
F+G+S	43.94±1.28^{c}	62.51±1.61^{a}	54.85±3.04^{b}	55.01±1.93^{b}
Total dietary fiber	12.70±0.30^{a}	4.50±0.11^{c}	9.50±0.33^{b}	8.30±0.00^{b}
Crude fat	0.47±0.04	0.44±0.02	0.64±0.04	0.51±0.04
Ash	4.26±0.34^{b}	7.62±0.33^{a}	9.06±0.11^{a}	8.88±0.08^{a}
FOS	6.72±0.37^{a}	1.78±0.13^{b}	1.61±0.06^{b}	1.29±0.06^{b}
Total phenolics	1.33±0.03^{a}	0.54±0.03^{b}	0.51±0.02^{b}	0.50±0.01^{b}
Live LAB	Not detected	Not detected	8.64±0.14	8.16±0.15

Results are shown as mean ± standard deviation (n=3). Means within a row with unlike superscript letters are shown to be significantly different ($p<0.05$). F+G+S, sum of fructose, glucose and saccharose; FOS, fructooligosaccharides (kestose, nystose, fructosyl nystose).

have noted that changes in gene expression are consistent with a pattern of decreased hepatic lipid synthesis and fat deposition, establishing a link between onion intake and alterations in liver gene expression related to fat metabolism [Chang *et al.*, 2022].

The results in **Table 3** show that the dietary addition of onion preparations determined 6–70% (ηp^2=0.064–0.696) of the variations in the expression of the analyzed genes in the liver tissue of rats. The moderate effect size was noted in the case of genes: *Ldlr*, *Olr1*, *Ptgs2*, *Stat3*, *Cyp8b1*, and *Fasn*. In the case of the remaining analyzed genes, the observed effect size was large. The highest expression of *Ahr* and *Pparg* genes in the liver was observed in the GDFb group ($p<0.05$ *vs.* all other groups). All four onion dietary treatments caused a significant decrease in hepatic *Hif1a*, *Scd1*, and *Acly* expression compared to changes observed in the control animals ($p<0.05$). The *Nfkb1* and *Nrlh4* expression was the highest in the onion untreated preparation group ($p<0.05$ *vs.* GD, GDFa groups and $p<0.05$ *vs.* other groups, respectively). The *Ppara* hepatic expression was elevated in the GDFb rats ($p<0.05$ *vs.* control and GDFa groups). The osmotic dehydration with a glucose-fructose syrup as well as subsequent lactic fermentation with selected bacterial strains (groups GD, GDFa, GDFb) caused a significant decrease in the hepatic *Srebf1* and *Tlr4* expression compared to the control groups (*Srebf1*) and control and G groups (*Tlr4*), respectively. The GDFa dietary treatment caused a significant increase in the *Lpl*, *Cyp27a1*, *Cyp7a1* expression in the liver as compared to all other treatments ($p<0.05$). The GD and GDFa groups excelled the G rats in terms of hepatic *Baat* expression ($p<0.05$). The G rats had the highest *Cpt1a* and *Fdft1* expressions in the liver ($p<0.05$ *vs.* GDFa and $p<0.05$ *vs.* all other groups, respectively).

The applied dietary treatments with onion preparations explained 7–17% of the variance in the liver fat (ηp^2=0.102; moderate effect), total cholesterol (ηp^2=0.065; moderate effect), and triglycerides (ηp^2=0.165; large effect) contents (**Table 4**). A one-way ANOVA analysis showed a near-significant trend to decreased liver TG in the GDFb group (p=0.080). The dietary addition of the dehydrated onion preparation caused a significant decrease in plasma AST activity ($p<0.05$ *vs.* C), while the GDFa and GDFb groups exhibited decreased ALT activity (in both cases $p<0.05$ *vs.* C). In the case of plasma ALP, there was a descending tendency in its activity in the plasma in the onion groups (p=0.063). The dietary onion addition explained 18%, 23% and 24% of the variance in ALP, AST, and ALT activity, respectively. In the case of total cholesterol (TC) and glucose concentration, the respective percentages were 20% and 29% (the effect sizes: ηp^2=0.195 and ηp^2=0.286, respectively). The lowest glucose and TC plasma concentrations were determined in the GDFb rats ($p<0.05$ *vs.* C and GD, and $p<0.05$ *vs.* C, respectively). The onion dietary treatments exerted moderate effects on HDL concentration in the plasma and the value of atherogenic coefficient (AC; ηp^2=0.068 and ηp^2=0.088, respectively). Such size of the effect on triglycerides (TG) and atherogenic index of plasma (AIP) was noted as a large effect (ηp^2=0.307 and ηp^2=0.247, respectively). The lowest TG concentration and AIP value were determined in the GD group ($p<0.05$ *vs.* C and GDFa and $p<0.05$ *vs.* C, respectively).

As demonstrated by the analysis of multiple liver tissue markers, the beneficial changes observed in the rats fed diets containing onion preparations, both directly in the liver tissue and in blood biochemical parameters, should be attributed to the modulation of the expression of several genes essential for metabolic regulation. However, it is necessary to distinguish which genes were activated or suppressed by the onion preparation itself and whether the applied technological processes

Table 2. Final body weight (BW), daily diet intake, and internal organ weight in rats fed diets with onion preparations and a control diet.

Parameter	C	G	GD	GDFa	GDFb	SEM	*p*-Value	Effect size ηp^2
Final BW (g)	327.1	330.2	329.5	334.5	326.4	2.281	0.832	0.031
Diet intake (g/day)	18.70	18.99	19.07	18.86	19.00	0.108	0.834	0.031
Fat (g)	38.55	35.46	30.98	35.43	34.55	0.994	0.203	0.121
Lean (g)	213.6	222.5	223.3	223.4	218.3	1.648	0.260	0.108
Fat (% BW)	11.76	10.72	9.462	10.63	10.57	0.300	0.206	0.121
Lean (% BW)	65.32^{b}	67.42^{ab}	67.77^{a}	66.79^{ab}	66.89^{ab}	0.268	0.034	0.201
eWAT (g/100 g BW)	2.867	2.508	2.632	2.668	2.680	0.065	0.545	0.064
Heart (g/100 g BW)	0.270	0.265	0.265	0.262	0.265	0.002	0.880	0.025
Spleen (g/100 g BW)	0.235	0.218	0.233	0.222	0.202	0.006	0.470	0.074
Liver (g/100 g BW)	3.651	3.614	3.656	3.500	3.467	0.044	0.552	0.063
Testes (g/100 g BW)	1.019	1.018	1.024	0.994	1.030	0.010	0.811	0.033
Kidneys (g/100 g BW)	0.649	0.651	0.671	0.631	0.637	0.007	0.512	0.068

Mean values within a row with unlike superscript letters are shown to be significantly different ($p<0.05$; post-hoc Tukey test); ηp^2, partial eta squared; SEM, pooled standard error of the mean (standard deviation for all rats divided by the square root of rat number, $n=50$); eWAT, epididymal white adipose tissue. 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* ŁOCK 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 3. Gene expression in the liver tissue in rats fed diets with onion preparations and a control diet.

Gene	C	G	GD	GDFa	GDFb	SEM	*p*-Value	Effect size ηp^2
Ahr	0.045^{b}	0.031^{b}	0.051^{b}	0.055^{b}	0.325^{a}	0.029	0.002	0.312
Hif1a	0.118^{a}	0.007^{b}	0.013^{b}	0.037^{b}	0.006^{b}	0.009	<0.001	0.432
Ldlr	0.238	0.243	0.304	0.305	0.150	0.032	0.553	0.064
Nfkb1	0.148^{ab}	0.351^{a}	0.118^{b}	0.062^{b}	0.168^{ab}	0.027	0.005	0.273
Nr1h4	0.080^{b}	0.207^{a}	0.043^{b}	0.034^{b}	0.088^{b}	0.014	<0.001	0.389
Olr1	0.071	0.011	0.010	0.061	0.033	0.008	0.061	0.177
Ppara	0.028^{b}	0.145^{ab}	0.036^{ab}	0.022^{b}	0.161^{a}	0.016	0.003	0.299
Pparg	0.178^{b}	0.004^{b}	<0.001^{b}	0.145^{b}	1.543^{a}	0.129	<0.001	0.425
Ptgs2	<0.001	<0.001	0.001	0.001	<0.001	<0.001	0.530	0.066
Srebf1	1.021^{a}	0.612^{ab}	0.355^{b}	0.183^{b}	0.228^{b}	0.067	<0.001	0.429
Stat3	0.128	0.177	0.071	0.060	0.112	0.016	0.151	0.136
Tlr4	0.470^{a}	0.475^{a}	<0.001^{b}	<0.001^{b}	<0.001^{b}	0.046	<0.001	0.520
Lpl	0.034^{b}	0.025^{b}	0.024^{b}	0.244^{a}	0.030^{b}	0.019	<0.001	0.435
Scd1	15.653^{a}	3.513^{b}	4.262^{b}	2.708^{b}	3.877^{b}	0.874	<0.001	0.629
Acly	0.339^{a}	<0.001^{b}	0.037^{b}	0.015^{b}	0.072^{b}	0.024	<0.001	0.554
Baat	1.718^{ab}	0.847^{b}	2.454^{a}	2.462^{a}	1.196^{ab}	0.203	0.027	0.211
Cpt1a	1.050^{ab}	2.787^{a}	1.509^{ab}	0.540^{b}	1.455^{ab}	0.217	0.013	0.241
Cyp27a1	0.132^{b}	0.203^{b}	0.127^{b}	1.286^{a}	0.185^{b}	0.090	<0.001	0.512
Cyp7a1	0.077^{b}	<0.001^{b}	0.031^{b}	0.938^{a}	0.029^{b}	0.079	<0.001	0.431

Table 3 cont. Gene expression in the liver tissue in rats fed diets with onion preparations and a control diet.

Gene	C	G	GD	GDFa	GDFb	SEM	*p*-Value	Effect size ηp^2
Cyp8b1	0.249	0.379	0.264	0.181	0.181	0.035	0.382	0.086
Fasn	0.312	0.285	0.136	0.282	0.092	0.040	0.303	0.099
Fdft1	0.020^{b}	0.520^{a}	0.006^{b}	0.050^{b}	0.024^{b}	0.034	<0.001	0.696

Mean values within a row with unlike superscript letters are shown to be significantly different ($p<0.05$; post-hoc Tukey test); ηp^2, partial eta squared; SEM, pooled standard error of the mean (standard deviation for all rats divided by the square root of rat number, $n=50$). Gene names: *Ahr*, aryl hydrocarbon receptor; *Hif1a*, hypoxia-inducible factor 1-alpha; *Ldlr*, low-density lipoprotein receptor; *Nfkb1*, nuclear factor kappa b subunit 1; *Nr1h4* (*Fxr*), nuclear receptor subfamily 1, group H, member 4 (Farsenoid x receptor); *Olr1* (*Lox-1*), oxidized low-density lipoprotein receptor 1 (Lectin-like oxidized LDL receptor-1); *Ppara*, peroxisome proliferator-activated receptor alpha; *Pparg*, peroxisome proliferator-activated receptor gamma; *Ptgs2*, prostaglandin-endoperoxidase synthase 2; *Srebf1*, sterol regulatory element binding transcription factor 1; *Stat3*, signal transducer and activator of transcription 3; *Tlr4*, toll-like receptor 4; *Lpl*, lipoprotein lipase; *Scd1*, steaoryl-CoA desaturase 1; *Acly*, ATP citrate lyase; *Baat*, bile acid-CoA: amino acid N-acyltransferase; *Cpt1a*, carnitine palmitoyltransferase 1a; Cyp27a1, cytochrom P450 family 27 subfamily a member 1; *Cyp7a1*, cytochrom P450 family 7 subfamily a member 1; *Cyp8b1*, cytochrom P450 family 8 subfamily b member 1; *Fasn*, fatty acid synthase; *Fdft1*, farnesyl-diphosphate farnesyltransferase 1. 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* ŁOCK 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 4. Liver fat content and biochemical blood plasma parameters in rats fed diets with onion preparations and a control diet.

Parameter	C	G	GD	GDFa	GDFb	SEM	*p*-Value	Effect size ηp^2
Liver								
Fat (g/100 g)	15.02	13.53	13.18	13.39	14.18	0.298	0.288	0.102
TC (mg/g)	4.457	4.588	4.534	4.269	4.027	0.115	0.543	0.065
TG (mg/g)	15.90	15.83	15.28	15.26	13.21	0.344	0.080	0.165
Plasma								
AST (U/L)	56.80^{a}	51.14^{ab}	48.32^{b}	53.18^{ab}	50.96^{ab}	0.836	0.016	0.232
ALT (U/L)	22.45^{a}	20.43^{ab}	19.87^{ab}	18.01^{b}	18.55^{b}	0.454	0.012	0.241
ALP (U/L)	176.7	166.0	164.7	162.7	156.6	2.218	0.063	0.176
GL (mmol/L)	18.63^{a}	16.40^{ab}	17.72^{a}	17.47^{ab}	15.19^{b}	0.315	0.003	0.286
TC (mmol/L)	2.119^{a}	1.682^{ab}	1.833^{ab}	1.879^{ab}	1.591^{b}	0.059	0.040	0.195
HDL (mmol/L	0.547	0.485	0.507	0.549	0.474	0.017	0.512	0.068
TC/HDL	3.858	3.626	3.695	3.440	3.435	0.077	0.374	0.088
AC	2.858	2.626	2.695	2.440	2.435	0.077	0.374	0.088
TG (mmol/L)	1.733^{a}	1.307^{ab}	1.022^{b}	1.508^{a}	1.295^{ab}	0.061	0.002	0.307
TG/HDL	3.253^{a}	2.791^{ab}	2.124^{b}	2.733^{ab}	2.779^{ab}	0.112	0.027	0.211
AIP	0.500^{a}	0.430^{ab}	0.303^{b}	0.428^{ab}	0.430^{ab}	0.018	0.010	0.247
ALB (μmol/L)	406.8	413.1	412.9	414.3	405.1	3.865	0.927	0.018
Urea (mmol/L)	3.921	4.118	3.726	3.717	4.121	0.080	0.298	0.101

Mean values within a row with unlike superscript letters are shown to be significantly different ($p<0.05$; post-hoc Tukey test); ηp^2, partial eta squared; SEM, pooled standard error of the mean (standard deviation for all rats divided by the square root of rat number, $n=50$); AC, atherogenic coefficient; AIP, atherogenic index; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GL, glucose; TC, total cholesterol; TG, triglycerides. 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* ŁOCK 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.

(osmotic dehydration with a glucose-fructose syrup and subsequent lactic acid fermentation with selected bacterial strains) exerted any additional effects. Accordingly, regardless of further modification of the onion preparation through dehydration and fermentation, a beneficial reduction in the expression of *Hif1a*, *Scd1*, and *Acly* was observed in all dietary groups receiving onion preparations. It is well known that the activation of *Hif1a via* downstream genes, such as *Srebp1* and *Fasn*, promotes lipogenesis, and that *Hif1a* is often excessively activated in pathological conditions, such as non-alcoholic fatty liver disease (NAFLD) [Zeng *et al*., 2025]. In turn, *Scd1* regulates the synthesis of triglycerides, phospholipids, and cholesterol, and increased expression of this gene promotes lipogenesis and may contribute to hepatic steatosis [Yu *et al*., 2025]. Similarly, elevated *Acly* activity enhances lipogenesis and lipid accumulation in hepatocytes [Rauckhorst *et al*., 2025]. Consistent with these findings, Son *et al*. [2024] reported that a dietary onion extract rich in quercetin modulated hepatic gene expression of key regulators of lipid metabolism, including *Srebp1*, *Fasn*, *Ppara*, and *Cpt1a*, and that these changes were accompanied by improved lipid profiles and reduced serum triglyceride and low-density lipoprotein (LDL) levels in rats fed a high-fat diet.

In the present study, the application of osmotic dehydration of onion (v. Allurion) in a glucose-fructose syrup resulted in a health-beneficial reduction in the hepatic expression of the *Nfkb1*, *Srebf1*, and *Tlr4* genes. Increased expression of the first of these genes is observed in chronic inflammatory states associated with non-alcoholic fatty liver disease (NAFLD) and enhanced immune activation; moreover, sustained activation of the *NF-κB* transcriptional complex (including *Nfkb1*) exacerbates liver tissue fibrosis [Cordero-Pérez *et al*., 2025]. In turn, the overexpression of *Srebf1* promotes lipogenesis in response to metabolic signaling and leads to increased lipid storage in hepatocytes [Yang *et al*., 2020]. Elevated *Tlr4* expression may trigger inflammatory responses and activate hepatic stellate cells, which are responsible for collagen production and the progression of liver fibrosis [Ding *et al*., 2025]. In our study, the most favorable changes in plasma biochemical parameters, namely AST activity, triglyceride (TG) concentration, and the atherogenic index of plasma (AIP), were observed in the rats fed the GD diet containing onion prepared by osmotic dehydration with a glucose-fructose syrup. Osmotic dehydration is a low-temperature pretreatment that partially removes water from plant tissues by immersion in a hypertonic solution. As this process does not involve high thermal exposure, it enables better preservation of heat-sensitive nutrients compared with conventional hot-air drying, particularly vitamins, polyphenols, and flavor compounds that are susceptible to degradation under conditions of elevated temperature and oxygen [Galus *et al*., 2025; Yadav & Singh, 2014]. Of course, the literature on the health-promoting effects of consuming fermented fruits and vegetables is extensive. However, these studies do not address the effects of consuming products that were first subjected to osmotic dehydration before fermentation. We aimed to fill this gap in knowledge with the present experiment. The combined application of dehydration and fermentation produced some effects that were not observed in the C, G, or GD groups. Specifically, beneficial changes were observed in hepatic gene expression: *Ahr*, *Ppara*, and *Pparg* (with the preparation fermented using the probiotic strain P27) and *Lpl*, *Cyp27a1*, and *Cyp7a1* (with the preparation fermented using strain 944). As suggested by Shang *et al*. [2023], endogenous *Ahr* activation *via* dietary or microbial ligands can confer hepatoprotection and reduce inflammation. Increased *Ppara* expression is associated with enhanced insulin sensitivity in tissues as well as enhanced fatty acid β-oxidation, decreased plasma triglycerides, anti-inflammatory effects (*e.g.*, inhibition of NF-κB activation), and anti-steatotic action in the liver. Conversely, increased *Pparg* expression can promote hepatic steatosis under certain conditions, such as NAFLD, but it can also attenuate inflammation (through NF-κB inhibition) and exert antifibrotic effects [Sepehri *et al*., 2025]. Indeed, the most favorable changes in parameters, such as liver triglycerides (TG) and plasma levels of alkaline phosphatase (ALP), glucose, and total cholesterol (TC), were observed in our study in the GDFb group.

The counts of total white blood cells (WBC) and lymphocytes (LYM) were affected by the onion dietary treatments in a moderate manner (**Table S2** in Supplementary Materials; ηp^2=0.085–0.117). The neutrophil (NEU) count and percentage were the highest in the G group ($p<0.05$ *vs*. all other onion groups, and $p<0.05$ *vs*. GDFb, respectively). The onion treatments explained 30 and 25% of the variance in NEU count and percentage, respectively. The addition of dietary onion preparations to rat diets exerted a moderate to large effect on their red blood cell count, hemoglobin level, hematocrit percentage, and red cell distribution width (ηp^2=0.100–0.140). The size of the effect of the dietary factor on platelet count and mean platelet volume was moderate (ηp^2=0.065–0.086), while a large effect was noted on platelet distribution width (ηp^2=0.175). The above hematological changes should be regarded as a physiological response within normal limits. The increased NEU level observed in group G (untreated onion preparation), in the absence of alterations in other blood parameters, should not be associated with elevated stress levels or infectious conditions [Herrero-Cervera *et al*., 2022].

The dietary addition of onion preparations explained 14% and 20% of the variance in relative cecal digesta mass and cecal digesta pH value, respectively, meaning that dietary manipulation had a strong influence (**Table 5**). The dietary treatment with untreated onion preparation (group G) significantly lowered digesta pH in the cecum as compared to the control rats ($p<0.035$). The applied dietary onion treatments had a moderate effect (ηp^2=0.068–0.088) on cecal acetic and valeric acid contents, while a large effect was noted for cecal butyric acid level (ηp^2=0.143). The butyric acid percentage in the SCFA profile was significantly higher in the GD group than in the C control animals (p=0.034; ηp^2=0.202). There was a significant difference between the GDFa and GDFb groups in the SCFA pool (31.4 *vs*. 53.3 μmol/100 g of body weight; p=0.033; ηp^2=0.203). The dietary

Table 5. Cecal parameters in rats fed diets with onion preparations and a control diet.

Parameter	C	G	GD	GDFa	GDFb	SEM	*p*-Value	Effect size ηp^{2}
Cecum								
Cecal tissue (g/100 g BW)	0.240	0.282	0.258	0.257	0.262	0.006	0.349	0.092
Cecal digesta (g/100 g BW)	0.501	0.635	0.600	0.479	0.692	0.031	0.144	0.138
Cecal pH	7.471^{a}	7.191^{b}	7.385^{ab}	7.335^{ab}	7.265^{ab}	0.031	0.035	0.200
SCFA (μmol/g digesta)								
Acetic	42.64	41.06	41.98	39.38	46.33	1.257	0.515	0.068
Propionic	18.01	18.37	18.61	16.68	17.65	0.629	0.894	0.023
Butyric	7.171	8.436	10.50	7.650	7.779	0.407	0.128	0.143
Isovaleric	1.459	1.329	1.470	1.428	1.613	0.061	0.701	0.046
Valeric	2.643	2.055	2.261	1.958	2.101	0.116	0.374	0.088
Isobutyric	1.560	1.376	1.460	1.442	1.498	0.056	0.893	0.023
PSCFA	5.663	4.760	5.191	4.828	5.212	0.206	0.667	0.050
Total SCFA	74.03	72.62	76.28	68.54	76.96	2.094	0.739	0.042
SCFA profile (%)								
Acetic	57.73	56.23	55.45	57.41	60.13	0.656	0.230	0.114
Propionic	24.29	25.60	24.09	24.22	22.96	0.561	0.709	0.045
Butyric	10.39^{ab}	11.41^{ab}	13.68^{a}	11.11^{ab}	10.15^{b}	0.398	0.034	0.202
SCFA pool (μmol/100 g BW)	36.28^{ab}	45.62^{ab}	45.53^{ab}	31.37^{b}	53.32^{a}	2.444	0.033	0.203

Mean values within a row with unlike superscript letters are shown to be significantly different ($p<0.05$; post-hoc Tukey test); ηp^{2}, partial eta squared; SEM, pooled standard error of the mean (standard deviation for all rats divided by the square root of rat number, $n=50$); BW, body weight; SCFA, short-chain fatty acid; PSCFA, putrefactive short-chain fatty acid. 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* ŁOCK 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.

onion preparation factor accounted for 14% to 18% of the variance observed in both the intracellular and total activity of bacterial α- and β-glucosidase in the cecal digesta (**Table S3** in Supplementary Materials). Additionally, a statistical trend indicating an increased release rate of these enzymes from bacterial cells into the cecal environment was noted in the groups fed diets containing onion, with *p*-values of 0.098 and 0.080, respectively. The onion preparation treatments had moderate effects on cecal bacterial extracellular, intracellular and total activities of α- and β-galactosidase (ηp^{2}=0.072–0.130). The highest extracellular and total activity of cecal bacterial β-glucuronidase was found in the GD group (**Table 6**; $p<0.05$ *vs.* all other groups, and $p<0.05$ *vs.* GDFa and GDFb groups, respectively; ηp^{2}=0.396 and ηp^{2}=0.291, respectively). The onion dietary treatments explained 13% of the variance in the percentage value of β-glucuronidase and α-arabinopyranosidase release rates (ηp^{2}=0.134 and 0.127, respectively). The dietary onion preparation factor had a large effect on intracellular and total α-arabinopyranosidase activity in the aecal digesta of rats (ηp^{2}=0.174 and 0.168, respectively). There was also a statistical tendency to an increase in intracellular and total α-arabinopyranosidase activity, especially in the G group ($p=0.065$ and $p=0.074$, respectively). The dietary addition of onion preparations had a moderate effect on extracellular and total β-xylosidase cecal activity (ηp^{2}=0.098 and 0.084, respectively), and a large effect on the release rate of that enzyme (ηp^{2}=0.179). The β-xylosidase release rate tended to increase in the dietary onion treatments as compared to the C rats ($p=0.058$). The inclusion of onion preparations in the diet did not induce highly significant changes in the metabolic activity of the rat cecal microbiota, indicating good gastrointestinal tolerance of this dietary supplementation. Positive effects entailed the significant acidification of the cecal digesta in the rats fed the diet containing untreated onion preparation, an increase in the butyric acid pool in group GD (dehydrated onion preparation), and an

Table 6. Microbial enzymatic activity in the cecal digesta in rats fed diets with onion preparations and a control diet.

Enzyme	C	G	GD	GDFa	GDFb	SEM	*p*-Value	Effect size ηp^2
β-Glucuronidase								
Extracellular (μmol/h/g digesta)	26.15^b	26.42^b	37.11^a	21.70^b	19.56^b	1.375	<0.001	0.396
Intracellular (μmol/h/g digesta)	14.22	11.62	12.22	10.58	11.54	0.720	0.603	0.057
Total (μmol/h/g digesta)	40.37^{ab}	38.04^{ab}	49.32^a	32.28^b	31.10^b	1.730	0.003	0.291
Release rate (%)	65.25	69.07	74.79	67.48	64.27	1.446	0.156	0.134
α-Arabinopyranosidase								
Extracellular (μmol/h/g digesta)	2.615	3.075	3.253	3.167	3.199	0.098	0.232	0.114
Intracellular (μmol/h/g digesta)	1.105	1.880	1.209	1.210	1.561	0.099	0.065	0.174
Total (μmol/h/g digesta)	3.720	4.955	4.462	4.377	4.760	0.147	0.074	0.168
Release rate (%)	70.78	63.14	73.06	71.77	68.14	1.407	0.181	0.127
β-Xylosidase								
Extracellular (μmol/h/g digesta)	3.361	3.396	4.362	3.707	4.226	0.189	0.310	0.098
Intracellular (μmol/h/g digesta)	1.843	2.217	1.760	1.385	1.872	0.205	0.808	0.034
Total (μmol/h/g digesta)	4.600	4.276	5.111	4.187	5.039	0.187	0.401	0.084
Release rate (%)	72.79	79.14	84.76	88.00	82.58	1.753	0.058	0.179

Mean values within a row with unlike superscript letters are shown to be significantly different ($p<0.05$; post-hoc Tukey test); ηp^2, partial eta squared; SEM, pooled standard error of the mean (standard deviation for all rats divided by the square root of rat number, $n=50$); 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* ŁOCK 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.

increase in the total short-chain fatty acid (SCFA) pool in the group fed a diet containing dehydrated/fermented onion preparation. The increased bacterial β-glucuronidase activity observed in group GD, an enzyme generally associated with less desirable gut microbiota [Rauf *et al.*, 2025], can be attributed to the lower fiber content and the higher simple sugar content of the onion osmotic-dehydrated in a glucose-fructose syrup. Fermentation of this preparation led to a reduction in both the total and extracellular activity of this enzyme.

The dietary onion factor had a moderate effect on the cecal hyodeoxycholic, tauro-α-muricholic, tauro-β-muricholic, glycocholic, and taurochenodeoxycholic acids (**Table 7**; $\eta p^2=0.065$–-0.129). The GDFa and GDFb treatments caused a significant decrease in the cecal lithocholic acid content as compared to C rats ($p<0.05$; $\eta p^2=0.272$). All onion groups had a significantly lowered β-hyodeoxycholic acid content in the cecal digesta *vs*. control group ($p<0.05$; $\eta p^2=0.481$). The tauro-ω-muricholic level in GDFa significantly excelled the GDFb group ($p<0.05$; $\eta p^2=0.180$). Changes in the bile acid content of the rat cecal digesta in response to dietary supplementation with onion preparations should be considered minor, similar to the case of SCFAs. The observed reduction in cecal lithocholic acid (LCA) in the dehydrated/fermented GDFa and GDFb groups is likely beneficial, as excessive LCA is cytotoxic and genotoxic. Moreover, lower LCA levels may reflect favorable modulation of the gut microbiota and its activity [Chen *et al.*, 2025].

The dietary application of onion preparations had a moderate effect size ($\eta p^2=0.065$–0.134; **Table S4** in Supplementary Materials) on the following plasma relative protein expression levels (NPX) related to immunological status of the rat's body: Aryl hydrocarbon receptor (Ahr), C-C motif chemokine 2 (Ccl2), C-X-C motif chemokine 9 (Cxcl9), follistatin (Fst), interleukin-1 alpha (Il1a), interleukin-6 (Il6), interleukin-17A (Il17a), plexin-A4 (Plxna4), tumor necrosis factor receptor superfamily member 11B (Tnfrsf11b), and V-set and immunoglobulin domain-containing protein 2 (Vsig2). A large effect size was noted in the case of transforming growth factor beta receptor type 3 level ($\eta p^2=0.146$). The diet composition factor explained 10% and 15% of the variance in forkhead box protein O1 (Foxo1) and NAD kinase (Nadk) expression levels (**Table S5** in Supplementary Materials). With

Table 7. Cecal bile acid (BA) content (mg/g digesta) in rats fed diets with onion preparations and a control diet.

	C	G	GD	GDFa	GDFb	SEM	*p*-Value	Effect size ηp^2
Primary BA	42.31	43.09	39.27	42.44	41.97	2.602	0.993	0.005
αMCA	12.08	13.17	11.82	15.05	13.25	0.886	0.813	0.033
βMCA	28.44	28.32	25.63	25.72	27.07	1.815	0.980	0.009
CA	1.717	1.457	1.655	1.501	1.460	0.189	0.989	0.006
CDCA	0.068	0.142	0.162	0.172	0.199	0.035	0.820	0.032
Secondary BA	30.41	22.47	26.05	22.06	24.60	2.126	0.800	0.035
ωMCA	7.835	10.33	7.846	8.370	8.474	0.676	0.780	0.037
UDCA	0.506	0.999	0.739	0.662	0.856	0.124	0.780	0.037
HDCA	0.393	0.275	0.259	0.176	0.244	0.037	0.468	0.074
DCA	1.639	1.741	1.487	1.560	1.844	0.183	0.978	0.009
LCA	2.219^{a}	1.600^{ab}	1.661^{ab}	1.103^{b}	0.919^{b}	0.125	0.005	0.272
HCA	16.52	12.35	13.70	10.03	12.07	1.323	0.639	0.053
βHDCA	1.297^{a}	0.162^{b}	0.343^{b}	0.169^{b}	0.200^{b}	0.090	<0.001	0.481
Conjugated BA	0.983	0.750	1.851	1.439	1.367	0.243	0.665	0.050
TαMCA	0.203	0.094	0.193	0.449	0.306	0.054	0.291	0.102
TωMCA	0.104^{ab}	0.156^{ab}	0.134^{ab}	0.421^{a}	0.056^{b}	0.043	0.049	0.180
TβMCA	0.580	0.447	1.444	0.533	0.822	0.179	0.407	0.083
GCA	0.057	0.036	0.061	0.016	0.136	0.023	0.535	0.065
TCDCA	0.040	0.017	0.019	0.020	0.046	0.005	0.172	0.129
Total BA	73.70	71.31	67.17	65.95	67.95	4.546	0.984	0.008

Mean values within a row with unlike superscript letters are shown to be significantly different ($p<0.05$; post-hoc Tukey test); ηp^2, partial eta squared; SEM, pooled standard error of the mean (standard deviation for all rats divided by the square root of rat number, $n=50$); MCA, muricholic acid; CA, cholic acid; HDCA, hyodeoxycholic acid; UDCA, ursodeoxycholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; TMCA, tauromuricholic acid; GCA, glycocholic acid; TCDCA, taurochenodeoxycholic acid; HCA, hyocholic acid. 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* ŁOCK 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.

regard to carbohydrate metabolism parameters, the plasma expression levels (NPX) of carboxypeptidase E (Cpe), delta-like 1 homolog (Dlk1), enolase 2 (Eno2), and follistatin-related protein 3 (Fstl3) were characterized with a moderate effect size (ηp^2=0.066–0.084). The lipoprotein lipase (Lpl) and perilipin-1 (Plin1) plasma relative expression levels, which are connected with lipid metabolism, were characterized with a moderate effect size (ηp^2=0.070–0.082). The dietary onion treatments explained 11% of the variance in the expression level of epithelial cell adhesion molecule (Epcam). Effect size estimates provide information on the magnitude of biological differences and may be informative even in the absence of statistical significance, particularly in studies with limited power. While our findings should be interpreted with caution, the observed effect sizes suggest that these proteins (connected with moderate to large effect size) may be biologically relevant and warrant further investigation in larger, adequately powered studies, or in dietary scenarios with higher supplementation doses.

CONCLUSIONS

In summary, the observed moderate-to-large effect sizes indicate that dietary onion supplementation plays a significant role in influencing body composition and metabolic response in rats. In all groups fed diets with onion preparations, there was a consistent downregulation of hepatic *Hif1a*, *Scd1*, and *Acly*, suggesting that onion intake exerts an anti-lipogenic effect that was independent of onion processing methods. Furthermore, osmotic dehydration in a glucose-fructose syrup enhanced these benefits, as evidenced by a reduction in the expression of inflammatory and lipogenic genes (*Nfkb1*, *Srebf1*, *Tlr4*) and improvement in plasma markers related to liver function and lipid metabolism. The subsequent fermentation process exerted additional, preparation-specific effects on hepatic genes involved in fatty acid oxidation, bile acid metabolism, and inflammatory regulation. Overall, dietary onion preparations appear to be valuable vehicles of these beneficial metabolic impacts, while the technological processing of onions can influence their specificity. These findings highlight the potential of appropriately processed onion preparations as functional dietary components that can promote metabolic health.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

ADDITIONAL INFORMATION

The animal study was approved by Local Animal Care and Use Committee in Olsztyn, Poland (Approval No. 41/2022; Olsztyn, Poland, 15.06.2022).

SUPPLEMENTARY MATERIALS

The following are available online at **https://journal.pan.olsztyn.pl/Impact-of-Osmotic-Dehydration-and-Lactic-Acid-Fermentation-of-Onion-on-the-Physiological,225064,0,2.html**; **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.

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