agriculture

MDPI

Article

Metabolome Analysis Reveals Potential Mechanisms of Mannan Oligosaccharides to Improve Health, Growth Performance, and Fatty Acid Deposition in *Hu* Lambs

Ting Liu [1], Fadi Li [2,3], Jianfeng Xu [1], Jing Wang [1], Zhenfeng Shen [1], Fan Zhang [1], Jiaqi Wang [1] and Chen Zheng [1,*]

[1] College of Animal Science and Technology, Gansu Agricultural University, Lanzhou 730070, China
[2] State Key Laboratory of Grassland Agro-Ecosystems, Key Laboratory of Grassland Livestock Industry Innovation, Ministry of Agriculture and Rural Affairs, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou 730020, China
[3] Engineering Laboratory of Mutton Sheep Breeding and Reproduction Biotechnology in Gansu Province, Minqin 733300, China
* Correspondence: zhengc@gsau.edu.cn; Tel.: +86-931-7631225

Abstract: The effect of mannan oligosaccharides (MOS) on health, growth performance, fatty acids deposition, serum, and urine metabolites, as well as the correlation between differential metabolites and other indexes, were investigated in *Hu* lambs. In total, 30 seven-day-old *Hu* male lambs were fed a milk replacer with or without 0.2% MOS (15 lambs in each). The lambs were placed on this diet until they were 28 days old. MOS significantly increased the apparent digestibility of organic matter (OM), crude protein (CP), ether extract (EE), calcium (Ca) and phosphorus (P), and unsaturated fatty acid (UFA) proportion, and decreased the diarrhea rate and saturated fatty acid (SFA) proportion in lambs ($p < 0.05$). MOS upregulated 20 metabolites in serum and 1 in urine and downregulated 11 metabolites in serum and 2 in urine ($p < 0.05$). Most of these metabolites comprised glycerophosphoethanolamines and glycerophosphocholines, which are significantly correlated with nutrient digestibility and fatty acid composition ($p < 0.05$). Overall, our results suggest that MOS benefited the health, nutrient utilization, and fatty acid profiles in *Hu* lambs via glycerolipid and glycerophospholipid metabolism pathways.

Keywords: fatty acid; growth performance; lamb; mannan oligosaccharides; metabolites; metabolome

Citation: Liu, T.; Li, F.; Xu, J.; Wang, J.; Shen, Z.; Zhang, F.; Wang, J.; Zheng, C. Metabolome Analysis Reveals Potential Mechanisms of Mannan Oligosaccharides to Improve Health, Growth Performance, and Fatty Acid Deposition in *Hu* Lambs. *Agriculture* **2022**, *12*, 1327. https://doi.org/10.3390/agriculture12091327

Academic Editors: Agnieszka Ludwiczak and Joanna Składanowska-Baryza

Received: 2 July 2022
Accepted: 25 August 2022
Published: 28 August 2022

1. Introduction

Mannan oligosaccharides (MOS) are a new class of active antigenic substances extracted from cell walls of yeast (*Saccharomyces cerevisiae*) and are widely used as prebiotic additives in livestock and poultry feed due to their gastrointestinal and immunological effects [1,2]. In several cases, dietary MOS supplementation has been found to improve the health status, growth, and productive performance of Charolaise heifers [3]. Notably, in ruminant feeding programs, while some scientists believe that ruminal microbes degrade the MOS and reduce their effectiveness, diets with MOS have been demonstrated to benefit the ruminant hosts. For instance, supplementing dietary MOS was shown to improve the nutrient utilization by *Hu* sheep and nitrogen metabolism in the rumen [4]. MOS can also decrease urine nitrogen excretion and energy released as methane [5], thus promoting nitrogen retention and antioxidant capacity in *Hu* sheep [6]. Furthermore, evidence has shown that they improve the average daily weight gain (ADG) and decrease the fecal *Escherichia coli* counts in Holstein heifer calves [7], as well as enhance the health of the ruminal epithelium and decrease the incidence and severity of hepatic abscesses in Dorper × Santa Ines crossbred lambs [8]. The potential mechanisms of these observations, however, remain unclear. Most studies have only concluded that MOS regulated the gastrointestinal microbiota to further improve the health status of the gastrointestinal tract and whole body,

immunity, growth, and productive performance. However, there is only a small amount of direct evidence to confirm the metabolism pathways and metabolites involved.

We, therefore, conducted a lamb feeding trial to investigate the effects of supplementing MOS in a milk replacer on the metabolome (i.e., all small-molecule chemicals in a biological sample) in early-stage lambs. Additionally, to elucidate the potential metabolic mechanisms of MOS in lambs, we explored the relationship between metabolites and the lambs' health, growth performance, and fatty acid deposition.

2. Materials and Methods

All experiments in this study were conducted following the approved guidelines of the Regulation Standing Committee of Gansu People's Congress. All experimental protocols and sample collections were approved by the Ethics Committee of the Gansu Agriculture University under permit number GAU-LC-2020-018.

2.1. Experimental Design, Animals, and Housing

We used a controlled experimental design wherein 30 twin male *Hu* lambs (Zhongtian Sheep Industry Co. LTD, Jinchang, China) from 15 *Hu* ewes were selected and divided into two treatments groups (control and MOS-supplemented, termed CON and MOS, respectively), with 15 lambs in each group, where each lamb was a replicate. At 1–3 days of age, the lambs suckled from their mothers. After 4 days, they were separated from their mothers and trained to suckle milk replacer from a feeding bottle. At 7 days, the twin lambs were blocked, ensuring no difference between treatments (CON: 4.09 $\pm$ 0.66 kg; MOS: 4.07 $\pm$ 0.61 kg).

Next, after 8 days, the lambs were fed a milk replacer with or without MOS according to the experimental design. The CON treatment lambs were fed control milk replacer (Beijing Precision Animal Nutrition Research Center, Beijing, China; the chemical composition of the milk replacer is shown in Table 1), and MOS treatment lambs were fed control milk replacer supplemented with 0.2% MOS (SCIPHAR®; Sciphar, Inc., Xi'an, Shaanxi Province, P. R. China; extracted from *Saccharomyces cerevisiae* yeast walls and MOS purity $\geq$ 99%). The lambs were fed four times each day (00:00, 06:00, 12:00, and 18:00 h), and the feeding quantity was 2% of the lamb's body weight [9,10]. The milk replacer was dissolved in warm water with a volume of five times that of the milk replacer. The lambs were fed individually in 1.45 m^2 cages with slatted floors and had ad libitum access to water. The feeding experiment lasted until the lambs were 28 days old.

Table 1. Chemical composition of lamb milk replacer (air-dried sample basis, %).

Ingredients	Concentration
Dry matter	95.68
Protein	24.89
Ether extract	17.50
Crude fiber	2.16
Crude ash	5.81
Calcium	1.02
Total phosphorus	0.59
Sodium chloride	0.1–0.2
Lysine	$\geq$2.2
Methionine	$\geq$1
Threonine	$\geq$1
Vitamin E (IU/kg)	$\geq$80
Fatty acid (percent of total fatty acid methyl esters quantified)	
UFA	32.483
SFA	54.476
MUFA	15.399
PUFA	17.083

Table 1. *Cont.*

Ingredients		Concentration
SFA:UFA		1.677
PUFA:SFA		0.314
MUFA:PUFA		0.901
n−3 PUFA		4.284
n−6 PUFA		12.799
n−6:n−3 PUFA		2.988
Butyric	C4:0	0.666
Caproic	C6:0	0.552
Octanoic	C8:0	0.417
Capric	C10:0	0.741
Lauric	C12:0	0.336
Tridecanoic	C13:0	3.660
Myristic	C14:0	0.400
Myristoleic	C14:1n5	3.537
Pentadecanoic	C15:0	0.154
Pentadecenoic	C15:1n5	0.181
Palmitic	C16:0	20.176
Palmitoleic	C16:1n7	0.487
Heptadecanoic	C17:0	0.210
Heptadecenoic	C17:1n7	0.484
Stearic	C18:0	24.571
Elaidic	C18:1n9t	0.352
Oleic	C18:1n9c	1.264
Linolelaidic	C18:2n6t	0.788
Linoleic	C18:2n6c	0.232
Arachidic	C20:0	1.479
γ-Linolenic	C18:3n6	0.358
α-Linolenic	C18:3n3	4.116
Heneicosanoic	C21:0	0.125
Behenic	C22:0	0.989
Eicosatrienoic	C20:3n6	0.127
Erucic	C22:1n9	8.828
Eicosatrienoic	C20:3n3	0.168
Arachidonic	C20:4n6	1.126
Docosadienoic	C22:2n6	10.168
Nervonic	C24:1n9	0.267

UFA: Unsaturated fatty acid. SFA: Saturated fatty acid. MUFA: Monounsaturated fatty acid. PUFA: Polyunsaturated fatty acid.

2.2. Sample Collection and Analysis

2.2.1. Lamb Growth Performance

At 7, 14, 21, and 28 days of age, the lambs were weighed before morning feeding, and the ADG was calculated. Additionally, the individual milk replacer intake and residue were recorded daily to calculate the average daily feed intake. The diarrhea of each lamb was monitored and recorded daily to calculate the diarrhea rate.

2.2.2. Digestion Trial

From 8–28 days of age, individual lamb feces were collected. Briefly, the fecal samples from each lamb were collected weekly, and sampling ratios were 20% of the total feces. The fecal samples were divided into two parts and prepared according to the Association of Official Analytical Chemists: One part was dried in a forced-air oven at 65 °C for 72 h to obtain a partial dry matter sample for measuring dry matter (DM, method 930.15), ether extract (EE, method 920.85), ash (method 942.05), calcium (Ca, method 978.02), and phosphorus (P, method 946.06); the remaining part was mixed with 10% sulfuric acid solution to fix nitrogen for measuring crude protein (CP, method 990.03) [11]. Finally,

nutrient contents in the milk replacer and feces were quantified to calculate the apparent nutrient digestibility.

2.2.3. Slaughter Trial

At 29 days of age, 12 lambs from each treatment were randomly selected, and 10 mL of blood and urine samples were collected for metabolite analysis. In brief, blood samples were collected by jugular vein into the non-anticoagulant tubes for preparing serum, and urine samples were collected by urine bags for 12 h and sub-collected 10 mL into sterile tubes. After weighing, eight lambs of near-average weight were randomly selected and euthanized according to the methods described by Chen et al. [12]. Thereafter, the *longissimus dorsi* (LD) muscle and abdominal adipose tissues were sampled and stored at −20 °C for fatty acid composition analysis.

2.2.4. Fatty Acid Composition Measurement

The fatty acid composition of the LD muscle and abdominal adipose tissues was determined via the gas chromatography method described by O'Fallon et al. [13]. Briefly, the samples were cut into 1.5-mm rectangular strips with a razor blade, and then 1 g was weighed into 15-mL tubes along with 0.7 mL of 10 M KOH and 5.3 mL of absolute methanol. The samples were then incubated in a 55 °C water bath for 1.5 h with vibrations for 5 s per 20 min. Thereafter, the tubes were cooled under running water until reaching room temperature, and 0.5 mL of 12 M sulfuric acid solution was added. The samples were inverted, mixed, and incubated again in a 55 °C water bath for 1.5 h with vibrations for 5 s per 20 min. After water-cooling the samples again, 3 mL of n-hexane was added, and the mixtures were homogenized using a vortex. The samples were centrifuged at 1610 × *g* for 5 min and filtered to obtain the liquid supernatant.

A 6890 N gas chromatography system (Agilent Technologies, Inc., Santa Clara, CA, USA) with a 100-m (0.25-mm i.d.) fused silica column (SP-2560; Sigma-Aldrich, Inc., St. Louis, MO, USA) was used to measure the fatty acid composition of both muscle and adipose tissue. The chromatography conditions were as follows: carrier gas: nitrogen; carrier gas flow: 1.2 mL/min; injection port temperature: 220 °C; split ratio: 100:1; injection volume: 1.0 μL; detector: flame ionization detector (FID); detector temperature: 250 °C; detector gas flow: air (450 mL/min), nitrogen (40 mL/min), and hydrogen (45 mL/min); and oven temperature programming: 140 °C for 5 min, 2 °C per min rise until 200 °C, maintaining for 5 min, 6 °C per min rise until 230 °C, maintaining for 20 min. Fatty acids were identified by comparing their retention times with the fatty acid methyl standards, and fatty acid percentages were computed according to the methods described by Pewan et al. [14] (Supplementary Material Dataset S1).

2.2.5. Metabolome Analysis of Serum and Urine Samples

Metabolome analysis was carried out by LipidALL Technologies Co., Ltd. (Changzhou, Jiangsu Province, P. R. China). In brief, serum (50 μL) and urine (100 μL) samples were thawed and transferred with 200 μL and 400 μL methanol, respectively, into a 1.5 mL centrifuge tube. The samples were centrifuged at 1204 × *g* and 4 °C for 10 min. Thereafter, the supernatants were transferred into new 1.5 mL centrifuge tubes, and methanol was added to obtain 80% methanol solutions (*v*/*v*). The solutions were incubated at −80 °C for 7 h and then centrifuged at 1404 × *g* and 4 °C for 10 min. The supernatants were transferred into new tubes and dried by a centrifugal concentrator. After that, 100 μL of 1% acetonitrile with an internal standard solution was added to redissolve samples.

Samples were analyzed via UHPLC-MS (5600 PLUS, AB SCIEX, Framingham, MA, USA) with ACQUITY UPLC HSS T3 columns (1.8 μm, 2.1 × 100 mm, Waters, Dublin, Ireland) under ESI mode. The chromatographic conditions were curtain gas: 35, ion spray voltage: 5500 V (positive ion mode), ion spray voltage: −4500 V (negative ion mode), temperature: 450 °C, ion source gas 1: 50, and ion source gas 2: 50.

2.3. Data Analysis

Statistical analyses of growth performance and nutrient apparent digestibility were performed in SPSS (IBM Corp. released 2019 and IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY, USA: IBM Corp.), using the linear model:

$$Y_{ij} = \mu + T_i + A_j + (T{:}A)_{ij} + \varepsilon_{ij}$$

where Y_{ij} is the value measured in treatment i at age j; μ is the overall mean; T_i is the fixed effect of the two treatments (CON and MOS; i = 1 and 2); A_j is the fixed effect of time over the three weeks (j = 1, 2, and 3); $(T{:}A)_{ij}$ is the fixed effect of the interaction between treatment and time; and ε_{ij} is the random residual error.

The data of fatty acids were analyzed by independent-sample t-tests using the following model:

$$t = \frac{\overline{x_1} - \overline{x_2}}{S_{\overline{x_1} - \overline{x_2}}}$$

where $\overline{x_1}$ and $\overline{x_2}$ are the means of the different treatments, and $S_{\overline{x_1} - \overline{x_2}}$ is the standard error of the mean difference.

Significance was determined at $p \leq 0.05$, and the tendency was at $0.05 < p \leq 0.10$, using Tukey's multiple comparison test.

After quality control, 143 and 103 metabolites were identified from serum and urine, respectively. We then used Principal Component Analysis (PCA) and Orthogonal partial least-squares discriminant analysis (OPLS-DA) to identify differential metabolites, where $p < 0.05$ indicated significant differential metabolites. Thereafter, the KEGG (Kyoto Encyclopedia of Genes and Genomes) databank was used to analyze metabolic pathways, find the enriched differential metabolites pathways, and calculate the pathway impact. Pearson correlation analysis (Prism 9.3, GraphPad Software, San Diego, CA, USA) was used to analyze the correlation between serum and urine differential metabolites and growth performance and fatty acid composition in LD muscle and abdominal adipose tissue. Significance was determined at $p \leq 0.05$.

3. Results

3.1. Growth Performance

Supplementation with MOS did not significantly influence the final body weight, ADG, or milk replacer intake of *Hu* lambs ($p > 0.05$). However, it significantly increased organic matter apparent digestibility (OMD), crude protein apparent digestibility (CPD), ether extract apparent digestibility (EED), calcium apparent digestibility (CaD), and phosphorus apparent digestibility (PD) at different growth stages ($p < 0.05$), and significantly decreased the diarrhea rate of *Hu* lambs ($p < 0.05$, Figure 1). During the whole experimental stage, time was the main factor that affected the growth performance and nutrient digestion significantly ($p < 0.05$), while the treatment and the interaction of treatment and time influenced the nutrient apparent digestibility more ($p < 0.05$, Table 2).

3.2. Fatty Acid Proportion in Longissimus Dorsi Muscle and Abdominal Adipose Tissue

Supplementation with MOS did not impact the fatty acid composition and percentage in LD muscle and abdominal adipose tissue of *Hu* lambs ($p > 0.05$, Figure 2). However, MOS significantly increased the proportion of monounsaturated fatty acid (MUFA) and n−3 polyunsaturated fatty acid (PUFA, $p < 0.05$) and significantly decreased the proportion of saturated fatty acid (SFA, $p < 0.05$) in LD muscle (Figure 3).

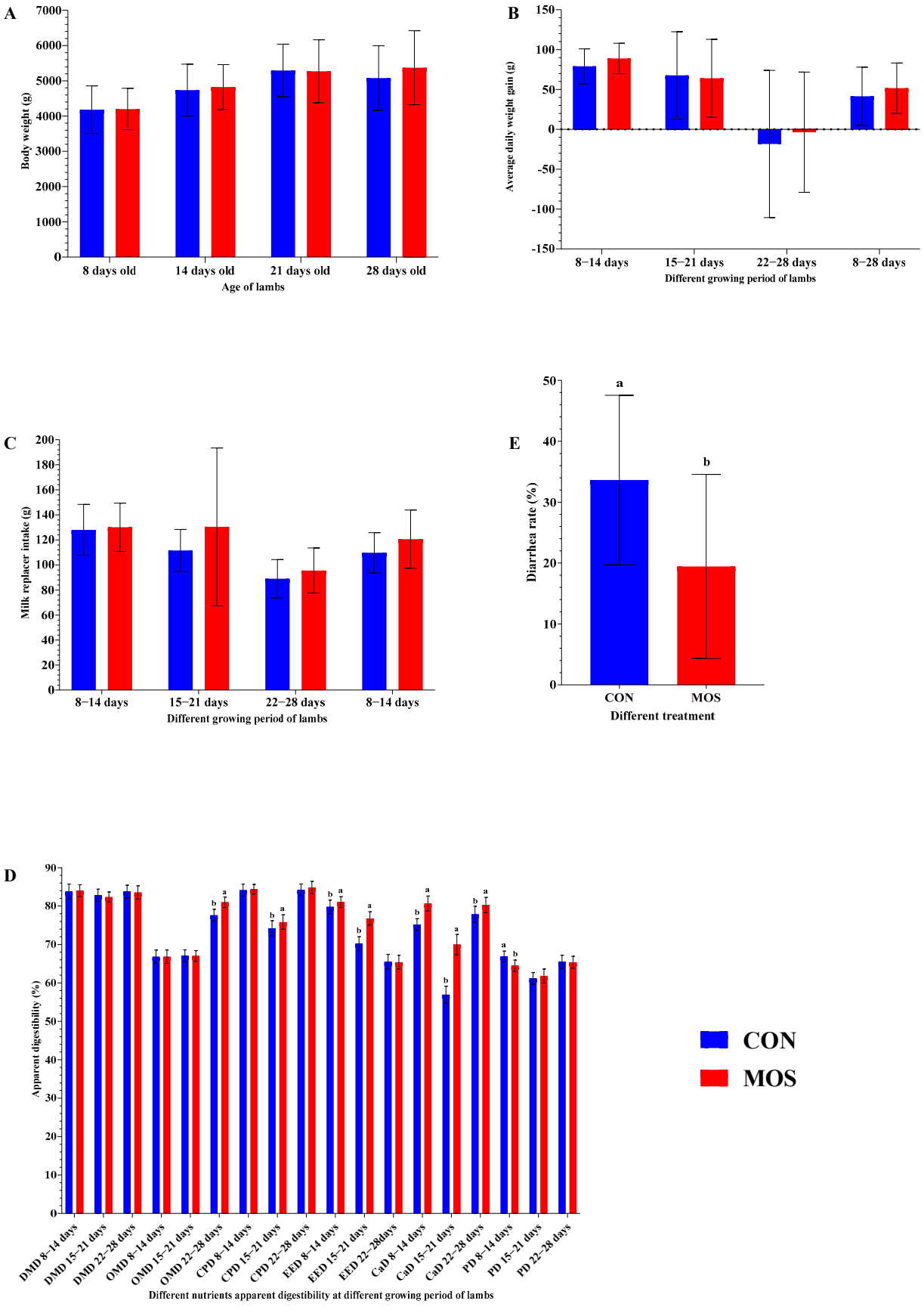

Figure 1. Growth performance of *Hu* lambs treated with either control or mannan oligosaccharides milk replacer. (**A**) Body weight of *Hu* lambs at different ages; (**B**) average daily weight gain of *Hu*

lambs at different growing periods; (**C**) milk replacer intake of *Hu* lambs at different growing periods; (**D**) nutrients' apparent digestibility of *Hu* lambs at different growing period; (**E**) diarrhea rate of *Hu* lambs. *Hu* lambs were fed milk replacers with or without 0.2% mannan oligosaccharides (MOS; n = 15 per treatment). The growth performances in 30 lambs divided into two groups based on MOS supplementation are shown for the 3−week collection phase of the study. DMD: Dry matter apparent digestibility; OMD: Organic matter apparent digestibility; CPD: Crude protein apparent digestibility; EED: Ether extract apparent digestibility; CaD: Calcium apparent digestibility; PD: Phosphorus apparent digestibility. ^{ab} Values above columns with different superscripts differ significantly at $p \leq 0.05$.

Table 2. Growth performance and nutrient digestibility of *Hu* lambs treated with either control or mannan oligosaccharides.

Item	CON	MOS	SEM	*p* Value		
				Treatment	Time	Treatment × Time
Body weight (g)	4412.64	5063.99	188.09	0.087	0.365	0.994
Average daily weight gain (g)	40.97	49.92	7.02	0.440	<0.001	0.975
Average daily milk replacer intake (g)	73.15	96.48	6.37	0.067	0.160	0.790
Dry matter digestibility (%)	83.47	83.29	0.18	0.621	0.007	0.715
Organic matter digestibility (%)	70.51 ^{b}	71.61 ^{a}	0.65	0.001	<0.001	<0.001
Crude protein digestibility (%)	80.88 ^{b}	81.69 ^{a}	0.50	0.023	<0.001	0.261
Ether extract digestibility (%)	71.84 ^{b}	74.41 ^{a}	0.70	<0.001	<0.001	<0.001
Calcium digestibility (%)	70.00 ^{b}	76.98 ^{a}	0.90	<0.001	<0.001	<0.001
Phosphorus digestibility (%)	64.52	63.90	0.27	0.065	<0.001	0.001

Lambs were fed milk replacers with or without 0.2% mannan oligosaccharides (MOS; n = 15 per treatment). The growth performance and nutrient digestibility in 30 lambs divided into two groups based on MOS supplementation are shown for the 3–week collection phase of the study. ^{ab} Values within a row with different superscripts differ significantly at $p \leq 0.05$.

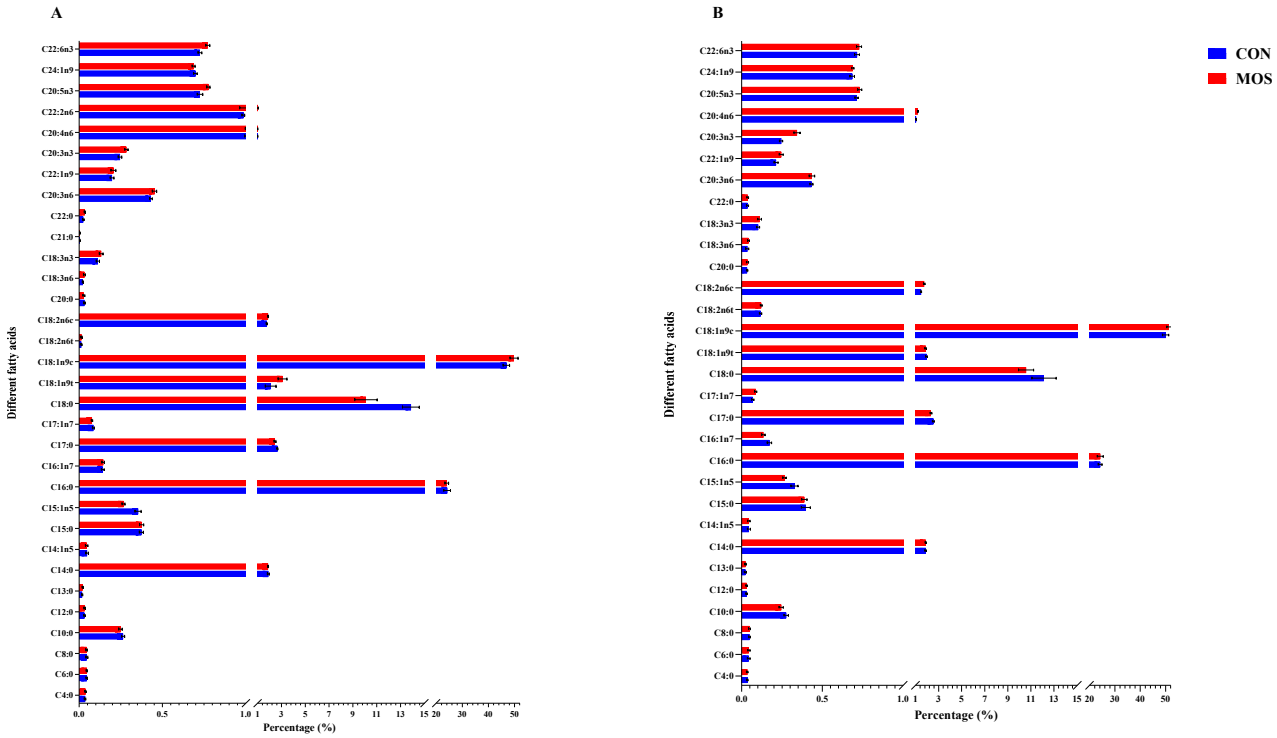

Figure 2. Fatty acids composition and proportion in muscle and adipose tissue of *Hu* lambs treated with either control or mannan oligosaccharides milk replacer. (**A**) *longissimus dorsi* muscle; (**B**) abdominal

adipose tissue. Lambs were fed milk replacers with or without 0.2% mannan oligosaccharides (MOS; n = 8 per treatment). The individual fatty acid percentages of total fatty acid methyl esters quantified in 16 lambs divided into two groups based on MOS supplementation are shown for the 3–week collection phase of the study.

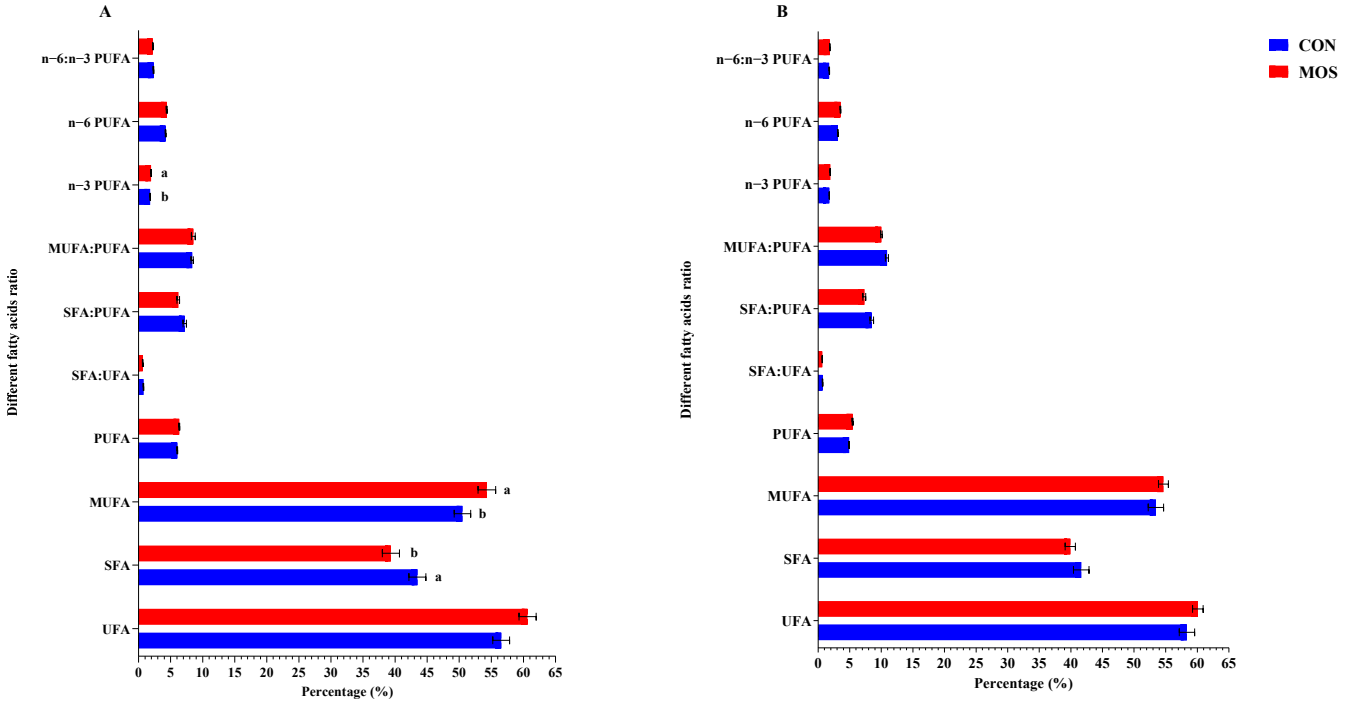

Figure 3. Different types of fatty acid proportion in muscle and adipose tissue of *Hu* lambs treated with either control or mannan oligosaccharides milk replacer. (**A**) *longissimus dorsi* muscle; (**B**) abdominal adipose tissue. Lambs were fed milk replacers with or without 0.2% mannan oligosaccharides (MOS; n = 8 per treatment). The individual types of fatty acid percent or their ratio in 16 lambs divided into two groups based on MOS supplementation are shown for the 3–week collection phase of the study. UFA: Unsaturated fatty acid. SFA: Saturated fatty acid. MUFA: Monounsaturated fatty acid. PUFA: Polyunsaturated fatty acid. ^{ab} Values on the right side of columns with different superscripts differ significantly at $p \leq 0.05$.

3.3. Differential Metabolites in Serum and Urine

PCA analysis revealed that samples from serum and urine were all within the 95% confidence interval, and relatively, the distribution of serum samples from lambs supplemented with MOS was more concentrated. The OPLS-DA analysis identified 31 differential metabolites from serum samples and three differential metabolites from urine samples, as indicated by their variable importance for the projection (VIP > 1.0) and Wilcoxon test ($p < 0.05$). This included 20 metabolites upregulating and 11 metabolites downregulating in serum. Additionally, one metabolite was upregulated and two metabolites were downregulated in urine (Table 3 and Figure 4, Supplementary Material Dataset S2).

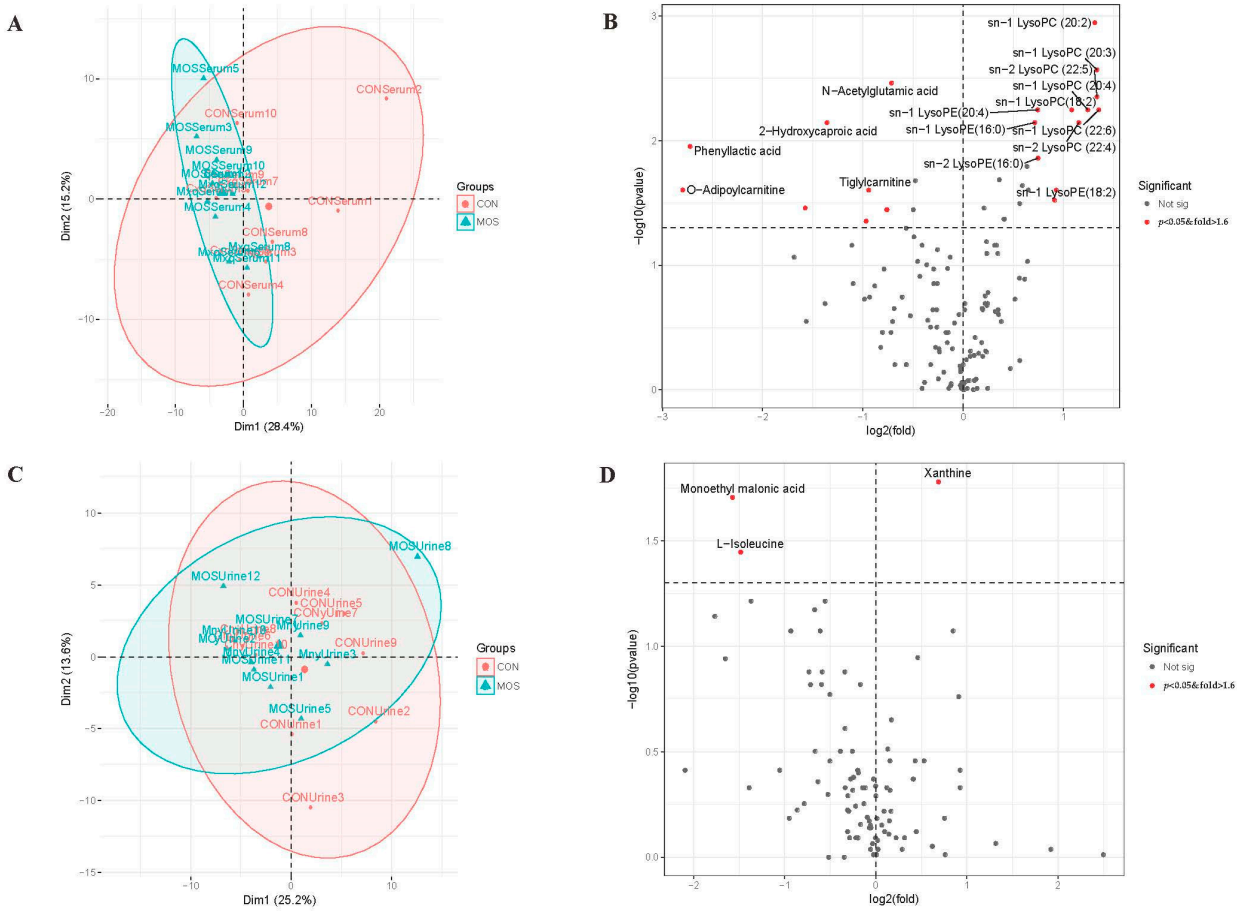

Figure 4. PCA and OLSA-DA analyses of serum and urine metabolites of *Hu* lambs treated with either control or mannan oligosaccharides milk replacer. (**A**) PCA analyses of serum metabolites; (**B**) volcano plot of serum metabolites; (**C**) PCA analyses of urine metabolites; (**D**) volcano plot of urine metabolites. Lambs were fed milk replacers with or without 0.2% mannan oligosaccharides (MOS; n = 12 per treatment). The serum and urine metabolites in 24 lambs divided into two groups based on MOS supplementation are shown for the 3–week collection phase of the study.

Table 3. Differential metabolites in serum and urine samples of *Hu* lambs treated with either control or mannan oligosaccharides.

Class	Metabolites	*p*-Value	FC
	Serum		
Polyols	Phenyllactic acid	0.009	0.151
Phenols	33–Hydroxyisovaleric acid	0.036	0.590
Indolyl carboxylic acids	Indolelactic acid	0.032	0.336
Imidazoles	Allantoin	0.036	0.708
Glycerophosphoethanolamines	sn–1 LysoPE(16:0)	0.007	1.642
	sn–1 LysoPE(18:2)	0.025	1.907
	sn–1 LysoPE(20:4)	0.006	1.676
	sn–2 LysoPE(16:0)	0.014	1.678
	sn–2 LysoPE(18:1)	0.014	1.553
	sn–2 LysoPE(18:2)	0.030	1.881
Glycerophosphocholines	sn–1 LysoPC(16:0)	0.021	1.505
	sn–1 LysoPC(16:1)	0.025	1.479
	sn–1 LysoPC(18:2)	0.006	2.116
	sn–1 LysoPC(20:2)	0.001	2.485
	sn–1 LysoPC(20:3)	0.002	2.521
	sn–1 LysoPC (20:4)	0.006	2.366

Table 3. *Cont.*

Class	Metabolites	*p*-Value	FC
	sn–1 LysoPC(22:6)	0.006	2.552
	sn–2 LysoPC(16:0)	0.025	1.569
	sn–2 LysoPC (22:4)	0.007	2.226
	sn–2 LysoPC (22:5)	0.004	2.524
Fatty acids	2–Hydroxy–2–methylbutyric acid	0.036	0.590
Carbohydrates	Glyceric acid	0.021	1.284
Benzoic acids	2–Hydroxycaproic acid	0.007	0.391
Amino acids	4–Hydroxyproline	0.043	1.326
	Betaine	0.032	1.155
	N–Acetylglutamic acid	0.003	0.609
	N–Acetyl–L–alanine	0.043	1.330
	Urea	0.017	0.720
Acyl carnitines	Butyrylcarnitine	0.043	0.511
	O–Adipoylcarnitine	0.025	0.144
	Tiglylcarnitine	0.025	0.520
	Urine		
Amino acids	L–Isoleucine	0.036	0.357
Carboxylic acids	Monoethyl malonic acid	0.020	0.336
Purines	Xanthine	0.016	1.612

Lambs were fed milk replacers with or without 0.2% mannan oligosaccharides (MOS; n = 12 per treatment). The serum and urine metabolites in 24 lambs divided into two groups based on MOS supplementation are shown for the 3–week collection phase of the study. FC: Fold change.

3.4. Functional Annotation of Differential Metabolites, Enrichment Analysis, and KEGG Metabolic Pathway Analysis

Among the 31 serum and 3 urine metabolites, 25 serum and 3 urine metabolites were identified in The Human Metabolome Database (HMDB), and 24 serum and 3 urine metabolites were included in the analysis (Table 4 and Figure 5). The 24 serum metabolites, including glycerolipid, glyoxylate, dicarboxylate; arginine, proline, glycine, serine, threonine, glycerophospholipid, and purine, were involved in various metabolism systems. The impact scores were higher in glycerolipid, arginine, proline, and glycerophospholipid metabolism. The three urine metabolites were involved in valine, leucine, and isoleucine degradation, valine, leucine, and isoleucine biosynthesis, purine metabolism, and aminoacyl–tRNA biosynthesis. The impact scores were higher in valine, leucine, and isoleucine biosynthesis.

Table 4. Pathway analysis of serum and urine samples of *Hu* lambs treated with either control or mannan oligosaccharides.

Pathway	−log(*p*)	Impact
Serum		
Glycerolipid metabolism	1.85630	0.10471
Glyoxylate and dicarboxylate metabolism	1.23730	0.00000
Arginine and proline metabolism	1.11890	0.04102
Glycine, serine and threonine metabolism	1.00840	0.00000
Glycerophospholipid metabolism	0.68863	0.04680
Purine metabolism	0.26797	0.00000
Urine		
Valine, leucine and isoleucine degradation	2.64820	0.00000
Valine, leucine and isoleucine biosynthesis	2.64820	0.33333
Purine metabolism	1.02960	0.02143
Aminoacyl–tRNA biosynthesis	0.70622	0.00000

Lambs were fed milk replacers with or without 0.2% mannan oligosaccharides (MOS; n = 12 per treatment). The serum- and urine-enriched pathways in 24 lambs divided into two groups based on MOS supplementation are shown for the 3–week collection phase of the study.

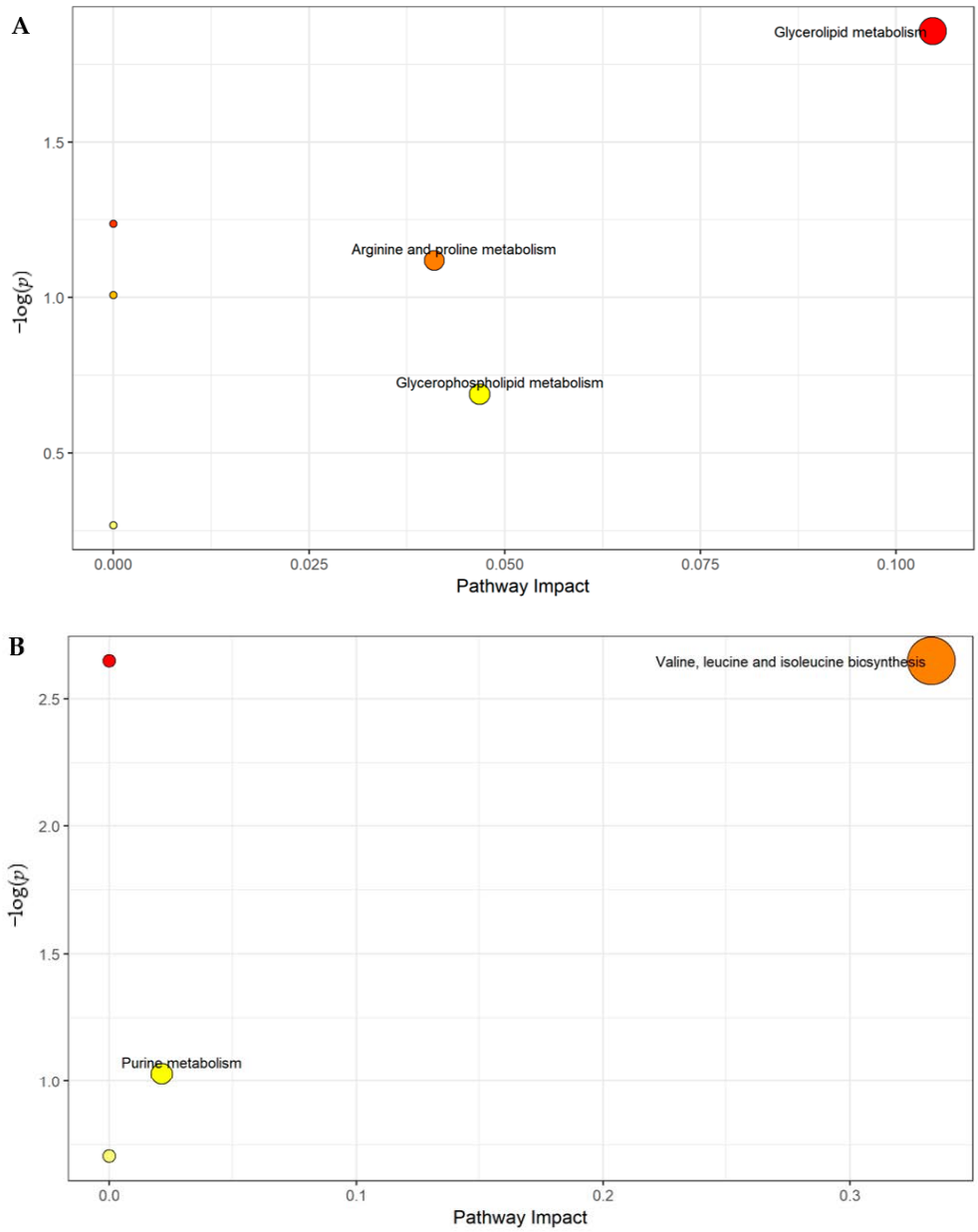

Figure 5. Pathway topology analyses of serum (**A**) and urine (**B**) metabolites of *Hu* lambs treated with either control or mannan oligosaccharides milk replacer. Lambs were fed milk replacers with or without 0.2% mannan oligosaccharides (MOS; $n = 12$ per treatment). The serum and urine metabolites in 24 lambs divided into two groups based on MOS supplementation are shown for the 3–week collection phase of the study.

3.5. Correlation between Growth Performance and Fatty Acid Proportion in Muscle and Adipose Tissue and Serum and Urine Metabolites

The serum and urine metabolites were significantly associated with growth performance and fatty acids proportion of *Hu* lambs ($p < 0.05$). For instance, among serum metabolites, glycerophosphocholines (sn−1 LysoPC(22:6)) were significantly positively correlated with body weight and ADG ($p < 0.05$). Further, several glycerophosphoethanolamines and glycerophosphocholines were significantly positively correlated with the CPD and EED at 8–14 days and negatively correlated with the PD at 15–21 days ($p < 0.05$). Allantoin, butyrylcarnitine, indolelactic acid, N–acetylglutamic acid, O–adipoylcarnitine, phenyllactic acid, and urea were significantly positively correlated, and betaine was negatively correlated, with the CPD at 15–21 days ($p < 0.05$, Figure 6). We found several glycerophos-

phoethanolamines and glycerophosphocholines that were significantly positively correlated with the proportion of unsaturated fatty acid (UFA), MUFA, PUFA, and n−3PUFA, and negatively related to the proportion of SFA, SFA:UFA, SFA:PUFA, and n−6:n−3PUFA in the LD muscle ($p < 0.05$, Figure 7). Several glycerophosphoethanolamines and glycerophosphocholines were also significantly positively correlated with the proportion of UFA, MUFA, PUFA, n−3PUFA, n−6PUFA, and n−6:n−3PUFA, and negatively correlated with the proportion of SFA, SFA:UFA, SFA:PUFA, and MUFA:PUFA in abdominal adipose tissue ($p < 0.05$, Figure 8). Among urine metabolites, L−isoleucine, monoethyl malonic acid, and xanthine were significantly positively correlated with the EED at 22–28 days, CPD at 8–14 days, and CaD at 8–14 days ($p < 0.05$, Figure 6). Additionally, monoethyl malonic acid was significantly positively correlated with the proportion of PUFA and n−3PUFA, and xanthine was significantly positively correlated with the proportion of n−6:n−3PUFA in the LD muscle ($p < 0.05$, Figure 7).

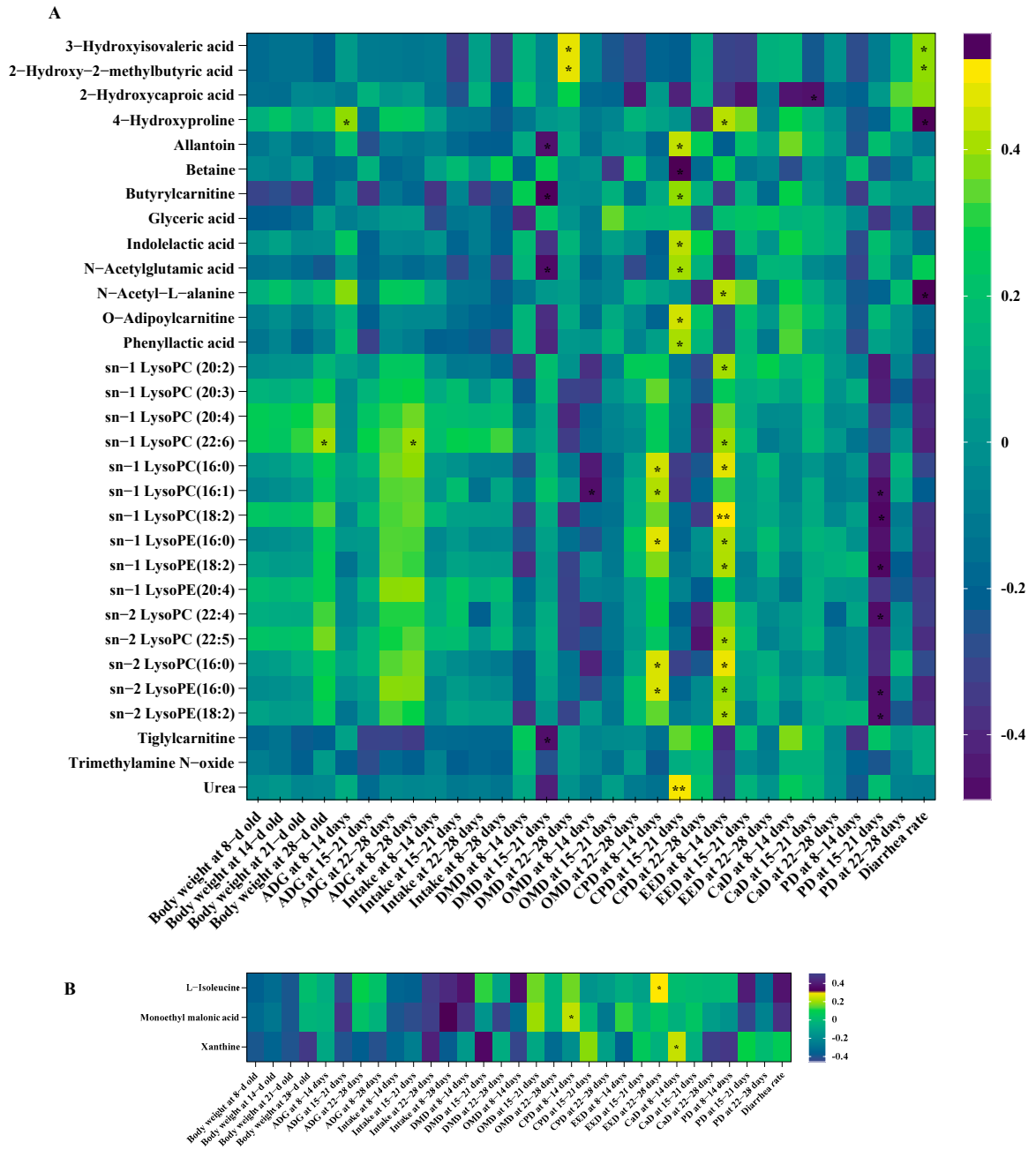

Figure 6. Correlation between serum (**A**) and urine (**B**) metabolites with growth performance of *Hu*

lambs treated with either control or mannan oligosaccharides milk replacer. Lambs were fed milk replacers with or without 0.2% mannan oligosaccharides (MOS; n = 15 per treatment in growth performance measurement; n = 12 per treatment in metabolome analyses). The growth performances in 30 lambs and the serum and urine metabolites in 24 lambs divided into two groups based on MOS supplementation are shown for the 3–week collection phase of the study. ADG: Average daily weight gain; DMD: Dry matter apparent digestibility; OMD: Organic matter apparent digestibility; CPD: Crude protein apparent digestibility; EED: Ether extract apparent digestibility; CaD: Calcium apparent digestibility; PD: Phosphorus apparent digestibility. * Values within cells differ significantly at $p \leq 0.05$, and ** values within cells differ significantly at $p \leq 0.01$.

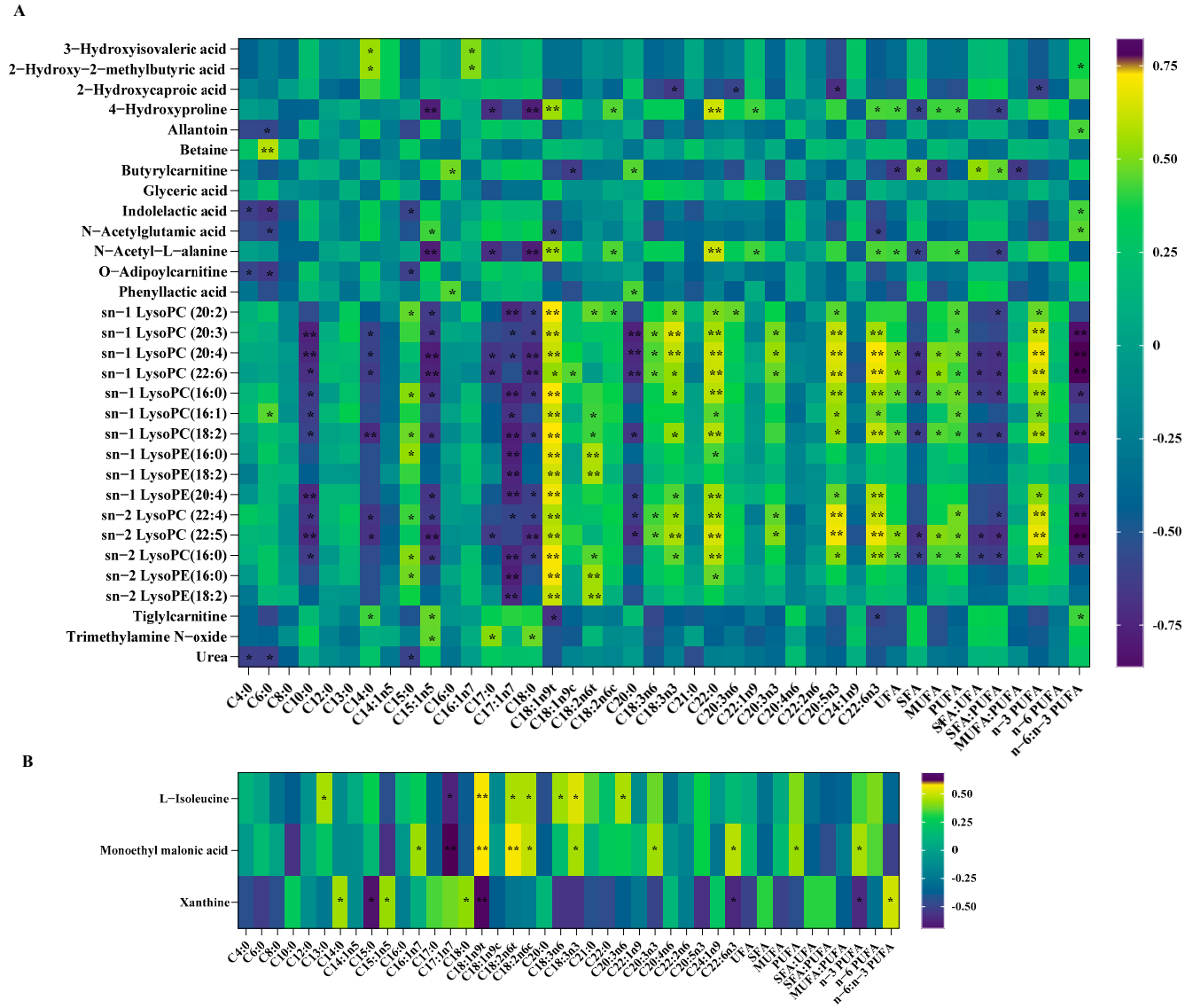

Figure 7. Correlation between serum (**A**) and urine (**B**) metabolites with fatty acid proportion in *longissimus dorsi* muscle of *Hu* lambs treated with either control or mannan oligosaccharides milk replacer. Lambs were fed milk replacers with or without 0.2% mannan oligosaccharides (MOS; n = 8 per treatment in fatty acid proportion analyses; n = 12 per treatment in metabolome analyses). The fatty acid proportions in muscle of 16 lambs and the serum and urine metabolites in 24 lambs divided into two groups based on MOS supplementation are shown for the 3−week collection phase of the study. UFA: Unsaturated fatty acid; SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid. * Values within cells differ significantly at $p \leq 0.05$, and ** values within cells differ significantly at $p \leq 0.01$.

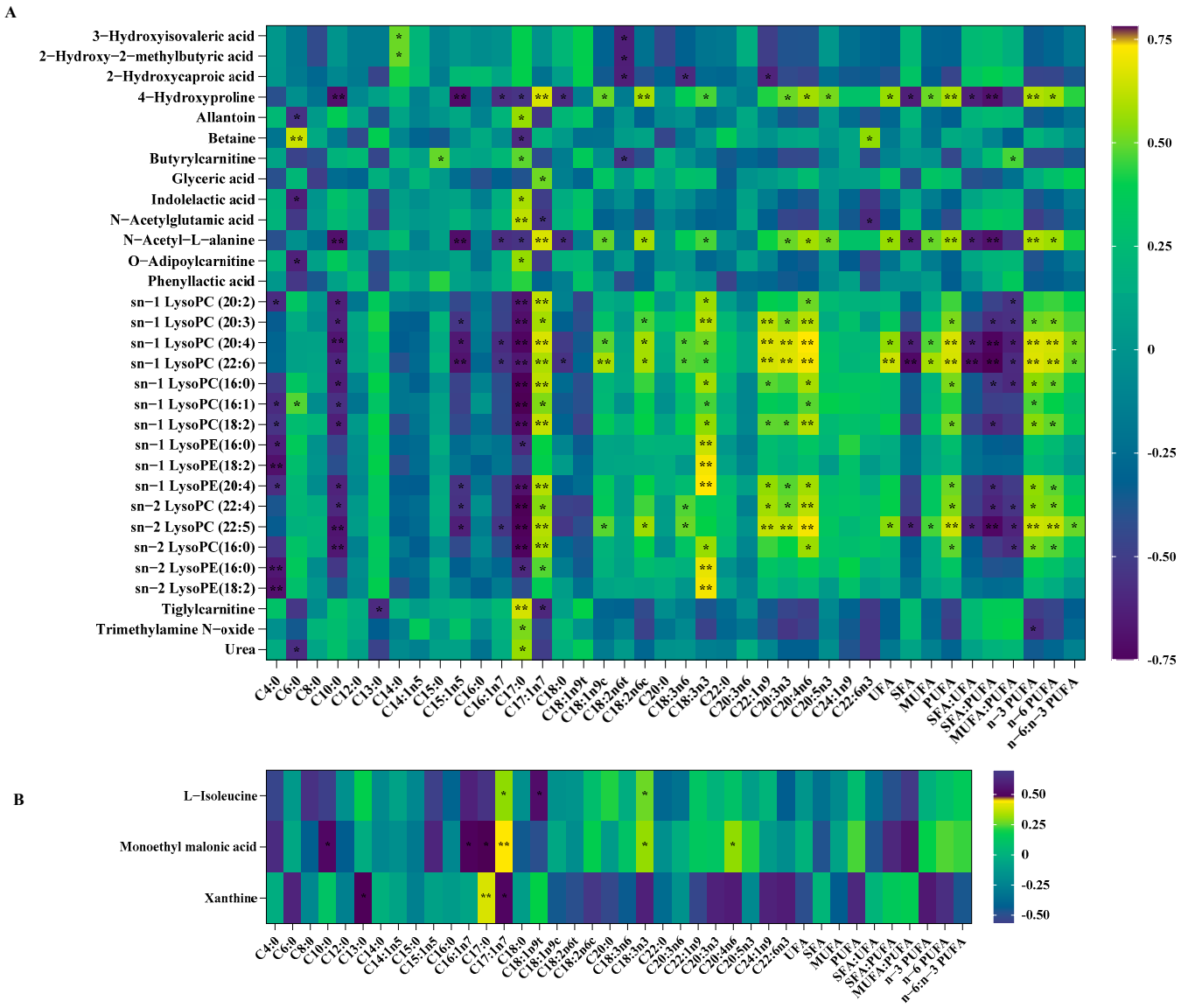

Figure 8. Correlation between serum (**A**) and urine (**B**) metabolites with fatty acid proportion in abdominal adipose tissue of *Hu* lambs treated with either control or mannan oligosaccharides milk replacer. Lambs were fed milk replacers with or without 0.2% mannan oligosaccharides (MOS; $n = 8$ per treatment in fatty acid proportion analyses; $n = 12$ per treatment in metabolome analyses). The fatty acid proportions in adipose tissue of 16 lambs and serum and urine metabolites in 24 lambs divided into two groups based on MOS supplementation are shown for the 3−week collection phase of the study. UFA: Unsaturated fatty acid; SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid. * Values within cells differ significantly at $p \leq 0.05$, and ** values within cells differ significantly at $p \leq 0.01$.

4. Discussion

As the development of lamb's rumen was hindered by insufficient stimulation from solid food during this trial, the effect of MOS on growth would likely have been driven by mechanisms similar to those in monogastric animals [15]. Mannan oligosaccharides have been found to bind to pathogenetic microbes with type−1 fimbriae, such as *Escherichia coli* and *Salmonella* species. Moreover, MOS increase the total anaerobic bacteria such as *Lactobacillus*, *Bifidobacterium*, and especially *Bacteroides*, which efficiently ferment indigestible polysaccharides to short-chain fatty acids (SCFAs) and, consequently, build up nutrient absorption and protect hosts from disease [15]. This indicates that SCFAs increase the parti-

tion of nutrients into the other tissues, are directly absorbed in the hindgut, and are used as an energy source in tissues. This may explain our observation that supplementing a milk replacer with MOS significantly increased the CPD, EED, CaD, and PD of *Hu* lambs, and decreased the diarrhea rate. In addition, previous research has shown that MOS produce longer villi in animals, which provides a larger surface area for absorbing nutrients in the intestine. This leads to efficient enzyme production and maturation of the intestinal cells due to a lower renewal rate [16,17], thereby increasing nutrient digestibility in the intestinal tract. These findings are in line with our previous study, which concluded that MOS improved histomorphology in the duodenum and ileum, maintained the intestinal barrier, reduced the apoptotic rate of intestinal epithelial cells, and provided benefits for nutrient absorption in lambs [18]. Additionally, MOS modulate immune responses in gut-associated lymphoid tissue, such as cecal tonsils, and enhance the titers of plasma antibodies IgM and IgG, cecum IgA levels, mucin mRNA expression, and intestinal immune functions [19]. Therefore, MOS supplementation in the diet is often associated with improved ADG, feed conversion ratio, and carcass weight [20]. Several previous studies provided evidence to support this association. For example, 4 g/day per calf MOS can promote DM intake, ADG, the feed conversion ratio, structural growth, neutral detergent fiber digestibility, and reduce *E. coli* count in the feces of buffalo calves [21]. In another study, 4 g/day per calf MOS added to warm whole milk tended to increase starter intake and body weight gain at 26–46 days of age in calves [22]. In addition, another study found that 1 g/kg MOS improved cellulose digestibility and acetate concentration, increased the amount of branched-chain fatty acids in cecal content, and decreased colonies of *Coliformis* in the gastrointestinal tract of rabbits during the fattening period [23]. Furthermore, 0.1% MOS increased the DMD and CPD in weanling pigs, which was likely due to the morphological improvements in the small intestine [24].

The ADG of both CON and MOS-treated lambs was negative during the third week, possibly due to the extreme feeding procedures adopted. Lambs were fed only the milk replacer during the whole experimental period and were not permitted to ingest solid food, such as starter, grass, or hay. Although the milk replacer provides nutrients to lambs, the deficiency of stimulation from solid food may have inhibited gastrointestinal physical development. These results suggest that earlier solid starter provision is an essential strategy in small ruminant feeding to successfully improve growth performance and productivity [25–27].

In the current study, most differential metabolites in serum and urine were glycerophospholipids and amino acids, suggesting that the MOS may regulate nitrogen and lipid metabolism in lambs. Enriched pathways of glycerolipid, glycerophospholipid, arginine, and proline metabolism, as well as valine, leucine, and isoleucine biosynthesis, and purine metabolism, also indicated that glycerophospholipids, arginine, proline, valine, leucine, and isoleucine could be biomarkers for MOS regulating metabolism in *Hu* lambs. In serum differential metabolites, several glycerophosphoethanolamines and glycerophosphocholines were significantly positively correlated with the CPD and EED and negatively correlated with the PD, while glycerophosphocholines (sn−1 LysoPC(22:6)) were significantly positively correlated with the body weight and ADG of *Hu* lambs. Glycerophosphoethanolamine and glycerophosphocholine are the main components of phospholipids. They are beneficial compounds that improve memory and cognition, promote brain development, inhibit cancer, and regulate lipid metabolism [28]. This may explain the positive correlation between growth performance and nutrient utilization of lambs and the enrichment of glycerolipid and glycerophospholipid metabolism pathways in this study. However, the digestion of phosphorus was negatively correlated with glycerophosphoethanolamines and glycerophosphocholines. Glycerophospholipids are rich in phosphorus elements and can inhibit the absorption of phosphorus in the intestinal tract; however, this mechanism needs further research. In addition, allantoin, butyrylcarnitine, indolelactic acid, N−acetylglutamic acid, O−adipoylcarnitine, phenyllactic acid, and urea were positively correlated with CPD, likely because all are involved in nitrogen

metabolism and enriched pathways of arginine and proline metabolism, and glycine, serine, and threonine metabolism.

SFA is associated with the increased production of cholesterol and, consequently, leads to a potential risk of cardiovascular diseases [29]. Therefore, the World Health Organization has recommended that total fat, SFA, and trans fatty acid should be 15–30%, < 10%, and < 1% of the total energy intake, respectively. Moreover, the European Food Safety Authority has recommended that total fat should only represent 20–35% of the energy intake and that the intake should be as low as possible for SFA and trans fatty acids [30]. As PUFA is beneficial for human health and can inhibit tumor angiogenesis and coronary arteriosclerosis, increasing its proportion in mutton to improve meat quality has attracted research interest [31].

The results showed that supplementing the milk replacer with MOS increased the MUFA and n−3 PUFA proportions in LD muscle. This suggests that MOS could increase UFA deposition in the muscle of lambs, especially n−3 PUFA, which would benefit both lambs and lamb-meat consumers. Previous studies have reported similar results. For example, 0.2% chitosan increased the oleic−cis−9 acid, linoleic acid, linolenic−trans−6 acid, arachidonic acid, and eicosapentaenoic acid contents in lamb meat [32]. Similarly, 0.5, 1.0, or 1.5 g/kg of MOS increased the oleic acid and MUFA contents in rabbit meat [23]. In addition, we found that the SFA:PUFA ranged from 6.01 to 8.85 in the muscle and adipose tissues. Except for abdominal adipose tissue of CON lambs, the ratios of SFA:PUFA were lower than the values obtained in the studies of Bezerra et al. [29] and Realini et al. [33], which were 12.5 and 7.69 for the muscle and adipose tissues, respectively, in lamb meat. Although a healthy SFA:PUFA ratio for humans should be < 2.5, the SFA:PUFA ratio of 6.67 in lambs is generally considered adequate [34,35], and it is difficult to decrease this ratio in lambs [36]. As the study period was relatively short, and the lambs did not consume solid food, a longer feeding trial is necessary to confirm the MOS-triggered fatty acid profile regulation efficiency we observed in lamb muscle and adipose tissues.

In serum metabolites, glycerophosphoethanolamines and glycerophosphocholines were correlated with most fatty acids in LD muscle and abdominal adipose tissues of *Hu* lambs, with significant positive associations with UFA, MUFA, PUFA, and n−3PUFA and a negative association with SFA.

In this study, supplementing MOS upregulated the concentration of glycerophosphoethanolamines and glycerophosphocholines in lambs. Glycerophosphocholines likely increased acetylcholine release, which accelerated the catecholamine-induced stimulation of the α2−adrenergic receptor, thus inhibiting the secretion of somatotropin release-inhibiting factor (SRIF) in the hypothalamus. The growth hormone (GH) was then stimulated, which may have induced the increase in triacylglycerols lipolysis in the liver and skeletal muscles [37], ultimately decreasing SFA proportion in muscle and adipose tissues. Moreover, glycerophosphoethanolamines are not only the main component of the cell membrane, but the precursor of many essential biological molecules including diacylglycerol (DAG), fatty acids, and phosphatidic acid (PA) [38], which are involved in glycerolipid metabolism and glycerophospholipid metabolism, in accordance with our enriched pathway analysis results. Notably, purine metabolism was identified in both serum and urine, and in this system, xanthine is transferred into uric acid by xanthine oxidase and generates reactive oxygen species, which in turn oxidizes uric acid into allantoin. Here, we found that the allantoin concentration in serum was decreased by MOS, while the xanthine concentration in urine was increased, suggesting that MOS may alleviate oxidative stress in lambs by reducing xanthine oxidase.

5. Conclusions

In conclusion, supplementing the milk replacer with MOS significantly increased OMD, CPD, EED, CaD, and PD and decreased the diarrhea rate of *Hu* lambs. Moreover, dietary MOS supplementation upregulated the serum concentration of glycerophosphoethanolamines and glycerophosphocholines, thereby increasing the UFA proportion in

muscle and adipose tissues via both glycerolipid metabolism and glycerophospholipid metabolism pathways. Overall, we found that supplementing the milk replacer with MOS has health benefits and can adjust the fatty acids composition in muscle and adipose tissues of *Hu* lambs during early growth.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12091327/s1, The details of fatty acids composition and percentage measurement are shown in Supplementary Material Dataset S1: Figure S1. Chromatogram of 37 fatty acid methyl ester standards. The plots of OPLS-DA model and permutation test in serum and urine samples are shown in Supplementary Material Dataset S2: Figure S1. Plot of OPLS-DA model and permutation test of serum sample of *Hu* lambs treated with either control or mannan oligosaccharides milk replacer. Figure S2. Plot of OPLS-DA model and permutation test of urine sample of *Hu* lambs treated with either control or mannan oligosaccharides milk replacer.

Author Contributions: T.L.: Conceptualization, methodology, data curation, writing, funding acquisition; F.L.: Conceptualization, methodology; J.X.: Investigation; J.W. (Jing Wang): Investigation; Z.S.: Investigation; F.Z.: Investigation; J.W. (Jiaqi Wang): Investigation; C.Z.: Conceptualization, methodology, supervision, funding acquisition. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the National Natural Science Foundation of China (grant numbers 31860657 and 32160803), and the Special Talent Introduction Program of Gansu Agricultural University (grant number GSAU-RCZX201710).

Institutional Review Board Statement: The study was conducted in accordance with the approved guidelines of the Regulation Standing Committee of Gansu People's Congress and approved by the Ethics Committee of the Gansu Agriculture University (protocol code GAU-LC-2020-018 and date of approval).

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: We would like to thank all the people who helped with this experiment.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Xu, B.; Wu, S.; Han, Q. Modulation of the growth performance and innate immunity of loaches (*Paramisgurnus dabryanus*) upon dietary mannan oligosaccharides. *3 Biotech* **2021**, *11*, 133. [CrossRef]
2. Sanguri, S.; Gupta, D. Prebiotic Mannan Oligosaccharide Pretreatment Improves Mice Survival Against Lethal Effects of Gamma Radiation by Protecting GI Tract and Hematopoietic Systems. *Front. Oncol.* **2021**, *11*, 677781. [CrossRef] [PubMed]
3. Grossi, S.; Dell'Anno, M.; Rossi, L.; Compiani, R.; Rossi, C.A.S. Supplementation of Live Yeast, Mannan Oligosaccharide, and Organic Selenium during the Adaptation Phase of Newly Arrived Beef Cattle: Effects on Health Status, Immune Functionality, and Growth Performance. *Antibiotics* **2021**, *10*, 1114. [CrossRef] [PubMed]
4. Zheng, C.; Zhou, J.; Zeng, Y.; Liu, T. Effects of mannan oligosaccharides on growth performance, nutrient digestibility, ruminal fermentation and hematological parameters in sheep. *PeerJ* **2021**, *9*, e11631. [CrossRef] [PubMed]
5. Zheng, C.; Ma, J.; Liu, T.; Wei, B.; Yang, H. Effects of Mannan Oligosaccharides on Gas Emission, Protein and Energy Utilization, and Fasting Metabolism in Sheep. *Animals* **2019**, *9*, 741. [CrossRef]
6. Zheng, C.; Li, F.; Hao, Z.; Liu, T. Effects of adding mannan oligosaccharides on digestibility and metabolism of nutrients, ruminal fermentation parameters, immunity, and antioxidant capacity of sheep. *J. Anim. Sci.* **2018**, *96*, 284–292. [CrossRef]
7. Lucey, P.M.; Lean, I.J.; Aly, S.S.; Golder, H.M.; Block, E.; Thompson, J.S.; Rossow, H.A. Effects of mannan-oligosaccharide and Bacillus subtilis supplementation to preweaning Holstein dairy heifers on body weight gain, diarrhea, and shedding of fecal pathogens. *J. Dairy Sci.* **2021**, *104*, 4290–4302. [CrossRef]
8. Diaz, T.G.; Branco, A.F.; Jacovaci, F.A.; Jobim, C.; Bolson, D.C.; Daniel, J. Inclusion of live yeast and mannan-oligosaccharides in high grain-based diets for sheep: Ruminal parameters, inflammatory response and rumen morphology. *PLoS ONE* **2018**, *13*, e0193313. [CrossRef]
9. Chagas, J.C.C.; Ferreira, M.A.; Faciola, A.P.; Machado, F.S.; Campos, M.M.; Entjes, M.R.; Donzele, J.L.; Marcondes, M.I. Effects of methionine plus cysteine inclusion on performance and body composition of liquid-fed crossbred calves fed a commercial milk replacer and no starter feed. *J. Dairy Sci.* **2018**, *101*, 6055–6065. [CrossRef]

10. Danso, A.S.; Morel, P.C.H.; Kenyon, P.R.; Blair, H.T. Effects of dietary protein and energy intake on growth, body composition and nutrient utilisation in lambs reared artificially with milk replacers and pellet feeds. *Anim. Feed Sci. Technol.* **2018**, *237*, 35–45. [CrossRef]
11. AOAC. *Official Methods of Analysis of AOAC International*, 21st ed.; AOAC: Gaithersburg, MD, USA, 2019.
12. Chen, H.; Mao, X.; He, J.; Yu, B.; Huang, Z.; Yu, J.; Zheng, P.; Chen, D. Dietary fibre affects intestinal mucosal barrier function and regulates intestinal bacteria in weaning piglets. *Br. J. Nutr.* **2013**, *110*, 1837–1848. [CrossRef]
13. O'Fallon, J.V.; Busboom, J.R.; Nelson, M.L.; Gaskins, C.T. A direct method for fatty acid methyl ester synthesis: Application to wet meat tissues, oils, and feedstuffs. *J. Anim. Sci.* **2007**, *85*, 1511–1521. [CrossRef] [PubMed]
14. Pewan, S.B.; Otto, J.R.; Kinobe, R.T.; Adegboye, O.A.; Malau-Aduli, A.E.O. MARGRA Lamb Eating Quality and Human Health-Promoting Omega-3 Long-Chain Polyunsaturated Fatty Acid Profiles of Tattykeel Australian White Sheep: Linebreeding and Gender Effects. *Antioxidants* **2020**, *9*, 1118. [CrossRef] [PubMed]
15. Teng, P.-Y.; Kim, W.K. Review: Roles of Prebiotics in Intestinal Ecosystem of Broilers. *Front. Vet. Sci.* **2018**, *5*, 245. [CrossRef] [PubMed]
16. Chacher, M.F.A.; Kamran, Z.; Ahsan, U.; Ahmad, S.; Koutoulis, K.; Din, H.Q.U.; Cengiz, Ö. Use of mannan oligosaccharide in broiler diets: An overview of underlying mechanisms. *World Poult. Sci. J.* **2017**, *73*, 831–844. [CrossRef]
17. Yang, Y.A.; Iji, P.; Choct, M. Dietary modulation of gut microflora in broiler chickens: A review of the role of six kinds of alternatives to in-feed antibiotics. *World Poult. Sci. J.* **2009**, *65*, 97–114. [CrossRef]
18. Zheng, C.; Li, F.D.; Li, F.; Zhou, J.W.; Duan, P.W.; Liu, H.H.; Fan, H.M.; Zhu, W.L.; Liu, T. Effects of Adding Mannan Oligosaccharides to Milk Replacer on the Development of Gastrointestinal Tract of 7–28 Days Old Hu Lambs. *Sci. Agric. Sin.* **2020**, *53*, 398–408. [CrossRef]
19. Adhikari, P.A.; Kim, W.K. Overview of Prebiotics and Probiotics: Focus on Performance, Gut Health and Immunity—A Review. *Ann. Anim. Sci.* **2017**, *17*, 949–966. [CrossRef]
20. Baurhoo, B.; Letellier, A.; Zhao, X.; Ruiz-Feria, C.A. Cecal Populations of *Lactobacilli* and *Bifidobacteria* and *Escherichia coli* Populations After In Vivo *Escherichia coli* Challenge in Birds Fed Diets with Purified Lignin or Mannanoligosaccharides. *Poult. Sci.* **2007**, *86*, 2509–2516. [CrossRef]
21. Sharma, A.N.; Kumar, S.; Tyagi, A.K. Effects of mannan-oligosaccharides and *Lactobacillus acidophilus* supplementation on growth performance, nutrient utilization and faecal characteristics in Murrah buffalo calves. *J. Anim. Physiol. Anim. Nutr.* **2018**, *102*, 679–689. [CrossRef]
22. Uzmay, C.; Kiliç, A.; Kaya, I.; Özkul, H.; Önenç, S.S.; Polat, M. Effect of mannan oligosaccharide addition to whole milk on growth and health of Holstein calves. *Arch. Anim. Breed.* **2011**, *54*, 127–136. [CrossRef]
23. Bovera, F.; Lestingi, A.; Iannaccone, F.; Tateo, A.; Nizza, A. Use of dietary mannanoligosaccharides during rabbit fattening period: Effects on growth performance, feed nutrient digestibility, carcass traits, and meat quality. *J. Anim. Sci.* **2012**, *90*, 3858–3866. [CrossRef] [PubMed]
24. Zhao, P.Y.; Jung, J.H.; Kim, I.H. Effect of mannan oligosaccharides and fructan on growth performance, nutrient digestibility, blood profile, and diarrhea score in weanling pigs. *J. Anim. Sci.* **2012**, *90*, 833–839. [CrossRef] [PubMed]
25. Aragona, K.M.; Suarez-Mena, F.X.; Dennis, T.S.; Quigley, J.D.; Hu, W.; Hill, T.M.; Schlotterbeck, R.L. Effect of starter form, starch concentration, and amount of forage fed on Holstein calf growth from 2 to 4 months of age. *J. Dairy Sci.* **2020**, *103*, 2324–2332. [CrossRef]
26. Bittar, C.M.M.; Gallo, M.P.; Silva, J.T.; de Paula, M.R.; Poczynek, M.; Mourão, G.B. Gradual weaning does not improve performance for calves with low starter intake at the beginning of the weaning process. *J. Dairy Sci.* **2020**, *103*, 4672–4680. [CrossRef]
27. Gelsinger, S.L.; Coblentz, W.K.; Zanton, G.I.; Ogden, R.K.; Akins, M.S. Physiological effects of starter-induced ruminal acidosis in calves before, during, and after weaning. *J. Dairy Sci.* **2020**, *103*, 2762–2772. [CrossRef]
28. Xie, H.-K.; Zhao, G.-H.; Wu, Z.-X.; Li, D.-Y.; Zhao, M.-T.; Li, A.; Liu, H.-L.; Zhou, D.-Y.; Zhu, B.-W. Differences in oxidative susceptibilities between glycerophosphocholine and glycerophosphoethanolamine in dried scallop (*Argopecten irradians*) adductor muscle during storage: An oxidation kinetic assessment. *J. Sci. Food Agric.* **2020**, *101*, 1554–1561. [CrossRef] [PubMed]
29. Bezerra, L.S.; Barbosa, A.M.; Carvalho, G.G.P.; Simionato, J.I.; Freitas, J.E.; Araújo, M.L.G.M.L.; Pereira, L.; Silva, R.R.; Lacerda, E.C.Q.; Carvalho, B.M.A. Meat quality of lambs fed diets with peanut cake. *Meat Sci.* **2016**, *121*, 88–95. [CrossRef] [PubMed]
30. Ferlay, A.; Bernard, L.; Meynadier, A.; Malpuech-Brugère, C. Production of trans and conjugated fatty acids in dairy ruminants and their putative effects on human health: A review. *Biochimie* **2017**, *141*, 107–120. [CrossRef]
31. Liang, Y.; Huang, X.; Zhang, Z.; Deng, K.; An, S.; Gao, X.; Wang, Z.; Liu, Z.; Wang, F.; Liu, D.; et al. Spirulina supplementation improves lipid metabolism and autophagic activities in the liver and muscle of Hu lambs fed a high-energy diet. *Arch. Anim. Nutr.* **2020**, *74*, 476–495. [CrossRef]
32. Pereira, T.L.; Fernandes, A.R.M.; Oliveira, E.R.; Cônsolo, N.R.B.; Marques, O.F.C.; Maciel, T.P.; Pordeus, N.M.; Barbosa, L.C.G.S.; Buarque, V.L.M.; Padilla, A.R.H.; et al. Serum metabolomic fingerprints of lambs fed chitosan and its association with performance and meat quality traits. *Animal* **2020**, *14*, 1987–1998. [CrossRef] [PubMed]
33. Realini, C.E.; Bianchi, G.; Bentancur, O.; Garibotto, G. Effect of supplementation with linseed or a blend of aromatic spices and time on feed on fatty acid composition, meat quality and consumer liking of meat from lambs fed dehydrated alfalfa or corn. *Meat Sci.* **2017**, *127*, 21–29. [CrossRef] [PubMed]

34. Abdallah, A.; Zhang, P.; Elemba, E.; Zhong, Q.; Sun, Z. Carcass characteristics, meat quality, and functional compound deposition in sheep fed diets supplemented with *Astragalus membranaceus* by-product. *Anim. Feed Sci. Technol.* **2020**, *259*, 114346. [CrossRef]
35. Demirel, G.; Ozpinar, H.; Nazli, B.; Keser, O. Fatty acids of lamb meat from two breeds fed different forage: Concentrate ratio. *Meat Sci.* **2006**, *72*, 229–235. [CrossRef]
36. Wood, J.D.; Richardson, R.I.; Nute, G.R.; Fisher, A.V.; Campo, M.M.; Kasapidou, E.; Sheard, P.R.; Enser, M. Effects of fatty acids on meat quality: A review. *Meat Sci.* **2004**, *66*, 21–32. [CrossRef]
37. Kawamura, T.; Okubo, T.; Sato, K.; Fujita, S.; Goto, K.; Hamaoka, T.; Iemitsu, M. Glycerophosphocholine enhances growth hormone secretion and fat oxidation in young adults. *Nutrition* **2012**, *28*, 1122–1126. [CrossRef] [PubMed]
38. Kondakova, T.; D'Heygère, F.; Feuilloley, M.J.; Orange, N.; Heipieper, H.J.; Poc, C.D. Glycerophospholipid synthesis and functions in Pseudomonas. *Chem. Phys. Lipids* **2015**, *190*, 27–42. [CrossRef]