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Virginia M. Artegoitia
University of Nebraska - Lincoln, virginia.artegoitia@food.au.dk

Andrew P. Foote
USDA, Agricultural Research Service

Ronald M. Lewis
University of Nebraska - Lincoln, ron.lewis@unl.edu

Harvey C. Freetly
USDA, Agricultural Research Service, harvey.freetly@ars.usda.gov

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Metabolomics Profile and Targeted Lipidomics in Multiple Tissues Associated with Feed Efficiency in Beef Steers

Virginia M. Artegoitia,*‡§ Andrew P. Foote,‡ Ronald M. Lewis,† and Harvey C. Freetly‡

*Department of Animal Science, University of Nebraska-Lincoln, Lincoln, Nebraska 68583, United States
†ARS, U.S. Meat Animal Research Center, USDA, Clay Center, Nebraska 68933, United States
‡ARS, U.S. Meat Animal Research Center, USDA, Clay Center, Nebraska 68933, United States
§Supporting Information

ABSTRACT: A study of multiple tissues was conducted to identify potential metabolic differences in cattle differing in feed efficiency. Individual feed intake and body weight was measured on 144 steers during 105 days on a high-concentrate ration. Steers were selected according to differences in average daily gain (ADG) with those with the greatest ADG ($n = 8$; $1.96 \pm 0.02$ kg/day) and least ADG ($n = 8$; $1.57 \pm 0.02$ kg/day), whose dry matter intake was within 0.32 SD of the mean intake ($10.10 \pm 0.05$ kg/day). Duodenum, liver, adipose, and longissimus-dorsi were collected at slaughter, and metabolomics profiles were performed by ultra performance liquid chromatography quadrupole-time-of-flight mass spectrometry. Principal components analyses, $t$-tests, and fold changes in tissues profile were used to identify differential metabolites between ADG groups. These were primarily involved in $\alpha$-linolenic metabolism, which was downregulated in the greatest ADG as compared to least-ADG group in duodenum, adipose, and longissimus-dorsi. However, taurine and glycerophospholipid metabolisms were both upregulated in the greatest ADG compared with least-ADG group in the liver. The phospholipids and cholesterol were quantified in the tissues. Lipid transport and oxidation were the main common metabolic mechanisms associated with cattle feed efficiency. Combining analyses of multiple tissues may offer a powerful approach for defining the molecular basis of differences in performance among cattle for key production attributes.

INTRODUCTION

Increased sustainability of livestock production is needed by the growing human population. Conversion of feed into meat is associated, among others, with emission of greenhouse gases and excretion of nitrogen and phosphorus. Thus, improving production efficiency by increasing beef produced per amount of feed offered would reduce both operating cost and waste from beef cattle production.

Through more than 50 years of research focused on nutritional management and genetic selection for beef cattle production, feed efficiency conversion rate has been improved on average from 10.2:1 to 5.4:1. Some of the traditional strategies are the use of high-concentrate diets and animal selection by moderately heritable variables such as intake and gain. However, large phenotypic differences regarding many complex traits of general biological interest in cattle such as growth, fat deposition, and energy partitioning are not completely understood. Application of new technologies, including “omics” selections that incorporate molecular breeding values, plays an important role in meeting these challenges. The use of genome-wide SNP markers is a promising predictor of the genetic merit of animals, despite genetics limiting to explain the differences of the phenotype. Thus, the final “omic” level in a biological system, metabolomics may provide the most “functional” information of the omics technologies, allowing the measure of the end products of the complex genetic epigenetic and environmental interactions. This analytical technique is increasingly used in livestock research for providing a distinctive insight into the biochemical activity and robustly correlate with animal production traits.

For the first time, we previously characterized metabolite changes in the rumen using the distinct ruminal profiling method, identifying 33 biomarkers related to differences in average daily gain (ADG). Linoleic/$\alpha$-linolenic acids (ALA) metabolism and biosynthesis of aromatic amino acids were the most altered functional pathways in the rumen associated with production efficiency of beef steers. In addition, fatty acids were quantified and evaluated as potential biomarkers to predict ADG in cattle.

An identification of a distinctive physiological process across multiple tissues could therefore usefully contribute for understanding the mechanism associated with cattle feed efficiency. We hypothesize that differences in ADG of cattle, with similar intake, are associated with common biochemical pathways at multiple levels of the tissues metabolism. A combination of metabolomics fingerprint and targeted lipidomics analysis was used to identify metabolites from animals that differed with feed efficiency in duodenum, liver, subcutaneous adipose, and longissimus-dorsi.

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RESULTS

Tissues Metabolomics Profiling. Differences in metabolites were identified between least-ADG and greatest-ADG animals \( (p < 0.05) \) in duodenum \( (n = 87) \), liver \( (n = 92) \); longissimus-dorsi (LD, \( n = 95 \)), and adipose \( (n = 52) \) tissue. The analysis of these metabolites revealed specific metabolic pathways to ADG groups in the duodenum, liver, adipose, and LD (Figures 1 and 2; see Supporting Information Table S2). The \( \alpha \)-linolenic acid (ALA) metabolism was the most relevant metabolic pathway downregulated in adipose, duodenum, and LD tissues (impact value = 1 and \( p < 0.05 \)), whereas taurine and glutathione
biosynthesis were upregulated in hepatic tissue (impact value = 0.5 and \( p < 0.08 \)) in greatest ADG compared to least-ADG animals.

No common identified metabolites were found in the four tissues evaluated (Figure 3). However, alanine and ALA were downregulated in duodenum, LD, and adipose tissues, whereas oleamide was upregulated in duodenum, LD, and liver tissues, in greatest ADG compared to least-ADG animals (Figure 3). In addition, phosphatidylcholine (PC) was upregulated in high-ADG animals in the four tissues but differed within fatty acids (Supporting Information Table S1).

Principal component analysis and score plot from supervised partial least square of discriminant analysis (PLS-DA) were conducted on the metabolites identified in the tissues. The analysis revealed a clear clustering between least-ADG and greatest-ADG animals (Figure 4), which suggest that biochemistry changed in the tissues according to cattle feed efficiency. The \( p \)-value for 1000 permutations was \( p < 0.05 \) in the tissues, indicating that the PLS-DA model was valid. For the first component of PLS-DA, descriptive statistics from model fitting by accuracy, estimates of the goodness of fit (\( R^2 \)), and estimates of goodness prediction (\( Q^2 \)) by tissue were: accuracy = 0.81, \( R^2 = 0.52 \), and \( Q^2 = 0.40 \) for LD; accuracy = 0.75, \( R^2 = 0.64 \), and \( Q^2 = 0.42 \) for adipose; accuracy = 0.94, \( R^2 = 0.94 \), and \( Q^2 = 0.62 \) for duodenum; accuracy = 0.77, \( Q^2 = 0.39 \) for liver.

### Phospholipids Quantification in Tissues.

On the basis of the findings of the nontargeted metabolomic analysis, phospholipids were quantified in liver (Table 1), duodenum (Table 2), adipose (Table 3), and LD (Table 4). The performance of phospholipids as biomarkers was assessed using receiver—operator characteristic (ROC) curves. According to the accepted classification of biomarker utility, the area under the curve (AUC) was the metric used to assess the metabolites as candidate markers.\(^\text{17}\)

The concentrations of compounds with the greatest sensitivity/specificity for ADG differences were cholesterol and LPC-18:1 in liver (AUC = 0.99, AUC = 0.98; FDR < 0.05; respectively) followed by PC-18:0/20:3 in LD (AUC = 0.86; FDR = 0.03). Steers with the greatest ADG had lower

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**Figure 2.** Integrated pathways metabolomics analysis in adipose, duodenum, longissimus-dorsi, and liver compare high-ADG vs low-ADG beef cattle with similar average dry matter intake according to Bos taurus KEGG pathway database. UPLC-q-ToF MS metabolites identified to differ between ADG groups by \( t \)-test (\( p < 0.05 \)).

**Figure 3.** Venn diagram showing the overlap across tissues of the metabolites identified that differ between ADG groups by \( t \)-test (\( p < 0.05 \)).
Figure 4. Tissues metabolomic profile of the steers with the greatest average daily gain (high-ADG; open red triangle) and the least ADG (low-ADG; green plus) with similar average dry matter intake. (A) Principal component analysis for UPLC-q-Tof MS metabolites identified to differ between ADG by t-test ($p < 0.05$). (B) Partial least square-discriminant analysis for UPLC-q-Tof MS metabolites identified to differ between ADG by t-test ($p < 0.1$). One data point represents one steer.
concentration of cholesterol and LPC-18:1 in liver and higher concentration of PC-18:20:3 in LD than least-ADG steers. No significant differences (p > 0.05) in phospholipids concentration were found in the duodenum and adipose tissues across ADG classification. However, liver cholesterol concentration was associated with cholesterol concentration in LD (r = 0.68; p < 0.01) and in duodenum (r = −0.67; p < 0.01), whereas liver cholesterol was not associated with cholesterol concentration in adipose tissue (r = 0.03; p = 0.40).

## DISCUSSION

This study represents the first comprehensive metabolome evaluation based on phenotypic differences in cattle efficiency for four tissues, duodenum, liver, adipose, and LD, combining metabolomics and targeted lipidomics approaches. Previously, rumen fluid was evaluated by UPLC-q-ToF MS metabolomics analysis associated with univariate and multivariate statistical methods to identify metabolites that differentiate steers with greatest ADG from least-ADG. On the basis of this previous approach, metabolomics analyses in multiple tissues on the same animals were conducted to provide evidence on physiological mechanisms involved in differences in weight-to-gain ratios in cattle with similar DMI. Furthermore, targeted lipidomics analysis was conducted to support the metabolomics results and provided valuable potential biomarkers for feed efficiency.

In the current study, the compounds identified according to differences in ADG, reflected the complexity and diversity of the metabolism in different tissues. For instance, the adipose tissues presented 51 compounds mainly associated with fatty acid metabolism, whereas in the liver 92 compounds were associated mainly with taurine and glutathione metabolism. Indeed, no common identified metabolites were found across the four tissues evaluated. However, ALA metabolism was downregulated in adipose, duodenum, and LD tissues in animals with greatest ADG. Lower level of ALA in more efficient animals might reflect more extensive ruminal biohydrogenation as reported before in ruminal fluid and plasma in the same animals. The ALA entering tissues is rapidly accumulated, although a certain percentage of ALA is subjected to desaturation or β-oxidation depending on the tissues needs. For instance, longer n-3 fatty acids, such EPA or DHA or others energetic carbons products, including ATP, monosaturated fatty acids, and phospholipids. In vivo, the two basic ALA metabolic fates are associated with the expressions of lipogenic enzymes, substrate level, and product inhibition. Therefore, considering that in this study the intake is similar between the two ADG groups, ALA levels in tissues probably reflected a predominant catabolic fate producing more energy in more efficient animals.

On the basis of fingerprint analysis, glycerophospholipid (GLP) compounds were the main lipid category, and their metabolism was displayed in the four tissues evaluated. According to differences on GLP head group, PC was the main phospholipid class in LD, liver, and adipose tissue, whereas the GLP acyl chain length was widely distributed across tissues. The specific functions of these GLP class/species are not full elucidated. However, the fatty acid pattern

| phospholipids and cholesterol | high-ADG | low-ADG | SEM | AUC<sup>b</sup> | p | FDR<sup>c</sup> |
|-------------------------------|---------|--------|-----|-------------|---|--------|
| phospatidylcholine            | 16/0:16:0 | 8.30 | 7.26 | 0.87 | 0.61 | 0.42 | 0.47 |
|                               | 16/0:18:1 | 7.09 | 5.82 | 0.89 | 0.66 | 0.33 | 0.47 |
|                               | 16/20:3 | 8.99 | 7.31 | 1.01 | 0.67 | 0.26 | 0.43 |
|                               | 16/0:20:4 | 13.1 | 10.2 | 1.57 | 0.63 | 0.21 | 0.38 |
|                               | 18/0:18:1 | 2.01 | 1.72 | 0.26 | 0.61 | 0.43 | 0.47 |
|                               | 18/0:18.2, 18:1/18:1 | 2.42 | 1.85 | 0.29 | 0.69 | 0.19 | 0.38 |
|                               | 18/0:20:3 | 1.12 | 0.55 | 0.16 | 0.86 | <0.01 | 0.03 |
|                               | 18/0:20:4 | 2.93 | 1.77 | 0.37 | 0.80 | 0.04 | 0.25 |
|                               | 18/0:22:5 | 1.16 | 0.52 | 0.21 | 0.81 | <0.01 | 0.05 |
|                               | 18/0:22:6, 18:1/22:5 | 2.53 | 1.72 | 0.31 | 0.75 | 0.09 | 0.26 |
|                               | 18:1/20:4, 18:0/20:5, 16:0/22:5 | 3.63 | 2.67 | 0.46 | 0.70 | 0.16 | 0.37 |
| phospatidylethanolamine        | 16/0:18:2 | 0.28 | 0.23 | 0.06 | 0.55 | 0.58 | 0.58 |
|                               | 18/0:18.0 | 0.76 | 0.52 | 0.12 | 0.66 | 0.16 | 0.37 |
|                               | 18/0:22:5 | 0.10 | 0.09 | 0.02 | 0.53 | 0.89 | 0.89 |
|                               | 18/0:22:6 | 0.28 | 0.22 | 0.05 | 0.56 | 0.45 | 0.47 |
| lysophosphatidylcholine        | 18:0 | 0.25 | 0.37 | 0.09 | 0.63 | 0.37 | 0.47 |
|                               | 18:1 | 0.19 | 0.20 | 0.06 | 0.52 | 0.94 | 0.94 |
|                               | 18:2 | 0.07 | 0.06 | 0.14 | 0.53 | 0.62 | 0.62 |
| lysophosphatidylethanolamine   | 16:0 | 0.04 | 0.05 | 0.01 | 0.61 | 0.43 | 0.47 |
|                               | 18:0 | 0.59 | 0.755 | 0.06 | 0.75 | 0.08 | 0.18 |
| cholesterol                   | 1.25 | 1.71 | 0.08 | 0.78 | 0.07 | 0.18 |

<sup>a</sup>Groups were least-ADG steers (n = 8), with the least average daily gain, and greatest-ADG steers (n = 8), with the greatest average daily gain, with groups having similar dry matter intake. <sup>b</sup>AUC = area under the curve calculated by receiver−operator characteristic curve analysis. <sup>c</sup>FDR = false discovery rate p-adjustment.
of GLP membrane together with the class distribution of phospholipids are major lipid-related factors that characterize the properties and functions of the membranes, including the regulation of transport processes, energy homeostasis, and signal transduction. Thus, the GLP profile is associated with diverse metabolic states. For instance, the PC acyl chain length was associated with the concentrations of glucose, insulin, leptin, and hepatic cholesterol in humans, and mice fed a high-fat diet. Furthermore, in our results, a fatty acid amine synthesized from phospholipids membrane, Oleamide, was upregulated in duodenum, liver, LD, and previously, in rumen fluid. The fatty acid amines belong to a group of endogenous lipid mediators, endocannabinoids, implicated on feed efficiency and carcass composition of beef cattle. Considering GLP is not only an energy-storage compound but also an interactive player in various metabolic processes, we sought to explore phospholipids and cholesterol concentrations in the liver and increased milk yield during the transition period in dairy ruminants. However, phosphatidylcholine has not been extensively studied in beef cattle. Certainly, dietary supplementation with lecithin improves the feed conversion rate in pigs on a high-fat diet during finishing period, and rumen-protected choline supplementation has been shown to promote weight gain and improve meat quality in young lambs. In addition, differential gene expression and transcripts already highlighted the importance of phospholipids metabolism in cattle feed efficiency. Despite dietary phospholipids are extensively degraded by the rumen and PC concentration depends almost exclusively upon endogenous synthesis, understanding of the requirements of PC is needed to improve feed efficiency in beef cattle.

The concentration of cholesterol is considered a robust biomarker of feed efficiency in cattle as consistent with our results, lower cholesterol in liver and blood is associated with more efficient beef cattle. Cholesterol concentrations in fact reflect the capacity of synthesis, transport, and oxidation by the liver. Particularly in ruminants, cholesterol is derived from hepatic de-novo biosynthesis, and its major catabolism represents the bile acid synthesis. In our results, cholesterol oxidation products, such as glycine or taurine conjugated, were in turn higher in the liver of animals with greater ADG. Therefore, the excretion and reabsorption of bile acid forms represent the basis of the enterohepatic circulation, an essential mechanism for the digestion and absorption of fatty acids, which likely relates to cattle feed efficiency.

By contrast, the less efficient animal presented higher hepatic concentration of cholesterol with an increase of glutathione metabolism. The liver is the primary organ for the synthesis of glutathione from glutamate, glycine, and cysteine and it is a major antioxidant, which regulates the homeostasis of free radicals. Perturbations of glutathione status are usually a consequence of oxidative stress and redox shift that impair liver function. Thus, the accumulation of

| phospholipids and cholesterol | low-ADG | high-ADG | SEM | AUC | p | FDR |
|-----------------------------|--------|---------|-----|-----|---|-----|
| phosphatidylcholine          |        |         |     |     |   |     |
| 16:0/16:0                   | 31.9   | 31.5    | 2.62| 0.59| 0.69| 0.68|
| 16:0/18:1                   | 6.19   | 5.01    | 0.13| 0.67| 0.14| 0.13|
| 16:0/20:3                   | 16.1   | 12.1    | 1.27| 0.79| 0.05| 0.07|
| 16:0/20:4                   | 16.9   | 14.1    | 1.25| 0.64| 0.13| 0.13|
| 18:0/18:1                   | 17.9   | 14.1    | 1.46| 0.81| 0.06| 0.09|
| 18:0/18:2, 18:1/18:1        | 5.24   | 4.08    | 0.25| 0.78| 0.09| 0.09|
| 18:0/20:3                   | 7.53   | 6.07    | 0.29| 0.64| 0.12| 0.12|
| 18:0/20:4                   | 10.6   | 8.63    | 0.88| 0.62| 0.12| 0.12|
| 18:0/22:5                   | 15.4   | 12.6    | 1.21| 0.64| 0.13| 0.13|
| 18:0/22:6, 18:1/22:5        | 12.0   | 9.71    | 0.99| 0.64| 0.12| 0.12|
| 18:1/20:4, 18:0/20:5, 16:0/22:5 | 13.9 | 11.8 | 0.95| 0.61| 0.13| 0.13|
| phosphatidylethanolamine    |        |         |     |     |   |     |
| 16:0/18:2                   | 0.20   | 0.21    | 0.02| 0.53| 0.92| 0.92|
| 18:0/18:0                   | 0.18   | 0.15    | 0.02| 0.72| 0.23| 0.24|
| 18:0/22:5                   | 0.16   | 0.18    | 0.01| 0.61| 0.27| 0.27|
| 18:0/22:6                   | 0.12   | 0.12    | 0.01| 0.61| 0.27| 0.27|
| lysophosphatidylcholine      |        |         |     |     |   |     |
| 18:0                       | 0.74   | 1.26    | 0.20| 0.89| 0.07| 0.07|
| 18:1                       | 1.55   | 2.47    | 0.17| 0.98| <0.01| 0.01|
| 18:2                       | 0.65   | 0.75    | 0.14| 0.63| 0.32| 0.32|
| lysophosphatidylethanolamine |        |         |     |     |   |     |
| 16:0                       | 1.54   | 2.14    | 0.29| 0.70| 0.16| 0.16|
| 18:0                       | 0.71   | 1.02    | 0.11| 0.78| 0.07| 0.07|
| cholesterol                 | 3.40   | 5.06    | 0.47| 0.99| <0.01| 0.02|

aGroups were least-ADG steers (n = 8), with the least average daily gain, and greatest-ADG steers (n = 8), with the greatest average daily gain, with groups having similar dry matter intake. AUC = area under the curve calculated by receiver–operator characteristic curve analysis. FDR = false discovery rate p-adjustment.

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cholesterol in the liver may result in part from a decrease in cholesterol oxidation and transport probably linked with hepatic performance.

In our results, the concentration of cholesterol was positively associated in liver and muscle. Despite the lack of information on body composition or carcass traits in this study, improving cattle feed efficiency potentially has a beneficial effect on carcass composition, particularly for the human health aspect. Furthermore, metabolomics results in LD from animals that are more efficient and presented a higher level of creatinine deficiency potentially has a beneficial effect on carcass composition, particularly for the human health aspect. The integration of the metabolomics profiles of tissues and metabolites points to the hepatic function as an important metabolic crossroad that has the potential to improve the gain-to-feed ratios.

Combining analyses of multiple tissues may offer a powerful approach for defining the molecular basis of differences in performance among cattle for key production attributes.

**EXPERIMENTAL PROCEDURES**

**Study Design and Samples Details.** This study was approved by the Institutional Animal Care and Use Committee (IACUC) at the U.S. Meat Animal Research Center.

After weaning, steers \( (n = 144\) Angus-sired) were housed in a facility with Calan Broadbent electronic headgates (American Calan, Inc., Northwood, NH) to measure individual feed intake. The diet consists on a DM basis, of 8% chopped alfalfa hay, 20% wet distillers grains with solubles, 67.75% dry-rolled corn, and 4.25% commercial vitamin and mineral supplement; the supplement contained monensin (Rumensin 80; Elanco Animal Health, Greenfield, IN) to meet the nutrient requirements (NRC, 2016) and provide a finished marketed carcass. Further details about the experimental design were provided previously. Briefly, feed intake was measured for daily gain.

**CONCLUSIONS**

Overall, lipid transport and oxidation were the main common metabolic mechanism involved in weight-gain differences at multiple metabolic levels of complexity in beef cattle notwithstanding the uniqueness of the molecular composition of each tissue. Both mechanisms were associated with the levels of ALA, phosphatidylcholine, and cholesterol that could be considered as useful biomarkers (in other sample formats e.g., blood, plasma) for feed efficiency in beef cattle.

**Table 3. Phospholipid and Cholesterol Concentrations in Adipose Tissue (mg/g) on Steers with the Low and High Average Daily Gain**

| phospholipids and cholesterol | high-ADG | low-ADG | SEM | AUC\(\text{b}\) | \(p\) | FDR\(\text{c}\) |
|-----------------------------|---------|---------|-----|-------------|-----|-----|
| **phosphatidylcholine**     |         |         |     |             |     |     |
| 16:0/16:0                   | 14.1    | 9.6     | 2.08| 0.70        | 0.14| 0.59|
| 16:0/18:1                   | 3.77    | 3.2     | 0.64| 0.62        | 0.55| 0.67|
| 16:0/20:3                   | 6.36    | 5.3     | 1.04| 0.52        | 0.80| 0.85|
| 16:0/20:4                   | 8.21    | 6.7     | 1.13| 0.58        | 0.35| 0.59|
| 18:0/18:1                   | 1.28    | 1.6     | 0.26| 0.67        | 0.44| 0.59|
| 18:0/18:2, 18:1/18:1        | 1.81    | 1.6     | 0.31| 0.67        | 0.40| 0.59|
| 18:0/20:3                   | 2.05    | 1.7     | 0.30| 0.63        | 0.35| 0.59|
| 18:0/20:4                   | 3.69    | 2.9     | 0.54| 0.55        | 0.31| 0.59|
| 18:0/22:5                   | 1.35    | 0.9     | 0.25| 0.62        | 0.20| 0.59|
| 18:0/22:6, 18:1/22:5        | 2.84    | 2.5     | 0.43| 0.68        | 0.21| 0.59|
| 18:1/20:4, 18:0/20:5, 16:0/22:5 | 3.65 | 2.9 | 0.50 | 0.63 | 0.30 | 0.59 |
| **phosphatidylethanolamine** |         |         |     |             |     |     |
| 16:0/18:2                   | 0.39    | 0.21    | 0.06| 0.77        | 0.06| 0.59|
| 18:0/18:0                   | 0.15    | 0.09    | 0.02| 0.71        | 0.14| 0.59|
| 18:0/22:5                   | 0.47    | 0.29    | 0.05| 0.69        | 0.19| 0.59|
| 18:0/22:6                   | 0.71    | 0.52    | 0.12| 0.63        | 0.30| 0.59|
| **lysophosphatidylcholine** |         |         |     |             |     |     |
| 18:0                       | 0.03    | 0.04    | 0.01| 0.61        | 0.45| 0.59|
| 18:1                       | 0.06    | 0.07    | 0.02| 0.52        | 0.89| 0.89|
| 18:2                       | 0.38    | 0.32    | 0.05| 0.53        | 0.81| 0.85|
| **lysophosphatidylethanolamine** |         |         |     |             |     |     |
| 16:0                       | 0.03    | 0.02    | 0.01| 0.55        | 0.45| 0.59|
| 18:0                       | 0.76    | 0.70    | 0.15| 0.60        | 0.28| 0.59|
| cholesterol                | 3.29    | 3.23    | 0.22| 0.53        | 0.78| 0.59|

\(\text{Groups were least-ADG steers (}\ n = 8\), with the least average daily gain, and greatest-ADG steers (}\ n = 8\), with the greatest average daily gain, with groups having similar dry matter intake. \(\text{AUC} = \text{area under the curve calculated by receiver–operator characteristic curve analysis. FDR} = \text{false discovery rate}\) \(p\)-adjustment. |
The extraction procedure was adapted from Artegitoia et al.\textsuperscript{12} Examples of each tissue were extracted for metabolomics analysis. The phospholipids and cholesterol of each tissue was weighed and diluted with 1 mL of chloroform/methanol/water (1:2:0.8 v/v), vortexed, and centrifuged at 16 000g for 10 min at 4 °C. The supernatant and solid precipitate were separated in different vials for aqueous (supernatant) and organic (precipitate) extractions, respectively. For the aqueous extraction, the supernatant was transferred to a new vial, dried under a nitrogen stream, and resuspended in 500 μL of acetonitrile/water (9:1 v/v). For the organic extraction, the solid precipitate was dissolved in 1 mL of dichloromethane/methanol (3:1 v/v), centrifuged (16 000g, 10 min at 4 °C), dried under a nitrogen stream, and resuspended in 500 μL in acetonitrile/water (9:1 v/v). The ultra-performance liquid-chromatography/mass spectrometry (UPLC/MS) analysis was carried out using a Waters ACQUITY ultra-performance liquid-chromatography (UPLC) system (Waters Corp., Milford, MA) equipped with an autosampler and coupled with a hybrid triple quadrupole-time of-flight mass spectrometry (XEVO-G2-S-qTOF; Waters Corp.). Details of instrument calibration, quality control samples, chromatogram separation, and MS system parameters were carried out as described before.\textsuperscript{15}

**Quantitative Lipidomics Analysis.** The phospholipids were quantified in duodenum, liver, longissimus-dorsi (LD), and adipose tissue by UPLC-triple quadrupole mass spectrometry (UPLC/MS) analysis using an adapted methodology.\textsuperscript{13} Briefly, phospholipids were extracted from 50 mg of tissues using the Bligh and Dyer method.\textsuperscript{14} A mixture (1 μL) of deuterated phospholipids (Avanti Polar 330707, Alabaster, AL) was used as an internal standard. The calibration curves for lysophosphatidylcholine, phosphatidylcholine, and phosphatidylethanolamine were carried out as described before.13

| phospholipids and cholesterol | group* | high-ADG | low-ADG | SEM | AUC\textsuperscript{b} | p | FDR\textsuperscript{c} |
|-------------------------------|--------|----------|---------|-----|-----------------|---|-----------|
| phosphatidylcholine           | 16:0/16:0 | 10.3     | 8.84    | 0.65 | 0.72            | 0.13 | 0.24     |
|                               | 16:0/18:1 | 1.04     | 0.87    | 0.07 | 0.75            | 0.10 | 0.21     |
|                               | 16:20:3   | 2.03     | 1.64    | 0.13 | 0.73            | 0.06 | 0.21     |
|                               | 16:0/20:4 | 2.57     | 2.08    | 0.16 | 0.77            | 0.06 | 0.21     |
|                               | 18:0/18:1 | 0.42     | 0.34    | 0.03 | 0.75            | 0.10 | 0.21     |
|                               | 18:0/18:2 | 0.63     | 0.50    | 0.05 | 0.77            | 0.07 | 0.21     |
|                               | 18:0/20:3 | 0.40     | 0.34    | 0.03 | 0.67            | 0.21 | 0.26     |
|                               | 18:0/20:4 | 0.86     | 0.74    | 0.06 | 0.70            | 0.17 | 0.25     |
|                               | 18:0/22:5 | 1.85     | 1.73    | 0.28 | 0.66            | 0.18 | 0.25     |
|                               | 18:0/22:6 | 1.81     | 1.39    | 0.17 | 0.75            | 0.10 | 0.21     |
|                               | 18:1/20:4 | 1.14     | 0.96    | 0.08 | 0.73            | 0.16 | 0.25     |
|                               | 18:0/22:5 | 1.42     | 0.39    | 0.06 | 0.56            | 0.76 | 0.76     |
|                               | 18:0/22:6 | 0.05     | 0.06    | 0.01 | 0.53            | 0.59 | 0.59     |
| lysophosphatidylcholine        | 18:0     | 0.92     | 0.81    | 0.08 | 0.67            | 0.38 | 0.43     |
|                               | 18:1     | 3.70     | 3.41    | 0.53 | 0.57            | 0.70 | 0.70     |
|                               | 18:2     | 21.1     | 21.8    | 4.94 | 0.56            | 0.91 | 0.91     |
| lysophosphatidylethanolamine   | 16:0     | 3.73     | 4.27    | 1.06 | 0.52            | 0.72 | 0.72     |
|                               | 18:0     | 1.23     | 1.07    | 0.09 | 0.73            | 0.84 | 0.84     |
| cholesterol                   | 1.46     | 1.20     | 0.09    | 0.62 | 0.10            | 0.21 | 0.21     |

*Groups were least-ADG steers (n = 8), with the least average daily gain, and greatest-ADG steers (n = 8), with the greatest average daily gain, with groups having similar dry matter intake. \( \text{AUC} = \text{area under the curve calculated by receiver–operator characteristic curve analysis.} \) \( \text{FDR} = \text{false discovery rate p-adjustment.} \)
phatidylcholine (LPC; Avanti Polar 83007, Alabaster, AL), lysophosphatidylamine (Avanti Polar 840081, Alabaster, AL), phosphatidylethanolamine (Avanti Polar 330707, Alabaster, AL), phosphatidylcholine (PC; Avanti Polar 840055, Alabaster, AL), and cholesterol (Avanti Polar 7000000, Alabaster, AL) were prepared by serial dilution (8-fold) in water/methanol (1:9 v/v).

The UPLC/MS analysis was carried out using a Waters ACQUITY ultra-performance liquid-chromatography (UPLC, Acquity BEH HILIC, 2.1 × 100 mm × 1.7 μm; Waters Corp., Milford, MA) equipped with an autosampler and coupled with a triple quadrupole mass spectrometry (XEVO-TQS; Waters Corp.). Details of instrument calibration, chromatogram separation, and MS system parameters were carried out as described before.\textsuperscript{13}

### Data and Statistical Analysis

Data processing and analysis were conducted individually for each tissue. Raw data from the fingerprinting analysis was aligned and normalized using total ion intensity using Progenesis QI v1.0 software (Waters Corp.). The bovine Metabolome Database (http://www.cowmetdb.ca/) was used to identify tissue’s compounds by using exact m/z values and retention times.

Data were tested for normality and log transformed and standardized using the Pareto scaling technique. The t-test, principal components analysis, and partial least square of discriminant analysis (PLS-DA) were conducted to identify and visualize differences of the compounds identified between least-ADG and greatest-ADG animals using MetaboAnalyst 3.0 software according to previously published recommended statistical procedure for metabolomics analysis.\textsuperscript{15} Raw p-values were adjust by PROC MULTTEST procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC) and reported as fold discovered rate (FDR) p-adjusted.

The Venn diagram of the identified metabolites between ADG groups was conducted across the four tissues.\textsuperscript{16} Pathway analysis was performed using a Bos taurus pathway library, which integrates global pathway enrichment analysis and relative between centrality pathway topology analysis from MetaboAnalyst 3.0 software. The identification and visualization of the top altered pathway were based on KEGG (http://www.genome.jp/kegg/) database sources.

The concentration of phospholipids was evaluated by t-test (p-adjusted), and a receiver-operator characteristic curve (ROC) used to evaluate the sensitivity and specificity of potential metabolic biomarkers.\textsuperscript{17} In addition, the association of cholesterol concentration among tissues was evaluated by PROC CORR procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC).

### ASSOCIATED CONTENT

1. Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b02494.

Identification of longissimus-dorsi, adipose, liver and duodenum metabolites for feed efficiency (Table S1); results from tissues pathway analysis (Table S2) (PDF)

### AUTHOR INFORMATION

**Corresponding Author**

*E-mail: virginia.artegoitia@food.au.dk.*

**ORCID**

Virginia M. Artegoitia: 0000-0001-5469-8908

**Present Address**

\textsuperscript{3}Department of Food Science, Aarhus University, Årslev 05792, Denmark (V.M.A.).

**Author Contributions**

A.P.F., H.C.F., and V.M.A. designed and planned the experiment. A.P.F. conducted the feeding and growth study. A.P.F. and V.M.A. collected tissue samples. V.M.A performed the LC-MS analysis, data analysis, and wrote the manuscript. R.M.L. consulted on data analysis and revised the manuscript.

**Notes**

The authors declare no competing financial interest.

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