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Effects of Byproduct Lactic Acid and Byproduct Betaine As Feed Additives on the Metabolomic Profiles of Blood, Meat, and Fat Tissue of Juvenile Bester Sturgeon (*Acipenser ruthenus* × *Huso huso*)

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Abstract The sturgeon is an ancient fish that grows slowly and matures sexually late. The diversity of chromosome numbers makes the biological study of sturgeon difficult. However, as an important aquaculture resource, the exploration of its culture conditions deserves in-depth study. This study reports the metabolomic profiles of the bester sturgeon's blood, meat, and fat when two food industrial byproducts rich in lactic acid and betaine (*N*,*N*,*N*-trimethylglycine), respectively, were used as feed additives using the metabolomic research method. The metabolomic results showed that a total of 388 metabolites were detected, of which 348 metabolites were classified and annotated in the Human Metabolome Database. The differential analysis showed that there were significant differences in the metabolomic profiles among blood, meat, and fat tissues. Screening of differential biomarker metabolites revealed that the main differential biomarker metabolites were derived from blood and that there were differences in the types of differential biomarker metabolites due to the two byproducts. This study provides new insights into the potential use of food industrial byproducts as feed additives and provides a theoretical basis for the improvement of sturgeon culture feeds.

Keywords: *metabolome, targeted metabolomics, metabolism, hybrid sturgeon*

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1. Introduction

In recent years, the concept of sustainable development has penetrated various industries. The sustainable development of aquaculture has gradually become more important [1]. It is an important goal to achieve sustainable development of aquaculture by reducing the cost of breeding and increasing the growth rate of cultured animals. Therefore, more and more byproducts are used in the development of aquaculture feeds, with the aim of reducing feed costs and improving the growth rate and quality of aquaculture products [2].

The growing demand for sustainable aquaculture practices has prompted the exploration of alternative feed additives that can improve the health, growth, and metabolic efficiency of farmed fish species. Sturgeon, a high-value aquaculture species, faces challenges in maintaining optimal growth and metabolic functions under controlled farming conditions. Currently, a large number of studies have shown that feed additives play a huge role in aquaculture, and more and more feed additives are being developed, researched, and used [3]. Therefore, enhancing their feed with novel additives that promote overall well-being could have significant economic and environmental benefits [4].

Lactic acid, a product of anaerobic fermentation [5], and betaine, a compound known for its role in cellular osmoregulation and methylation [6], are bioactive substances with promising effects on the metabolic regulation of aquatic species [7]. Recent studies have shown that lactic acid can influence gut microbiota and intestinal health [8]. At the same time, betaine has been linked to improved growth, stress tolerance, and disease resistance in various aquaculture species [9]. Sturgeons belong to the family *Acipenseridae*, and there are currently 27 known species of sturgeon [10]. Due to overfishing and habitat destruction caused by human activities, sturgeon are facing the risk of extinction [11]. Combining these two compounds in a feed additive may offer synergistic effects that could enhance the metabolic

performance of sturgeon.

Metabolomics, the comprehensive study of small molecules in biological systems [12], provides an ideal approach to investigate the biochemical impact of feed additives on sturgeon. This powerful tool allows for a holistic view of metabolomic alterations, identifying key biomarkers and pathways affected by dietary interventions [13]. Understanding how a lactic acid and betaine-rich feed additive influences the sturgeon's metabolomic profile will not only deepen the understanding of its physiological effects but also contribute to the development of more efficient and sustainable feeding strategies in sturgeon aquaculture.

This study aims to use metabolomics to understand the metabolomic characteristics of blood, meat, and fat tissue of bester sturgeon when food industrial byproducts rich in lactic acid and betaine (*N*,*N*,*N*-trimethylglycine) are used as feed additives, as well as the differences in metabolomic profiles between different tissues, and to reveal the differences in metabolomic characteristics caused by different byproducts. The research results will provide a theoretical basis for the reuse of industrial food byproducts, the formulation of sturgeon farming feeds, and the development of sustainable aquaculture.

2. Materials and Methods

2.1. Fish and Diet

Metabolomics analysis was performed using six hybrid juvenile bester sturgeon (*Acipenser ruthenus* × *Huso huso*), which were fed with commercial byproducts rich in lactic acid and betaine as feed additives for 294 days. The feeding time started on February 23, 2023, and tissue samples were collected on December 14, 2023. The water temperature in the pool averaged around 10°C one week before and after the sample collection time. Six bester sturgeon individuals were equally divided into two groups, and each group had three biological replicates. One group was classified as a lactic acid group, and the other group was classified as a betaine group. The fish were cultured in recirculating pools (each 2.09 m × 1.08 m × 0.60 m deep, 1.35 m^3) in the "patio" of the Hiroshima University campus. The water exchange rate was >1/3 of the pond volume per day, pH was 7-8, and dissolved oxygen levels were >6 mg L^{-1}.

The same basic feed was used for sturgeon in the lactic acid group and the betaine group. The basic feed was a fast-sinking cylindrical pellet feed from Scientific Feed Laboratory Co. Ltd. (Tokyo, Japan). The main contents of the basic feed were fish meal, wheat flour, soybean oil meal, corn gluten meal, rice bran oil meal, calcium phosphate, feed yeast, and calcium carbonate. The main nutrients of the basic diet were crude protein, >45.0%; crude fat, >6.0%; crude fiber, <3.0%; crude ash, <15.0%; calcium, >1.60%; and phosphorus, >1.20%. The daily feeding amount was generally 0.2-1.5% of the fish's body weight.

The feed of the lactic acid group was supplemented with a byproduct rich in lactic acid added to the basic diet. The byproduct lactic acid was the autoclaved centrifugation supernatant of a commercial culture of lactic acid bacteria prepared and provided by Hokkaido Sugar Co., Ltd. (Sapporo, Hokkaido, Japan). The feed of the betaine group was supplemented with a byproduct rich in betaine added to the basic diet. The byproduct betaine was the second-stage molasses derived from sugar production from sugar beet (*Beta vulgaris*), which was also prepared and provided by Hokkaido Sugar Co., Ltd. Supplementation of the basic diet with the additive byproducts was done in the same way as described in the previous studies [14]. The feed pellets for the experimental subgroups were sprayed with solution of byproduct rich in lactic acid or betaine, to prepare added pellets. The added pellets were stirred by roll-shaking for homogenization until they were dry.

The chemical compositions of the byproduct lactic acid and byproduct betaine were analyzed by liquid chromatography. Byproduct lactic acid was analyzed by anion chromatography using a 930 Compact IC Flex (Metrohm, Herisau, Switzerland) with the separation column Shodex SI-52 4E (Resonac Corporation, Tokyo Japan) at 40°C and 0.7 mL min^{-1} flow rate of the effluent, 3.6 mM Na_2CO_3. Byproduct betaine was analyzed by HPLC using a Prominence HPLC (Shimadzu Corporation, Kyoto, Japan) with the analytical column Shodex Asahipak NH2P-50 4E (Resonac; 4.6mmI.D.×250 mm) and the Refractive Index Detector RID-10A (Shimadzu) at 40°C and at 1.0 mL min^{-1} flow rate of the mobile phase, H_2O/CH_3CN = 25/75 (vol./vol.) isocratic mode. The feed was subsequently analyzed by metabolomics, and the raw data were provided as original data.

2.2. Tissue Sample Collection and Metabolomic Analysis

Blood, fat, and meat tissue samples were collected from the bester sturgeon. Sturgeon were humanely killed by a combination of impact and nerve stimulation to avoid unnecessary pain to the fish [15]. Sturgeon specimens were dissected, and tissue samples were extracted from the site. Blood, meat, and fat tissue samples were taken from each of the six sturgeon individuals. A total of 18 sturgeon samples were collected for metabolomics analysis. The collected blood, meat, and fat samples were stored in a -70°C freezer for subsequent metabolomics analysis. Finally, tissue samples were transported to BGI company (formerly Beijing Genomics Institute, Shenzhen, China; https://www.bgi.com/global/home) on dry ice for metabolomics analysis. Metabolite molecules were separated by ultra-performance liquid chromatography (UPLC), and metabolite identification was performed by tandem mass spectrometry (MS/MS) (details described in [16]). All the tissue samples were analyzed for targeted metabolomics using the HM700 panel. At the same time, the study also performed the same metabolomics analysis on the basal feed, feed from the lactic acid group, and feed from the betaine group.

2.3. Data Acquisition and Preprocessing

Using skyline (MacCoss Lab Software, https://skyline.ms/ project/home/software/Skyline/begin.view) to perform metabolite identification and quantification with default parameters, as well as manual inspection, is

assisted. Then, a data matrix containing information such as metabolite identification results and quantitative results was obtained, and the table was further processed for bioinformatic analyses.

2.4. Statistical and Bioinformatic Analyses

Metabolomic data were standardized or normalized to improve their normality before statistical analysis. To visualize the relatedness of the metabolomic profiles of blood samples, principal component analysis (PCA) and orthogonal partial least squares discrimination analysis (OPLS-DA) were performed at Metware Cloud, a public online platform for data analysis (https://cloud.metware.cn). PCA is an unsupervised method of data dimensionality reduction analysis with no grouping information when calculating. OPLS-DA is a supervised multivariate statistical analysis method that groups information when calculating. It combines the regression model between metabolite changes and experimental groups while reducing the dimensionality and uses a certain discrimination threshold to perform discriminant analysis on the regression results. Compared with PCA, OPLS-DA analysis can further show the differences between groups.

To screen differential metabolites as biomarkers among high-dimensional datasets, linear discriminant analysis (LDA) effect size analysis (LEfSe) [17] was performed online at the Hutternhower Lab, Biostatistics Department, Harvard T. H. Chan School of Public Health (https://huttenhower.sph.harvard.edu/lefse/). Differential biomarker metabolites were further projected on the metabolic pathway maps generated by the Kyoto Encyclopedia of Genes and Genomes, or KEGG (https://www.genome.jp/kegg/pathway.html).

2.5. Animal Ethics

This study on bester sturgeon was carried out following the guidelines established by the Animal Care and Use Committee of Hiroshima University (approval number F24-2) and under the guidelines and regulations relevant to the Sustainable Aquaculture Production Assurance Act, Japan (https:// www. japaneselawtranslation. go.jp/en/laws/view/3674).

3. Results

3.1. Chemical Compositions of the Byproduct Lactic Acid and Byproduct Betaine As Feed Additives

The anion composition of the byproduct lactic acid, i.e., autoclaved supernatant of commercial culture of lactic acid bacteria, was analyzed by anion chromatography and is shown in Table 1. Lactic acid was the most abundant anion, and smaller amounts of acetic acid, succinic acid, malonic acid, and inorganic ions were detected; other anions were present below the detection limit, i.e., <0.005 ppm.

Being a zwitterion or an inner salt, betaine (glycine betaine) was detected with a refractive index detector. The betaine content of the byproduct betaine, i.e., the second-stage molasses from the sugar beet processing, was 5.5±0.8%(w/v).

Table 1. Anion composition of the byproduct lactic acid that was used to supplement the basic diet

Anion	mg L^{-1}
Lactic acid	96361.6
Acetic acid	3482.3
Succinic acid	131.8
Malonic acid	32.1
Cl^-	163.6
PO_4^{3-}	126.6
SO_4^{2-}	61.6
NO_3^-	9.5

In addition to these chromatographic data, metabolomic profiles of the basic diet, byproduct lactic acid, and byproduct betaine are available (see Data Availability Statement below).

3.2. Metabolomic Profiles of Meat, Fat, and Blood Tissues of the Bester Sturgeon

The meat, fat, and blood samples were collected, and metabolomics analysis was performed simultaneously. One dataset containing metabolomics data of the three tissues was finally obtained. A total of 388 metabolites were detected in the meat, fat, and blood tissues of the bester sturgeon. Of these, 348 metabolites were annotated into nine *super-classes*, 35 *classes*, and 60 *sub-classes* based on the Human Metabolome Database (HMDB) (https://hmdb.ca/; [18]) (Figure 1 and Table S1); the remaining 13 metabolites were not categorically annotated with the HMDB database. In the *super-class* category, 129 metabolites were annotated to organic acids and derivatives (33%), and 95 metabolites were annotated to lipids and lipid-like molecules (24%). In the *class* category, 101 metabolites were annotated to carboxylic acids and derivatives (26%), and 82 metabolites were annotated to fatty acyls (21%). In the *sub-class* category, 84 metabolites were annotated to amino acids, peptides, and analogs (22%) and fatty acids and conjugates (14%), respectively.

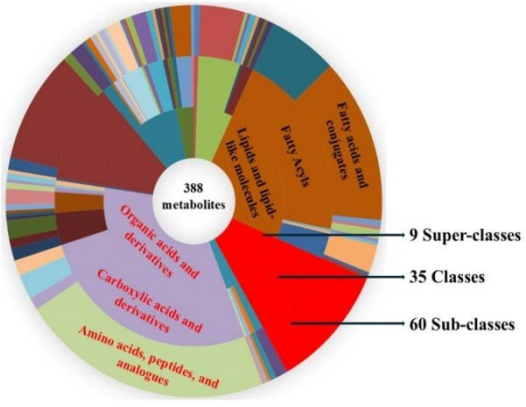

Figure 1. A sunburst plot visualized the statistics and hierarchical assignment of the 388 metabolites. The innermost, middle, and outermost rings represent the *super-class*, *class*, and *subclass* categories based on the Human Metabolite Database (HMDB; https://hmdb.ca/metabolite), respectively. "NA" represents "not annotated."

3.3. Multivariate Statistical Analyses

Principal component analysis (PCA) is a multidimensional data statistical analysis method with unsupervised pattern recognition, which aims to reveal the internal structure of multiple variables through a few principal components using a dimensionality reduction method. PCA analysis reflects the characteristics of metabolomics under multidimensional data through a number of principal components so that the dispersion of replicates within a group, as well as the differences between different groups, can be clearly observed by PCA analysis. The PCA results showed that the samples tended to be divided into two groups. The blood samples were clearly separated from the meat and fat samples, while the separation tendency between meat tissue and fat tissue was not obvious. In the PCA plot (Figure 2A), the first principal component (PC1) explained 38.35% of the total variation in the original data set; the second principal component (PC2) explained 18.11% of the total variation in the original data set. In the PCA plot (Figure 2B), the first principal component (PC1) explained 42.68% of the total variation in the original data set; the second principal component (PC2) explained 16.17% of the total variation in the original data set. The PCA plot also shows that the within-group replicates have a high degree of cohesion and even partial overlap, indicating that the within-group dispersion is extremely low and the data reliability is extremely high. The study also performed PCA analysis on the metabolomics data of the feeds, and the analysis results (Figure 2C) showed that there were obvious differences among the three feeds.

Different from the principal component analysis (PCA), Orthogonal partial least squares discrimination analysis (OPLS-DA) is a multivariate statistical analysis method with supervised pattern recognition that can effectively remove study-irrelevant effects and thus achieve the prediction of sample categories. In the OPLSDA analysis model, R^2X and R^2Y represent the explanatory rate of the built model to the X and Y matrices; the closer the value is to 1, the better the fit of the model. Q^2 represents the predictive ability of the model, and the value of Q^2 should be higher than 0.5, and the results could indicate that the constructed model is suitable. The results of the OPLS-DA analysis showed that the samples were divided into three regions (Figure 3), which indicates that there are obvious metabolomic differences among different tissues. Moreover, there are also obvious metabolomic differences among different feeds.

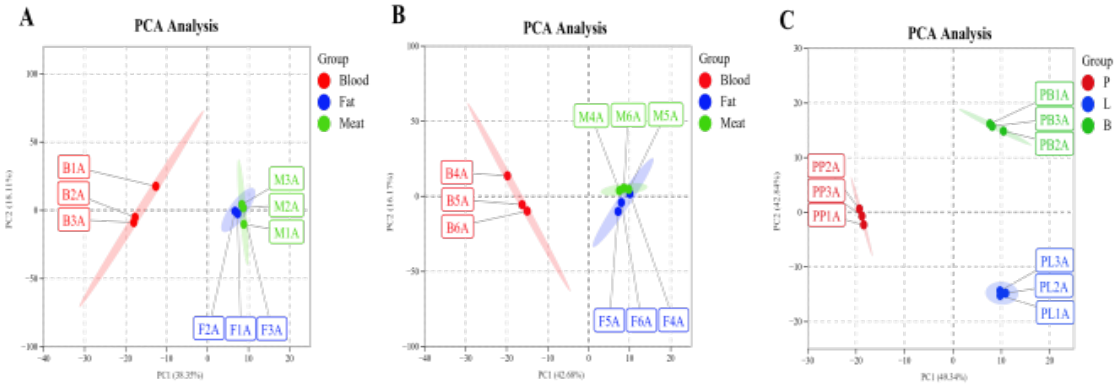

Figure 2. PCA analysis of metabolites in meat, fat, and blood of: **A**, lactic acid group; **B**, betaine group; and **C**, basic diet P and additives (byproduct lactic acid L and byproduct betaine B). PP1A, PP2A, and PP3A are three replicates of the basic diet. PL1A, PL2A, and PL3A are three replicates of the basic diet supplemented with a byproduct rich in lactic acid. PB1A, PB2A, and PB3A are three replicates of the basic diet supplemented with a byproduct rich in betaine. There is an obvious separation trend between blood tissue and the other two tissues, while the separation between meat tissue and fat tissue is not obvious. There is an obvious separation trend among the basic diet and the additives

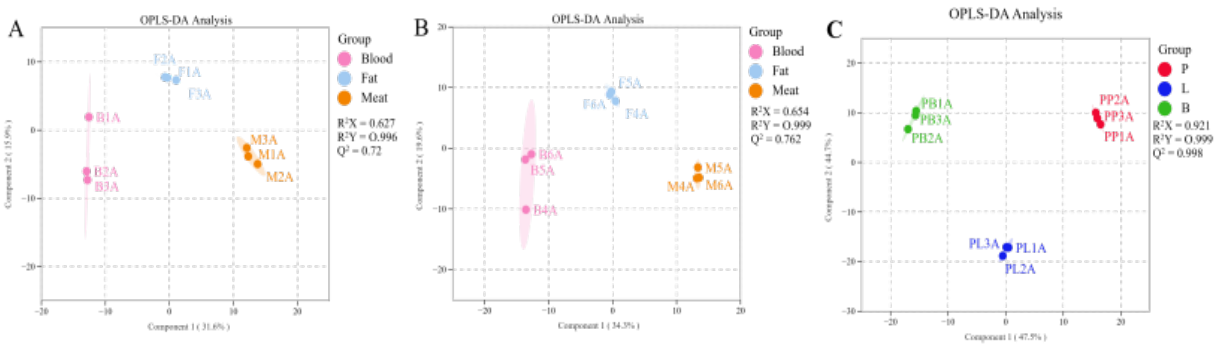

Figure 3. OPLS-DA analysis of metabolites in meat, fat, and blood: **A**, lactic acid group; **B**, betaine group; and **C**, basic diet P and additives (byproduct lactic acid L and byproduct betaine B). PP1A, PP2A, and PP3A are three replicates of the basic diet. PL1A, PL2A, and PL3A are three replicates of the basic diet supplemented with a byproduct rich in lactic acid. PB1A, PB2A, and PB3A are three replicates of the basic diet supplemented with a byproduct rich in betaine. R^2X, R^2Y, and Q^2 indicated significant differences among the three tissues. Furthermore, there is an obvious separation between the basic diet and the additives

3.4. Differential Metabolite Screening

Differential metabolite screening of meat, fat, and blood tissues was performed using the linear discriminant analysis (LDA) Effect Size (LEfSe). A total of 46 differential metabolites with LDA scores >1.5 were screened (Figure 4 and Table S2). In the lactic acid group (Figure 4A), two differential metabolites were from meat, two differential metabolites were from fat, and 23 differential metabolites were from blood. In the betaine group (Figure 4B), three differential metabolites were from meat, one differential metabolite was from fat, and 33 differential metabolites were from blood. The differential metabolites screened in the lactic acid group were mainly classified into amino acids, peptides and analogs, organooxygen compounds, and fatty acyls. The metabolites screened by the betaine group were mainly classified into fatty acyls and amino acids, peptides, and analogs.

At the same time, the study also screened the main differential metabolites in the feed (Figure 4C and Table S3) with LDA Score > 3 and screened a total of 32 differential metabolites. Fourteen differential metabolites came from the basal feed, seven differential metabolites came from the lactic acid group feed, and 11 differential metabolites came from the betaine group feed.

3.5. Differences in Metabolic Profiles of Bester Sturgeon Induced by Pure Feed Additives and Commercial By-Product Feed Additives

In previous studies, the effects of pure lactic acid and betaine as feed additives on the metabolic profiles of blood, meat, and fat tissue of the bester sturgeon were analyzed. In this study, LEfSe was used to perform differential metabolite biomarker screening on the metabolomics profiles of different tissues of bester sturgeon fed with commercial by-products as feed additives and those of different tissues of bester sturgeon fed with pure lactic acid and betaine as feed additives.

For blood samples (Figure 5), blood samples were collected once from bester sturgeons fed with commercial by-products as feed additives, and the average water temperature was 10°C one week before and after the sample collection. For bester sturgeons fed with pure lactic acid and betaine as feed additives, blood samples were collected twice. The average water temperature was 3°C and 10°C one week before and after the sample collection. This study conducted LEfSe analysis at both temperatures. Whether in the lactic acid group or the betaine group, commercial by-products as feed additives caused more biomarkers, and the biomarkers screened highly overlapped between different temperatures. Compared with commercial by-products such as feed additives, pure lactic acid and betaine cause the enrichment of DHA in bester sturgeon blood tissue. Compared with the lactic acid group, the betaine group caused more biomarkers, and the biomarkers screened in the lactic acid group can basically be found in the biomarkers in the betaine group.

For meat (Figure 6) and fat (Figure 7) samples, pure lactic acid and betaine as feed additives induced more biomarkers than commercial by-products as feed additives. Comparative analysis of biomarkers screened by the lactic acid group and betaine group showed that these biomarkers were highly overlapped.

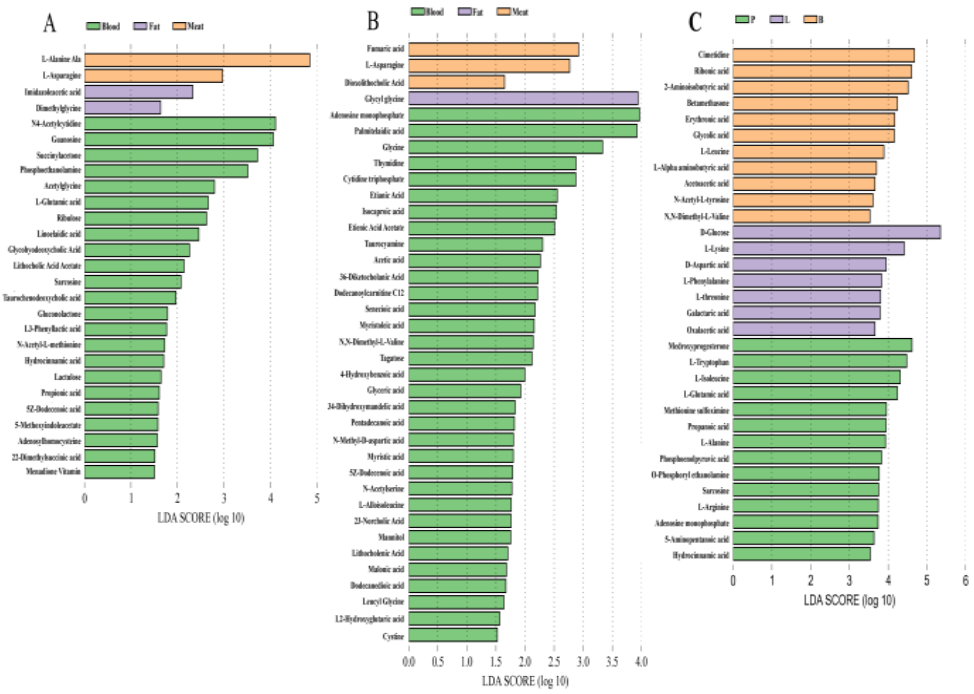

Figure 4. Differential metabolites were screened with LDA scores >1.5 in blood, fat, and meat tissues of: **A**, lactic acid group; **B**, betaine group; and **C**, basic diet P and additives (byproduct lactic acid L and byproduct betaine B). Differential biomarker metabolites were screened with LDA scores >3 in the feed group

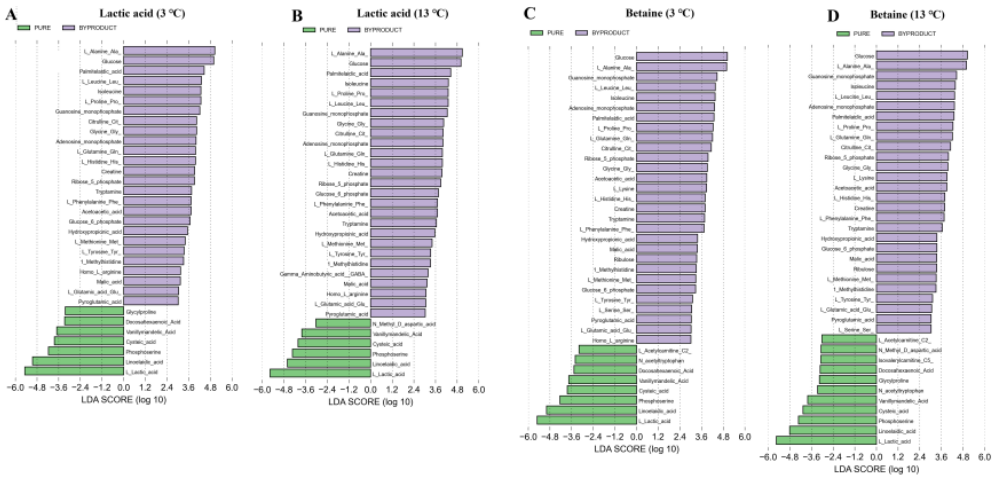

Figure 5. Biomarkers screened between blood samples of bester sturgeons fed pure feed additives and commercial by-product feed additives. According to the type of feed additive, **A** and **B** are from the lactic acid group, and **C** and **D** are from the betaine group. In **A** and **C**, the blood samples of bester sturgeons fed with pure betaine and lactic acid as feed additives were collected at 3°C. In **B** and **D**, the blood samples of bester sturgeons fed with pure betaine and lactic acid as feed additives were collected at 13°C

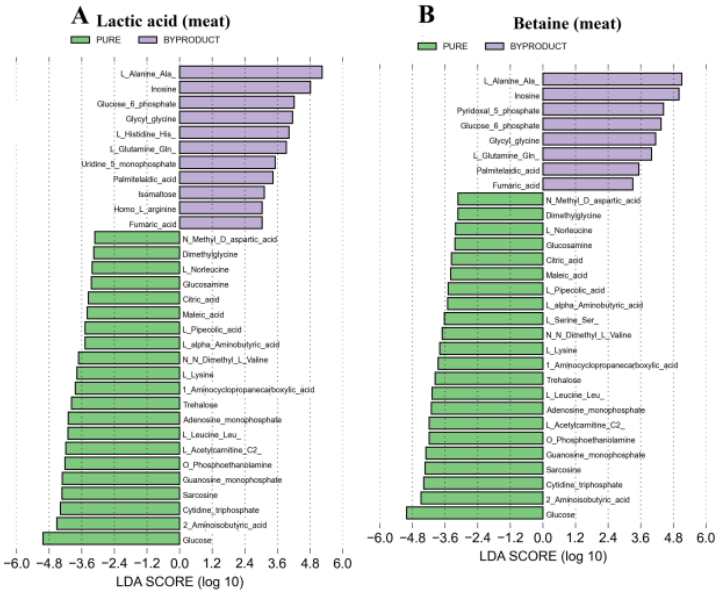

Figure 6. Biomarkers screened between meat samples of bester sturgeons fed pure feed additives and commercial by-product feed additives. According to the type of feed additive, **A** presented the results of the lactic acid group, and B presented the results of the betaine group

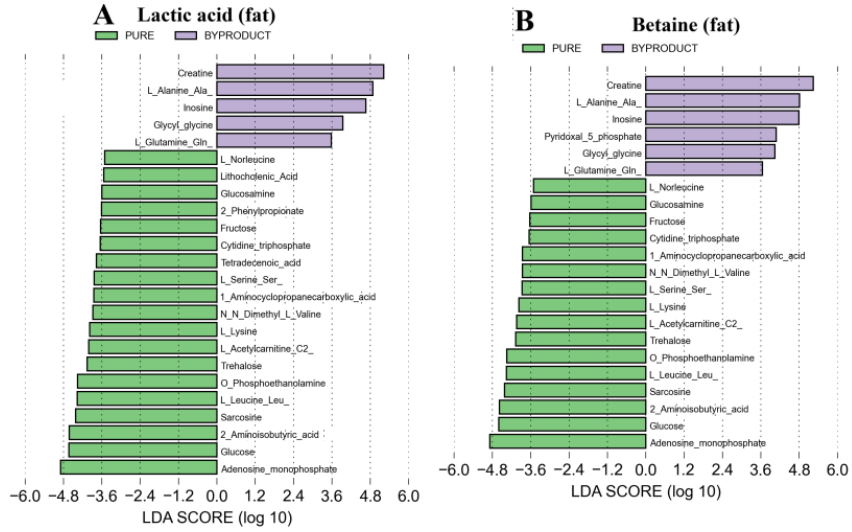

Figure 7. Biomarkers screened between fat samples of bester sturgeons fed pure feed additives and commercial by-product feed additives. According to the type of feed additive, **A** presented the results of the lactic acid group, and B presented the results of the betaine group

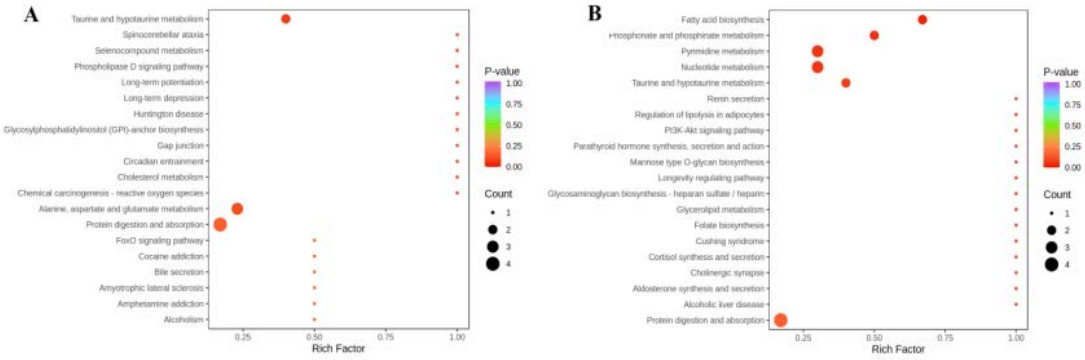

Figure 8. KEGG enrichment analysis of lactic acid group (**A**) and betaine group (**B**). Rich factor refers to the ratio of the differential metabolites in corresponding pathways to the total number of metabolites detected in this pathway. The size of the bubbles in the figure represents the number of differential metabolites enriched in the metabolomic concentration of the pathway, and the color of the bubbles represents the magnitude of different enrichment significance P-values

3.6. Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Analysis of Differential Metabolites

KEGG metabolic pathway enrichment analysis was performed on the screened differential metabolites. The results of the KEGG metabolic pathway enrichment analysis of differential metabolites from the lactic acid group showed that all 27 differential metabolites in the three tissue samples were involved in 66 metabolic pathways, of which 20 metabolic pathways with high significance were displayed in the form of bubble charts (Figure 8A). According to the P value, the most abundant metabolic pathways are protein digestion and absorption, taurine and hypotaurine metabolism, seleno-compound metabolism, glycosylphosphatidylinositol (GPI)-anchor biosynthesis, alanine, aspartate, and glutamate metabolism, nitrogen metabolism, primary bile acid biosynthesis, glycerophospholipid metabolism, sphingolipid metabolism, histidine metabolism, arginine and proline metabolism.

The results of the KEGG metabolic pathway enrichment analysis of differential metabolites from the betaine group showed that all 37 differential metabolites in the three tissue samples were involved in 74 metabolic pathways, of which 20 metabolic pathways with high significance were displayed in the form of bubble charts (Figure 8B). According to the P value, the most abundant metabolic pathways are fatty acid biosynthesis, phosphonate, and phosphinate metabolism, taurine and hypotaurine metabolism, glycerolipid metabolism, mannose type O-glycan biosynthesis, glycosaminoglycan biosynthesis-heparan sulfate/heparin, folate biosynthesis, pyruvate metabolism, carbon metabolism, fatty acid metabolism, oxidative phosphorylation, primary bile acid biosynthesis, ubiquinone and other terpenoid-quinone biosynthesis.

The main metabolic pathways enriched by characteristic metabolites induced by the supernatant of the commercial culture of lactic acid bacteria as feed additive were protein digestion and absorption, alanine, aspartate, and glutamate metabolism, and taurine and hypotaurine metabolism. The main metabolic pathways enriched by the characteristic metabolites caused by sugar beet processing byproducts as feed additives are fatty acid biosynthesis, phosphonate and phosphonate metabolism, pyrimidine metabolism, nucleotide metabolism, and protein digestion and absorption.

4. Discussion

Animal feed additives are important ingredients in feed production as they can improve feed utilization, animal health, and metabolism [19]. These additives are widely used worldwide and benefit a variety of animals and birds, including poultry [20]. They help improve growth performance, increase feed palatability, provide essential nutrients, and optimize feed utilization [21]. With increasing feed standards, growing consumer awareness, and demand for healthy animal byproducts, feed additive manufacturers are looking for residue-free and more natural alternatives to traditional additives. Some byproducts are used in aquaculture for their nutritional value, and these ingredients are rich in essential fatty acids, proteins, and bioactive compounds, making them effective and sustainable additives [22]. Polysaccharides from green algae and yeast-derived beta-glucans have been shown to enhance immunity and growth in species such as tilapia and shrimp [23]. Functional feed additives derived from agricultural and food industry byproducts (e.g., fruit peels and plant residues) have gained attention for improving health, growth, and water quality in aquaculture systems [24]. Probiotic-enriched feeds using microbial byproducts have also been successful in reducing harmful nitrogen compounds in the water, enhancing sustainability, and supporting fish health [25]. This study revealed a significant impact of feed additives such as the supernatant of the commercial culture of lactic acid bacteria and betaine-rich byproducts from sugar beet processing on the metabolomic profile of bester sturgeon using a metabolomics approach. By studying blood, meat, and fat tissue, a comprehensive understanding of how these feed additives modulate metabolic pathways, affect sturgeon physiology, and potentially improve aquaculture efficiency was gained in this study.

The differential metabolites of the feed containing the supernatant of the commercial culture of lactic acid bacteria were *D*-glucose, *L*-lysine, *D*-aspartic acid, *L*-phenylalanine, *L*-threonine, galactaric acid, and oxalacetic acid. The differential metabolites of the feed containing sugar beet processing byproducts were cimetidine, ribonic acid, 2-aminoisobutyric acid, betamethasone, erythronic

acid, glycolic acid, *L*-leucine, *L*-alpha aminobutyric acid, acetoacetic acid, *N*-acetyl-l-tyrosine and *N*, *N*-dimethyl-l-valine. The characteristic metabolites of different tissues of sturgeon were different due to different compositions of feed. Both lactic acid and betaine groups showed significant changes in blood metabolomic profiles, and a greater number of differential metabolites were identified compared with meat and fat tissue. In the previous study [14], the use of pure lactic acid and betaine as feed additives induced changes in the metabolomic profiles of blood tissues of sturgeon, with lactic acid as a feed additive causing upregulation of differential metabolites.

In contrast, betaine caused the downregulation of differential metabolites. The dominance of blood metabolites, particularly in stress-related pathways, underscores the systemic impact of dietary intervention [26]. Blood is a highly dynamic tissue that reflects rapid physiological changes and is an efficient medium for assessing metabolic responses [27]. Differential metabolites in the lactate group, such as acetic acid, malonic acid, and dodecanedioic acid, are part of energy and fatty acid metabolism [28,29,30]. Lithocholenic acid and 23-norcholic acid indicate liver function and lipid digestion [31]. Adenosine monophosphate (AMP) and cytidine triphosphate (CTP) are nucleotides essential for energy transfer and signaling [32]. Glycine, leucyl glycine, and N-methyl-D-aspartic acid play roles in neurotransmission and protein metabolism [33]. Dodecanoylcarnitine, myristic acid, myristoleic acid, and pentadecanoic acid suggest relevance to lipid metabolism [34,35,36]. Differential metabolites in the betaine group, such as acetylglycine, *N*-acetyl-l-methionine, and *S*-adenosylhomocysteine (SAH), are involved in methylation and acetylation pathways, which regulate gene expression and protein function [37]. Glycohyodeoxycholic acid and taurochenodeoxycholic acid are conjugated bile acids, indicative of more specialized lipid processing or stress on the liver [38]. Linoelaidic acid, a trans-fatty acid, may point to oxidative stress or diet-derived influences [39]. The feed ingredients did not cause too many unique metabolites in meat and fat tissue. The lactic acid group mainly caused an increase in amino acids (alanine, asparagine) in meat tissue, which may be related to the participation of lactic acid in amino acid metabolism [40].

Commercial byproducts rich in lactic acid and betaine as feed additives and pure lactic acid and betaine as feed additives caused different changes in the metabolic profiles of different tissues of bester sturgeon. This may be related to other metabolite components in commercial byproducts. In blood samples, the number of biomarkers induced by commercial byproducts as feed additives was much greater than that of pure lactic acid and betaine. In both the lactic acid group and the betaine group, the top two biomarkers induced by commercial byproducts were alanine and glucose. Alanine is a non-essential amino acid that participates in carbohydrate metabolism and can maintain blood sugar levels [41]. Compared with commercial byproducts, the top two biomarkers induced by pure lactic acid and betaine were lactic acid and linoelaidic acid. Lactic acid is an important metabolic intermediate that can participate in various metabolic pathways, such as the TCA cycle [42]. Linoelaidic acid is a trans-unsaturated fatty acid and a cis-trans isomer of linoleic acid. Trans fatty acids are usually converted into other forms of fatty acids in the body through fatty acid metabolism pathways. However, long-term intake of these trans fatty acids can lead to changes in the fatty acid composition of the human body, which may affect cell membrane structure, cardiovascular health, and metabolism [43].

Compared with blood tissue, the number of biomarkers induced by commercial by-products as feed additives in meat and fat tissues was less than that of pure lactic acid and betaine. In meat tissue, the top two biomarkers induced by commercial by-products were alanine and inosine. Inosine is a naturally occurring nucleoside composed of guanine and ribose. It is an important intermediate in nucleic acid metabolism, especially in the purine metabolic pathway. Inosine can also be converted to adenosine or guanosine, thereby participating in the synthesis of RNA and DNA [44]. The top two biomarkers induced by pure lactic acid and betaine were glucose and aminoisobutyric acid. Aminoisobutyric acid is a derivative of amino acids that can be converted into other amino acids through transamination. Its metabolism involves the interaction between amino acids and energy metabolism, especially playing a role in the regulation of intracellular metabolism. Aminoisobutyric acid is sometimes considered an important factor in promoting protein synthesis, although it is not an essential amino acid [45]. Some studies have shown that aminoisobutyric acid may help muscle growth and recovery or be related to the amino acid transport and synthesis process in muscle metabolism [46]. This may contribute to the improvement of bester sturgeon protein content and quality. In fat tissue, the first two biomarkers induced by commercial byproducts are alanine and creatine. Creatine is an amino acid composed of three amino acids: arginine, glycine, and methionine. It is mainly stored in the muscle in the form of creatine phosphate. The main function of creatine is to help muscles quickly produce energy in a short period [47]. Creatine may stimulate protein synthesis and promote muscle growth by promoting hydration in muscle cells and increasing cell volume. The first two biomarkers induced by pure lactate and betaine are glucose and adenosine monophosphate. Adenosine monophosphate is an important nucleotide that is widely present in cells and is involved in a variety of physiological processes, especially in energy metabolism and cell signaling [48]. Adenosine monophosphate is a key intermediate in intracellular energy metabolism. As a signaling molecule, Adenosine monophosphate can activate some metabolic pathways (e.g., AMP-activated protein kinase and AMPK) to help restore energy balance. Adenosine monophosphate plays an important role by activating AMP kinase (AMPK), which is an intracellular "energy sensor" that can regulate energy metabolism, including promoting glucose uptake, fatty acid oxidation, and reducing synthesis reactions, thereby helping cells cope with energy-deficient states. The increase in AMP triggers the energy regulation mechanism in cells and affects the metabolism of fat and sugar. After AMPK is activated, it can increase cellular energy production, improve metabolic levels, and even play a role in some diseases (e.g., obesity, diabetes, and metabolic syndrome) [49].

5. Conclusion

This study shows that food industrial byproducts can modulate the metabolomic profile of bester sturgeon, with important implications for the sustainability and efficiency of aquaculture. Supernatants of commercial cultures of lactic acid bacteria as feed additives and byproducts from sugar beet processing as feed additives induce different metabolomic profiles in sturgeon, and the differential metabolites involve different metabolic pathways. By elucidating the pathways involved, these findings pave the way for more targeted and effective dietary strategies to improve fish health and performance.

Supplementary Materials: The following supporting information can be downloaded at:

Table S1: Summary of the 388 metabolites of these blood, meat, and fat samples. "NA" represents "not annotated," and the NA metabolites were excluded from the category counts.

Table S2: Biomarkers screened from different tissues.

Table S3: Biomarkers screened from different feeds.

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Institutional Review Board Statement: The Animal Care and Use Committee of Hiroshima University authorized all animal experiments (permit number F24-2).

Data Availability Statement: Original data for analysis are available at:

https://www.sturgeon-metabolome.hiroshima-u.ac.jp/h0888_HM700_By-products.ods for blood, meat, and fat; and

https://www.sturgeon-metabolome.hiroshima-u.ac.jp/h0888_HM700_Feed-additives.ods for the basic feed, byproduct lactic acid, and byproduct betaine.

Conflicts of Interest: The authors declare no conflict of interest. The funder had no role in the design of the study, in the collection, analysis, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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References

[1] Subasinghe, R., Soto, D. and Jia, J., "Global aquaculture and its role in sustainable development," Reviews in Aquaculture, 1(1), 2-9, Feb 2009.

[2] Dawood, M.A.O., Habotta, O.A.E., Elsabagh, M., Azra, M. N., Van Doan, H., Kari, Z.A. and Sewilam, H., "Fruit processing byproducts in the aquafeed industry: A feasible strategy for aquaculture sustainability," Reviews in Aquaculture, 14(4), 1945-1965, May 2022.

[3] Dawood, M.A.O., Koshio, S. and Esteban, M.Á., "Beneficial roles of feed additives as immunostimulants in aquaculture: a review," Reviews in Aquaculture, 10(4), 950-974, Dec 2018.

[4] Vlaicu, P.A., Untea, A.E., Varzaru, I., Saracila, M. and Oancea, A.G., "Designing nutrition for health—incorporating dietary byproducts into poultry feeds to create functional foods with insights into health benefits, risks, bioactive compounds, food component functionality, and safety regulations. Foods, 12(21), Nov 2023.

[5] Reddy, G., Altaf, M., Naveena, B.J., Venkateshwar, M. and Kumar, E.V., "Amylolytic bacterial lactic acid fermentation — A review," Biotechnology Advances, 26(1), 22-34, Feb 2008.

[6] Dobrijević, D., Pastor, K., Nastić, N., Özogul, F., Krulj, J., Kokić, B., Bartkiene, E., Rocha, J.M. and Kojić, J., "Betaine as a functional ingredient: metabolism, health-promoting attributes, food sources, applications and analysis methods," Molecules, 28(12), Jun 2023.

[7] Lim, L.S., Chor, W.K., Tuzan, A.D., Shapawi, R. and Kawamura, G., "Betaine is a feed enhancer for juvenile grouper (Epinephelus fuscoguttatus) as determined behaviourally," Journal of Applied Animal Research, 44(1), 415-418, Oct 2015.

[8] Pessione, E., "Lactic acid bacteria contribution to gut microbiota complexity: lights and shadows," Frontiers in Cellular and Infection Microbiology, 2, Jun 2012.

[9] Mugwanya, M., Dawood, M.A.O., Kimera, F. and Sewilam, H., "A meta-analysis on the influence of dietary betaine on the growth performance and feed utilization in aquatic animals," Aquaculture Reports, 37, 102200, Jun 2024.

[10] Liu, Q. and Naganuma, T., "Metabolomics in sturgeon research: a mini-review," Fish Physiology and Biochemistry, 50 (4), 1895-1910, Aug 2008.

[11] Pfleger, M.O., Rider, S.J., Johnston, C.E. and Janosik, A.M., "Saving the doomed: Using eDNA to aid in detection of rare sturgeon for conservation (Acipenseridae)," Global Ecology and Conservation, 8, 99-107, Oct 2016.

[12] Wang, J.H., Byun, J. and Pennathur, S., "Analytical approaches to metabolomics and applications to systems biology," Seminars in Nephrology, 30(5), 500-511, Sep 2010.

[13] Picó, C., Serra, F., Rodríguez, A.M., Keijer, J. and Palou, A., "Biomarkers of nutrition and health: New tools for new approaches," Nutrients, 11(5), May 2019.

[14] Liu, Q. and Naganuma, T., "Effects of lactic acid and betaine as feed additives on metabolomic profiles of juvenile bester sturgeon (Acipenser ruthenus × Huso huso)," Journal of Food and Nutrition Research, 13(1), 18-33, Jun 2025. https:// pubs.sciepub.com/ jfnr/13/1/3.

[15] Poli, B.M., Parisi, G., Scappini, F. and Zampacavallo, G., "Fish welfare and quality as affected by pre-slaughter and slaughter management," Aquaculture International, 13 (1), 29-49, Jan 2005.

[16] Liu, Q. and Naganuma, T., "Tissue-specific biomarker metabolites of meat, fat and egg of Siberian sturgeon," Journal of Food and Nutrition Research, 12 (1), 1-13, Feb 2024.

[17] Chang, F., He, S.S. and Dang, C.Y., "Assisted selection of biomarkers by linear discriminant analysis effect size (LEfSe) in Microbiome Data," JOVE-JOURNAL OF VISUALIZED EXPERIMENTS, (183), May 2022.

[18] Wishart, D.S., Guo, A., Oler, E., Wang, F., Anjum, A., Peters, H., Dizon, R., Sayeeda, Z., Tian, S., Lee, Brian L., Berjanskii, M., Mah, R., Yamamoto, M., Jovel, J., Torres-Calzada, C., Hiebert-Giesbrecht, M., Lui, Vicki W., Varshavi, D., Varshavi, D., Allen, D., Arndt, D., Khetarpal, N., Sivakumaran, A., Harford, K., Sanford, S., Yee, K., Cao, X., Budinski, Z., Liigand, J., Zhang, L., Zheng, J., Mandal, R., Karu, N., Dambrova, M., Schiöth, Helgi B., Greiner, R. and Gautam, V., "HMDB 5.0: the Human Metabolome Database for 2022," Nucleic Acids Research, 50(D1), D622-D631, Jan 2022.

[19] Van der Aar, P.J., Molist, F. and van der Klis, J.D., "The central role of intestinal health on the effect of feed additives on feed intake in swine and poultry," Animal Feed Science and Technology, 233, 64-75, Nov 2017.

[20] Pandey, A.K., Kumar, P. and Saxena, M.J., "Feed additives in animal health. In R. C. Gupta, A. Srivastava, & R. Lall (Eds.)," Nutraceuticals in Veterinary Medicine (pp. 345-362), Springer International Publishing, May 2019.

[21] Bai, S.C., Hamidoghli, A. and Bae, J., "7-Feed additives: an

overview," In D. A. Davis (Ed.), Feed and Feeding Practices in Aquaculture (Second Edition) (pp. 195-229), Woodhead Publishing, May 2022.

[22] López-Pedrouso, M., Lorenzo, J.M., Cantalapiedra, J., Zapata, C., Franco, J.M. and Franco, D, "Chapter five-aquaculture and byproducts: Challenges and opportunities in the use of alternative protein sources and bioactive compounds," In J. M. Lorenzo & F. J. Barba (Eds.), Advances in Food and Nutrition Research (Vol. 92, pp. 127-185), Academic Press, Jan 2019.

[23] Holdt, S.L. and Kraan, S., "Bioactive compounds in seaweed: functional food applications and legislation," Journal of Applied Phycology, 23(3), 543-597, Feb 2011.

[24] Ajila, C.M., Brar, S.K., Verma, M., Tyagi, R.D., Godbout, S. and Valéro, J.R. "Bio-processing of agro-byproducts to animal feed," Critical Reviews in Biotechnology, 32(4), 382-400, Mar 2012.

[25] Torres-Maravilla, E., Parra, M., Maisey, K., Vargas, R.A., Cabezas-Cruz, A., Gonzalez, A., Tello, M. and Bermúdez-Humarán, L.G., "Importance of probiotics in fish aquaculture: Towards the identification and design of novel probiotics," Microorganisms, 12(3), Mar 2024.

[26] Kivimäki, M., Bartolomucci, A. and Kawachi, I., "The multiple roles of life stress in metabolic disorders," Nature Reviews Endocrinology, 19(1), 10-27, Oct 2022.

[27] Wikoff, W.R., Anfora, A.T., Liu, J., Schultz, P.G., Lesley, S.A., Peters, E.C. and Siuzdak, G., "Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites," Proceedings of the National Academy of Sciences, 106(10), 3698-3703, Mar 2009.

[28] Ng, W.K. and Koh, C.B., "The utilization and mode of action of organic acids in the feeds of cultured aquatic animals," Reviews in Aquaculture, 9(4), 342-368, Dec 2017.

[29] Yang, M.J., Cheng, Z.X., Jiang, M., Zeng, Z.H., Peng, B., Peng, X.X. and Li, H., "Boosted TCA cycle enhances survival of zebrafish to Vibrio alginolyticus infection," Virulence, 9(1), 634-644, Feb 2018.

[30] Ranea-Robles, P. and Houten, S.M., "The biochemistry and physiology of long-chain dicarboxylic acid metabolism," Biochemical Journal, 480(9), 607-627, May 2023.

[31] Guzior, D.V. and Quinn, R.A., "Review: microbial transformations of human bile acids," Microbiome, 9(1), 140, Jun 2021.

[32] Pietrowska-Borek, M., Dobrogojski, J., Sobieszczuk-Nowicka, E. and Borek, S., "New insight into plant signaling: Extracellular ATP and uncommon nucleotides," Cells, 9(2), Feb 2020.

[33] Kaushik, S.J. and Seiliez, I., "Protein and amino acid nutrition and metabolism in fish: current knowledge and future needs," Aquaculture Research, 41(3), 322-332. Feb 2010.

[34] Ochs, R.S. and Harris, R.A., "Mechanism for the oleate stimulation of gluconeogenesis from dihydroxyacetone by hepatocytes from fasted rats," Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 886(1), 40-47, Apr 1986.

[35] Yoon, D.S., Kim, D.H., Kim, J.H., Sakakura, Y., Hagiwara, A., Park, H.G., Lee, M.C. and Lee, J.S., "Interactions between lipid metabolism and the microbiome in aquatic organisms: A review," Marine Pollution Bulletin, 207, 116858, Oct 2024.

[36] Jenkins, B., West, J.A. and Koulman, A., "A review of odd-chain fatty acid metabolism and the role of pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0) in health and disease," Molecules, 20(2), 2425-2444, Jan 2015.

[37] Fagundes, M.A., Yang, S.Y., Eun, J.S., Hall, J.O., Moon, J.O. and Park, J.S., "Influence of supplementing a methionine derivative, N-acetyl-l-methionine, in dairy diets on production and ruminal fermentation by lactating cows during early to mid lactation," Journal of Dairy Science, 101(8), 7082-7094, Aug 2018.

[38] Prinville, V., Ohlund, L. and Sleno, L., "Targeted analysis of 46 bile acids to study the effect of acetaminophen in rat by LC-MS/MS," METABOLITES, 10(1), Jan 2020.

[39] Copeman, L.A., Parrish, C.C., Brown, J.A. and Harel, M., "Effects of docosahexaenoic, eicosapentaenoic, and arachidonic acids on the early growth, survival, lipid composition and pigmentation of yellowtail flounder (Limanda ferruginea): a live food enrichment experiment," Aquaculture, 210(1), 285-304, Jul 2002.

[40] Hui, S., Ghergurovich, J.M., Morscher, R.J., Jang, C., Teng, X., Lu, W., Esparza, L.A., Reya, T., Le, Z., Yanxiang Guo, J., White, E. and Rabinowitz, J.D., "Glucose feeds the TCA cycle via circulating lactate," Nature, 551(7678), 115-118, Oct 2017.

[41] Felig, P., "The glucose-alanine cycle," Metabolism, 22(2), 179-207, Feb 1973.

[42] Castillo Martinez, F.A., Balciunas, E.M., Salgado, J.M., Domínguez González, J.M., Converti, A. and Oliveira, R.P.d.S., "Lactic acid properties, applications and production: A review," Trends in Food Science & Technology, 30(1), 70-83, Mar 2013.

[43] Kwon, Y., "Effect of trans–fatty acids on lipid metabolism: Mechanisms for their adverse health effects," Food Reviews International, 32(3), 323-339, Mar 2016.

[44] Srinivasan, S., Torres, A.G. and Ribas de Pouplana, L., "Inosine in biology and disease," Genes, 12(4), Apr 2021.

[45] Tanianskii, D.A., Jarzebska, N., Birkenfeld, A.L., O'Sullivan, J.F. and Rodionov, R.N., "Beta-aminoisobutyric acid as a novel regulator of carbohydrate and lipid metabolism. Nutrients, 11(3), Feb 2019.

[46] Kamei, Y., Hatazawa, Y., Uchitomi, R., Yoshimura, R. and Miura, S., "Regulation of skeletal muscle function by amino acids. Nutrients, 12(1), Jan 2020.

[47] Kraemer, W.J. and Volek, J.S. "CREATINE SUPPLEMENTATION: Its role in human performance," Clinics in Sports Medicine, 18(3), 651-666, Jul 1999.

[48] Dzeja, P. and Terzic, A., "Adenylate kinase and AMP signaling networks: Metabolic monitoring, signal communication and body energy sensing," International Journal of Molecular Sciences, 10(4), 1729-1772, Apr 2009.

[49] Gruzman, A., Babai, G. and Sasson, S., "Adenosine monophosphate-activated protein kinase (AMPK) as a new target for antidiabetic drugs: A review on metabolic, pharmacological and chemical considerations," Rev Diabet Stud, 6(1), 13-36, May 2009.