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Non ruminant nutrition

The effects of dietary essential fatty acid ratios and energy level on growth performance, lipid metabolism, and inflammation in grow-finish pigs

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Abstract

The objective of this study was to investigate the effects of dietary metabolizable energy (ME) level and the ratio of linoleic acid:α-linolenic acid (LA:ALA) on the growth performance, lipid metabolism, circulatory and joint inflammatory status, and synovial fluid proteome of grow-finish pigs. A total of 224 pigs (BW = 41.5 ± 6.1 kg; PIC Genus 337 × 1050, Hendersonville, TN) were randomly assigned to either a high (3.55 Mcal/kg; HE) or low (3.29 Mcal/kg; LE) ME dietary treatment with a high (23:1) or low (12:1) LA:ALA in a 2 × 2 factorial arrangement. Diets were fed across three 28-d phases. Pigs were housed either four barrows or four gilts per pen. Blood samples were collected on days 0, 21, 42, and 84. Synovial fluid was collected from the hock and carpus joints on days 0 and 84. Liver and adipose tissue samples were collected on day 84. Data were analyzed as repeated measures using PROC MIXED (SAS 9.4) with pen as the experimental unit and energy level, essential fatty acid ratio, sex, phase, and their interactions as fixed effects. Compared to LE, HE increased days 28, 56, and 84 body weight (BW; $P = 0.005$). For the overall period, HE increased average daily gain (ADG) compared to LE ($P < 0.001$) and improved feed efficiency ($P = 0.001$), while LE increased feed intake compared to HE ($P < 0.001$). Gilts receiving diets with low LA:ALA had similar final BW to barrows receiving a low LA:ALA at days 28, 56, and 84 ($P = 0.024$), resulting from improved overall days 0–84 ADG compared to gilts receiving the high LA:ALA ($P = 0.031$). In the liver, HE decreased the mRNA abundance of acetyl CoA carboxylase (*ACACA*; $P = 0.004$), cluster of differentiation 36 ($P = 0.034$), and tended to decrease fatty acid synthase (*FASN;* $P = 0.056$). In adipose tissue, HE decreased *ACACA* ($P = 0.001$) and *FASN* ($P = 0.017$). Plasma inflammatory markers C-reactive protein (CRP) and tumor necrosis factor-α (TNFα) were reduced on day 84 compared to day 0 ($P \leq 0.014$). In the hock and carpus synovial fluid, LE tended to reduce CRP and TNFα ($P \leq 0.096$). Hock and carpus synovial fluid CRP were also reduced on day 84 compared to day 0 ($P = 0.001$). Age of the pig impacted serum and hock synovial fluid protein abundance, but not energy level, LA:ALA, or their interactions ($P < 0.05$). To conclude, the high and low LA:ALA ratios utilized in this study can be fed at varying energy levels without impacting growth. Additionally, LA:ALA ratios can differentially impact the growth of barrows and gilts.

Lay Summary

In pig diets, it has been established that added fat can improve growth and feed efficiency; however, insufficient research has been reported evaluating specific essential fatty acids found in commonly available fat sources. Essential fatty acids are important in several biological functions in the body, including growth, inflammation, and immune function. Given shared metabolism between essential fatty acids linoleic acid and α-linolenic acid, it has been suggested that their dietary ratio is critical to balance inflammatory responses. In the present research, a 12:1 dietary linoleic:linolenic acid ratio improved gilt, but not barrow, daily gain and did not impact inflammation. Pro-inflammatory responses were reduced over time, both in the blood and joint fluid. High-fat diets also improved growth performance, suppressed genes involved in fatty acid synthesis, and tended to increase joint inflammation. There was no interaction between dietary fat level and essential fatty acid ratio for any variable. Overall, dietary essential fatty acid ratios impact the growth of gilts, regardless of dietary fat inclusion, with no apparent effects on inflammation.

Key words: fatty acid ratio, grow-finish pigs, inflammation, joint, proteomics, synovial fluid

Abbreviations: ACACA, acetyl CoA carboxylase; ADG, average daily gain; ADFI, average daily feed intake; aEE, acid ether extract; ALA, α-linolenic acid; ARA, arachidonic acid; BW, body weight; CD36, cluster of differentiation 36/fatty acid translocase; cDNA, complementary DNA; CPT1A, carnitine palmitoyl transferase 1 A; CRP, C-reactive protein; Ct, cycle threshold; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; EDTA, ethylenediaminetetraacetic acid; EFA, essential fatty acids; EPA, eicosapentaenoic acid; FABP1, fatty acid binding protein 1; FABP4, fatty acid binding protein 4; FASN, fatty acid synthase; G:F, gain to feed ratio; GE, gross energy; GLA, γ-linolenic acid; HCD, higher energy collision dissociation; HSL, hormone sensitive lipase; IL-6, interleukin-6; LA, linoleic acid; LPL, lipoprotein lipase; ME, metabolizable energy; mRNA, messenger RNA; MS, mass spectrometer; N, nitrogen; PCR, polymerase chain reaction; PCV2, porcine circovirus Type 2; PPAR-α, peroxisome proliferator-activated receptor alpha; PPAR-γ, peroxisome proliferator-activated receptor gamma; PRRSv, porcine reproductive and respiratory syndrome virus; RPL-19, ribosomal protein-19; SID, standardized ileal digestible; STTD, standardized total tract digestible; TEAB, triethylammonium bicarbonate buffer; TMT, tandem mass tag; TNFα, tumor necrosis factor-α

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Introduction

Dietary fat inclusion provides the pig with a highly digestible energy source, as well as the essential fatty acids (EFA) necessary for many biological functions. Lipids have a low heat increment due to digestion and absorption compared to carbohydrates or proteins (Forbes and Swift, 1944; Stahley, 1984), making them valuable in diet formulation when external barn temperatures are high and feed intake is reduced. In recent years, EFA supplementation has been increasingly investigated in many food animal species. It is well established that dietary fat source directly impacts pork fat quality and fatty acid composition (Gatlin et al., 2002; Kellner et al., 2014). There is a growing interest in improving immune function and health with EFA supplementation, which has been reported in human and rodent studies (Yaqoob and Calder, 2007). Linoleic acid (LA) and α-linolenic acid (ALA) are two EFAs required in swine diets because they cannot be synthesized by the animal due to the lack of necessary enzymes. Linoleic acid (18:2n-6) is an 18-carbon fatty acid with two double bonds (Gurr et al., 2002). The first bond of LA is located on the sixth carbon from the methyl end, also known as the omega end. Alpha-linolenic acid (18:3n-3) is an 18-carbon fatty acid with three double bonds, the first being located on the third carbon from the omega end (Gurr et al., 2002). This nomenclature system has led to the use of the terms "omega-6" or "n-6" and "omega-3" or "n-3" fatty acids in literature; however, these can also refer to other long-chain fatty acids, such as γ-linolenic acid (GLA; 18:3n-6), dihomo-γ-linolenic acid (DGLA; 20:3n-6), arachidonic acid (ARA; 20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3), and docosahexaenoic acid (DHA; 22:6n-3).

The LA and ALA metabolic pathways utilize the same enzymes for elongation to ARA, EPA, DHA, and downstream metabolite production; thus, competition exists between the two pathways for the enzymes necessary to produce various compounds, including both pro- and anti-inflammatory proteins (Wolfe, 1982; Sprecher et al., 1995). Given this competition, it has been suggested that the ratio of LA:ALA is critically important for maintaining a balance of inflammatory metabolite production (Mariamenatu and Abdu, 2021). Typical corn and soybean meal-based swine diets have an LA:ALA ratio of approximately 25:1. This ratio can be even higher depending on the dietary lipid source utilized, resulting in LA being the primary substrate for the pathway. Substantial research exists evaluating how dietary fat source impacts growth, lipid metabolism, and inflammation in pigs across production phases (Liu, 2015, Kellner et al., 2017; Lauridsen, 2020; Wang et al., 2022). In swine production, lameness represents a significant cost during the grow-finish phases, as well as in gilt development, and ultimately in the breeding herd. Lameness leads to premature culling and has a significant impact on animal welfare in all production phases (Canning et al., 2019; Hallowell and Pierdon, 2022). Previous research has shown improvements in bone development and reduction of inflammation with dietary n-3 polyunsaturated fatty acid supplementation (Watkins et al., 2000; Duan et al., 2014). Research evaluating the n-6:n-3 ratio largely includes dietary supplementation with fish oil, a lipid source specifically rich in the n-3 fatty acids EPA and DHA. Insufficient research in growing pigs has been reported formulating diets to specific n-6:n-3 ratios using the parent n-6 and n-3 fatty acid compounds of LA and ALA. Dietary LA inclusion is currently recommended in gestating and lactating sow diets at 2.1 g/d and 6 g/d, respectively (NRC, 2012). Given that swine diets in the United States are often corn or wheat-based, the content of dietary LA is naturally high without supplementation of fat sources. Furthermore, these studies described above have been conducted using a wide range of dietary energy inclusions. Currently, there is no recommendation for dietary ALA in swine diets in the NRC (2012); however, Rosero et al. (2016) recommends 10 g/d in lactating sow diets. Therefore, the objective of this study was to investigate the effects of dietary LA:ALA and metabolizable energy (ME) level on the growth performance, lipid metabolism, circulatory and joint inflammation, and synovial fluid proteome in grow-finish pigs.

Materials and Methods

All experimental procedures adhered to guidelines for the ethical and humane use of animals for research according to the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010) and were approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC #19-354).

Animals, diets, and experimental design

A total of 224 growing pigs (41.5 ± 6.1 kg body weight (BW); PIC Genus 337 × 1,050, Hendersonville, TN) were individually weighed and allotted to single sex pens with four pigs per pen, for a total of 56 pens (n = 14). Pigs were housed at the Iowa State University Swine Nutrition Research Farm. Pens had partially slatted, concrete flooring with dimensions of 1.83 m × 1.93 m. Pigs originated from a porcine reproductive and respiratory syndrome virus (PRRSv) vaccinated herd. One pig per pen was vaccinated for *Lawsonia intercellularis* (Porcilis ILEITIS; Merck Animal Health, Madison, New Jersey), porcine circovirus Type 2 (PCV2), and *Mycoplasma hyopneumoniae* (Circumvent PCV-M G2; Merck Animal Health, Madison, New Jersey) according to the manufacturer's recommendations to replicate commercial gilt developer practices and stimulate an immune response. Pig health was monitored daily. All pigs were observed and evaluated daily in the pen for signs of lameness, including the inability to bear weight on all four legs, abnormal gait, or standing posture and physical evidence of trauma, such as swelling and malformation of the leg or joint.

Pens were randomly assigned to either a high (3.55 Mcal/kg; HE) or low (3.29 Mcal/kg; LE) metabolizable energy (ME) dietary treatment with a high (23:1) or low (12:1) LA:ALA in a 2 × 2 factorial arrangement. Dietary lipid sources included corn oil, flaxseed oil, choice white grease, and beef tallow. Diets were fed for three 28-d phases and were balanced for predicted average daily intake of linoleic acid within each dietary energy level. Diets were presented in mash form and primarily based on corn and soybean meal (Tables 1–3). A two-space galvanized steel feeder (width = 76 cm) with hinged lids and two nipple drinkers was used to provide ad libitum access to feed and water throughout the study. The diets were formulated to meet or exceed NRC (2012) nutrient recommendations for growing pigs and did not contain antibiotics or pharmacological levels of copper or zinc.

Sample collection

Pigs and feeders were individually weighed on days 0, 28, 56, and 84. Feed disappearance was recorded to calculate average daily gain (ADG), average daily feed intake (ADFI), and

Table 1. Ingredient and nutrient composition of the grow-finish experimental diets to evaluate dietary essential fatty acid ratios and energy level, phase 1 (as-fed basis)

Item	High energy		Low energy	
	High ratio	Low ratio	High ratio	Low ratio
Ingredient, %				
Corn	66.09	66.11	72.27	72.17
Soybean meal, 47%	23.62	23.62	23.62	23.62
Beef tallow	1.34	1.36	–	–
Choice white grease	5.15	5.00	–	–
Corn oil	–	–	0.66	0.63
Flaxseed oil	–	0.13	0.02	0.14
DL-methionine	0.15	0.15	0.09	0.09
L-lysine HCL	0.45	0.45	0.34	0.34
L-threonine	0.17	0.17	0.11	0.11
L-tryptophan	0.02	0.02	0.01	0.01
L-valine	0.08	0.05	–	0.00
Premix[1]	0.35	0.35	0.35	0.35
Limestone	0.96	0.96	0.97	0.97
Monocalcium phosphate	1.11	1.11	1.08	1.08
Salt	0.50	0.50	0.50	0.50
Calculated nutrient levels[2]				
ME, Mcal/kg	3.55	3.55	3.29	3.30
Ether extract, %	9.16	9.16	3.55	3.64
Linoleic acid, %	1.80	1.80	1.61	1.61
α-linolenic acid, %	0.08	0.15	0.07	0.13
Total P, %	0.58	0.58	0.59	0.59
STTD P, %	0.35	0.35	0.35	0.35
Calcium, %	0.70	0.70	0.70	0.70
SID Lysine, %	1.10	1.10	1.02	1.02
SID Met + Cys:Lys	0.57	0.57	0.57	0.57
SID Thr:Lys	0.62	0.62	0.62	0.62
SID Trp:Lys	0.18	0.18	0.18	0.18
SID Val:Lys	0.65	0.65	0.67	0.67
SID Ile:Lys	0.55	0.55	0.60	0.60
SID Lys/ME (g/kg)/Mcal	3.10	3.10	3.10	3.10
Analyzed nutrient levels[3]				
Dry matter, %	87.66	87.39	86.73	86.52
GE, Mcal/kg	4.54	4.54	4.13	4.24
Crude protein, %	15.57	14.70	15.20	15.46
aEE, %	10.49	10.51	4.18	4.17

[1]Provided 6,614 IU vitamin A, 827 IU vitamin D, 26 IU vitamin E, 2.6 mg vitamin K, 29.8 mg niacin, 16.5 mg pantothenic acid, 5.0 mg riboflavin, and 0.023 mg vitamin B12, 165 mg Zn (zinc sulfate), 165 mg Fe (iron sulfate), 39 mg Mn (manganese sulfate), 17 mg Cu (copper sulfate), 0.3 mg I (calcium iodate), and 0.3 mg Se (sodium selenite) per kg of diet.
[2]ME, metabolizable energy; STTD, standardized total tract digestible; SID, standardized ileal digestible.
[3]aEE, acid ether extract; GE, gross energy.

gain-to-feed ratio (G:F) for each phase. On days 0, 21, 42, and 84, blood samples were collected from three vaccinated barrows and three vaccinated gilts from each treatment via sterile, jugular venipuncture into an ethylenediaminetetraacetic acid (EDTA) treated vacutainer tube and a serum vacutainer tube (Becton Dickinson, Franklin Lakes, NJ). Serum and plasma samples were separated by centrifugation (2000 × *g* for 10 min at 4 °C), divided into two aliquots, and stored at -80 °C for future analyses.

Synovial fluid was collected according to Canning et al. (2018). Briefly, the same 24 sample pigs described above received an intramuscular injection of tiletamine hydrochloride and zolazepam hydrochloride (4.4 mg/kg), ketamine hydrochloride (2.2 mg/kg), and xylazine hydrochloride (4.4 mg/kg). Sedation was considered successful once a pig was anesthetized and had a negative palpebral response and toe withdrawal response prior to synovial fluid collection. Each pig was monitored closely throughout the collection procedure

Table 2. Ingredient and nutrient composition of grow-finish experimental diets to evaluate dietary essential fatty acid ratios and energy level, phase 2 (as-fed basis)

Item	High energy		Low Energy	
	High ratio	Low ratio	High ratio	Low ratio
Ingredient, %				
Corn	72.23	72.25	78.42	78.32
Soybean meal, 47%	17.51	17.51	17.51	17.51
Beef tallow	2.26	1.96	–	–
Choice white grease	4.14	4.34	–	–
Corn oil	0.06	–	0.55	0.52
Flaxseed oil	–	0.13	0.03	0.14
DL-methionine	0.11	0.11	0.05	0.06
L-lysine HCL	0.44	0.44	0.34	0.34
L-threonine	0.16	0.16	0.11	0.11
L-tryptophan	0.03	0.03	0.01	0.01
L-valine	0.05	0.05	–	–
Premix[1]	0.35	0.35	0.35	0.35
Limestone	0.97	0.97	0.98	0.98
Monocalcium phosphate	1.19	1.19	1.16	1.16
Salt	0.50	0.50	0.50	0.50
Calculated nutrient levels[2]				
ME, Mcal/kg	3.55	3.55	3.29	3.29
Ether extract, %	9.24	9.21	3.57	3.66
Linoleic acid, %	1.80	1.80	1.61	1.61
α-linolenic acid, %	0.08	0.15	0.07	0.13
Total P, %	0.57	0.57	0.58	0.58
STTD P, %	0.35	0.35	0.35	0.35
Calcium, %	0.70	0.70	0.70	0.70
SID Lysine, %	0.94	0.94	0.87	0.87
SID Met + Cys:Lys	0.57	0.57	0.57	0.57
SID Thr:Lys	0.63	0.63	0.63	0.63
SID Trp:Lys	0.18	0.18	0.18	0.18
SID Val:Lys	0.65	0.65	0.67	0.67
SID Ile:Lys	0.53	0.53	0.59	0.59
SID Lys/ME (g/kg)/Mcal	2.65	2.65	2.65	2.65
Analyzed nutrient levels[3]				
Dry matter, %	87.24	87.17	86.54	85.49
GE, Mcal/kg	4.39	4.53	4.19	4.19
Crude protein, %	13.05	12.91	13.43	13.50
aEE, %	10.99	11.02	4.55	4.52

[1]Provided 6,125 IU vitamin A, 700 IU vitamin D, 50 IU vitamin E, 3.0 mg vitamin K, 56.0 mg niacin, 27.0 mg pantothenic acid, 11.0 mg riboflavin, and 0.050 mg vitamin B12, 165 mg Zn (zinc sulfate), 165 mg Fe (iron sulfate), 39 mg Mn (manganese sulfate), 16.5 mg Cu (copper sulfate), 0.3 mg I (calcium iodate), and 0.3 mg Se (sodium selenite) per kg of diet.
[2]ME, metabolizable energy; STTD, standardized total tract digestible; SID, standardized ileal digestible.
[3]aEE, acid ether extract; GE: gross energy.

by recording heart rate, respiratory rate, rectal temperature, and depth of sedation every 10 min during sedation and at 30-min to 1-h intervals during recovery from anesthesia. Animals were monitored until they were able to stand unassisted.

Once anesthetized, each pig was positioned into dorsal recumbency. One tarsus and carpus of each pig were randomly selected, shaved, and further prepared with a chlorhexidine soap scrub (VetOne, Boise, ID), a 70% alcohol scrub, and final application of a tincture of 2% chlorhexidine. The chlorhexidine soap and alcohol scrubs were repeated once to ensure asepsis of the centesis sites. A sterile 18-gauge, 1.5-inch needle attached to a 12-mL sterile syringe was inserted into the dorsolateral aspect of the carpus or tarsus, and negative pressure was used to aspirate synovial fluid into the syringe.

On day 84, the 24 sample pigs were euthanized by captive bolt stunning followed by exsanguination. A 5 cm^2 section of subcutaneous adipose tissue from the tenth rib consisting of the outer, middle, and inner layers of subcutaneous adipose and a 5 cm^2 section of liver from the right lateral lobe

Table 3. Ingredient and nutrient composition of grow-finish experimental diets to evaluate dietary essential fatty acid ratios and energy level, phase 3 (as-fed basis)

Item	High energy		Low energy	
	High ratio	Low ratio	High ratio	Low ratio
Ingredient, %				
Corn	77.01	77.04	83.20	83.10
Soybean meal, 47%	12.75	12.75	12.75	12.75
Beef tallow	3.00	2.41	–	–
Choice white grease	3.30	3.83	–	––
Corn oil	0.12	–	0.46	0.44
Flaxseed oil	–	0.14	0.03	0.15
DL-methionine	0.08	0.08	0.03	0.03
L-lysine HCL	0.43	0.43	0.34	0.34
L-threonine	0.15	0.15	0.10	0.10
L-tryptophan	0.03	0.03	0.02	0.02
L-valine	0.05	0.05	0.00	0.00
Premix[1]	0.35	0.35	0.35	0.35
Limestone	0.98	0.98	0.99	0.99
Monocalcium phosphate	1.26	1.26	1.23	1.23
Salt	0.50	0.50	0.50	0.50
Calculated nutrient levels[2]				
ME, Mcal/kg	3.55	3.55	3.29	3.29
Ether extract, %	9.29	9.25	3.58	3.67
Linoleic acid, %	1.80	1.80	1.61	1.61
α-linolenic acid, %	0.08	0.15	0.07	0.13
Total P, %	0.56	0.56	0.57	0.57
STTD P, %	0.35	0.35	0.35	0.35
Calcium, %	0.70	0.70	0.70	0.70
SID Lysine, %	0.82	0.82	0.76	0.76
SID Met + Cys:Lys	0.57	0.57	0.57	0.57
SID Thr:Lys	0.63	0.63	0.63	0.63
SID Trp:Lys	0.18	0.18	0.18	0.18
SID Val:Lys	0.65	0.65	0.67	0.67
SID Ile:Lys	0.51	0.51	0.57	0.57
SID Lys/ME (g/kg)/Mcal	2.30	2.30	2.30	2.30
Analyzed nutrient levels[3]				
Dry matter, %	86.97	87.52	86.73	86.65
GE, Mcal/kg	4.55	4.54	4.21	4.21
Crude protein, %	11.37	11.93	11.53	11.26
aEE, %	10.15	9.28	4.76	4.74

[1]Provided 6,125 IU vitamin A, 700 IU vitamin D, 50 IU vitamin E, 3.0 mg vitamin K, 56.0 mg niacin, 27.0 mg pantothenic acid, 11.0 mg riboflavin, and 0.05 mg vitamin B12, 165 mg Zn (zinc sulfate), 165 mg Fe (iron sulfate), 39 mg Mn (manganese sulfate), 17 mg Cu (copper sulfate), 0.3 mg I (calcium iodate), and 0.3 mg Se (sodium selenite) per kg of diet.
[2]ME, metabolizable energy; STTD, standardized total tract digestible; SID, standardized ileal digestible.
[3]aEE, acid ether extract; GE: gross energy.

were removed, snap frozen in liquid N, and stored at −80 °C for subsequent analysis. One carpus and one hock joint were opened for collection of synovial fluid. Synovial fluid samples were stored at −80 °C until further analysis.

Chemical analysis

Diets were ground to 1 mm particle size with a Wiley Mill (Variable Speed Digital ED-5 Wiley Mill; Thomas Scientific, Swedesboro, NJ) and analyzed in duplicate for dry matter [method 930.15 (AOAC, 2007)], acid-hydrolyzed ether extract [aEE; method 2003.06; (AOAC, 2007)], and nitrogen [N, method 990.03 (AOAC, 2007); TruMac; LECO Corp., St. Joseph, MI]. An EDTA sample (9.56% N) was used as the standard for calibration and was determined to contain 9.55 ± 0.01% N. Crude protein was calculated as N × 6.25. Gross energy (GE) was determined in duplicate using an isoperibolic bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL). Benzoic acid (6,318 kcal GE/kg) was used as the standard for calibration and was determined to contain 6,319 ± 0.8 kcal GE/kg.

Total lipids in the dietary lipid sources were extracted by using a chloroform and methanol mixture (Folch et al., 1957). The lipids were methylated directly with acetyl chloride and methanol (Christie, 1972). Fatty acid methyl esters were quantified by a gas chromatograph (Varian 3800, Agilent Technologies, Palo Alto, CA) equipped with a Supelco Sp-2380 column and a flame ionization detector. Gas chromatograph conditions were as follows: initial column temp, 70 °C with a hold time of 4 min, temperature ramp was 13 °C per minute with a final column temperature of 215 °C. Peaks were identified by using commercially available fatty acid methyl ester standards (Nu-Chek-Prep Inc., Elysian, MN).

RNA isolation and quantitative PCR

Approximately 30 mg of liver tissue were homogenized using the QIAGEN Tissuelyser II (Germantown, MD, USA). Total RNA was isolated using the QIAGEN RNeasy Mini Kit (QIAGEN Group; Germantown, MD, USA) according to the manufacturer's recommendations. Approximately 100 mg of adipose tissue were homogenized using the Qiagen Tissuelyser II (QIAGEN Group; Germantown, MD, USA). Total RNA was isolated using the QIAGEN RNeasy Lipid Tissue Mini Kit (QIAGEN Group; Germantown, MD, USA) according to the manufacturer's recommendations. The concentration of RNA was quantified using a spectrophotometer (BioTek Cytation 5; Agilent Technologies, Santa Clara, CA). All samples had 260:280 nm ratios above 1.8. The QuantiTect Reverse Transcription Kit (QIAGEN Group; Germantown, MD, USA) was used according to the manufacturer's instructions to synthesize complementary DNA (cDNA) from 0.8 µg of the isolated liver RNA and 0.5 µg of the isolated adipose RNA. All cDNA samples were diluted 10-fold with nuclease-free water.

Real-time quantitative polymerase chain reaction (PCR) was performed using iQ SYBR Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA). The gene-specific primers for lipid metabolism, shown in Table 4, were diluted to 10 µM with nuclease-free water. *Ribosomal protein—L19 (RPL19)* was included as an endogenous reference gene. Each reaction included 10 µL of SYBR Green Supermix, 1 µL of each forward and reverse primer (Integrated DNA Technologies; Coralville, IA), 5 µL of nuclease-free water (Life Technologies; Austin, TX), and 3 µL of cDNA, for a total of 20 µL reaction volume. Each 96-well plate contained a no-reverse transcriptase negative control and a pooled cDNA reference sample. Samples were assayed in duplicate. Fluorescence of SYBR Green was quantified with a Real-time PCR Detection System (iQ5; Bio-Rad Laboratories Inc., Hercules, CA). Cycling conditions were as follows: 5-min initial denaturation at 95 °C followed by 40 PCR cycles (95 °C for 30 s, variable temperature for 30 s, and 72 °C for 30 s; Table 4) and a dissociation curve to verify the amplification of a single PCR product. Optical detection was performed at the appropriate temperature for each gene. Analyses of amplification plots were performed with an Optical System Software version 2.0 (iQ5; Bio-Rad Laboratories Inc., Hercules, CA), and cycle threshold (Ct) values for each reaction were obtained. The messenger RNA (mRNA)

Table 4. Primer sequences used for quantitative PCR to assess fatty acid metabolism

Gene[1]	Primer sequence (5′–3′)[2]	Annealing temperature, °C	Reference
ACACA	F: ATGGATGAACCGTCTCCC	51	Kellner et al. (2017)
	R: TGTAAGGCCAAGCCATCC		
CD36	F: CTGGTGCTGTCATTGGAGCAGT	55	Li et al. (2017)
	R: CTGTCTGTAAACTTCCGTGCCTGTT		
CPT1A	F: GCATTTGTCCCATCTTTCGT	55	Varady et al. (2012)
	R: GCACTGGTCCTTCTGGGATA		
FABP-1	F: ACATCAAGGGGACATCGG	55	Kellner et al. (2017)
	R: GTCTCCATCTCACACTCC		
FABP-4	F: CAGGAAAGTCAAGAGCACCA	55	Zhao et al. (2010)
	R: TCGGGACAATACATCCAACA		
FASN	F: CACAACTCCAAAGACACG	50	Kellner et al., (2017)
	R: AGGAACTCGGACATAGCG		
HSL	F: AACGCAATGAAACAGGCC	50	Kellner et al. (2017)
	R: TGTATGATCCGCTCAACTCG		
LPL	F: CCCTATACAAGAGGGAACCGGAT	55	Li et al. (2017)
	R: CCGCCATCCAGTCGATAAACGT		
PPAR-ϫ	F: GTGGAGACCGCCCAGGTTTG	55	Li et al. (2017)
	R: GGGAGGACTCTGGGTGGTTCA		
PPAR-α	F: AACGGCATCCAGAACAAG	50	Kellner et al. (2017)
	R: CATCACAGAGGACAGCATGG		
RPL-19	F: AACTCCCGTCAGCAGATCC	55	Li et al. (2019)
	R: GTACCCTTCCGCTTACCG		

[1]ACACA, acetyl CoA carboxylase; *CD36*, cluster of differentiation 36/fatty acid translocase; *CPT1A*, carnitine palmitoyl transferase 1 A; *FABP1*, fatty acid binding protein 1; *FASN*, fatty acid synthase; *HSL*, hormone sensitive lipase; *LPL*, lipoprotein lipase; *PPAR-α*, peroxisome proliferator-activated receptor alpha; *PPAR-ϫ*, peroxisome proliferator-activated receptor gamma; *RPL-19*, ribosomal protein-19.
[2]Direction of primer (F, forward; R, reverse).

abundance for each sample was normalized to *RPL19* and the pooled sample, and fold change was calculated using the $2^{-\Delta\Delta CT}$method (Livak and Schmittgen, 2001).

Inflammatory markers

Plasma and synovial fluid C-reactive protein (CRP), tumor necrosis factor-α (TNFα), and interleukin-6 (IL-6) concentrations were quantified using porcine-specific enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (R&D Systems; Minneapolis, MN). Plasma and synovial fluid TNFα and IL-6 were analyzed undiluted. Plasma and synovial fluid samples for CRP analysis were diluted 1:20,000 and 1:500, respectively with provided assay buffer. Plates were read at a wavelength of 450 nm (BioTek Cytation 5; Agilent Technologies, Santa Clara, CA), and a coefficient of variation of under 8% between duplicates was deemed acceptable.

Protein concentration and normalization

Total protein concentration for days 0 and 84 serum and hock synovial fluid samples (serum diluted 1:100, synovial fluid diluted 1:10 in ultrapure water) was determined for normalization of sample material using the Pierce bicinchoninic acid (BCA) Protein Assay kit (Thermo Fischer Scientific, Waltham, MA) according to the manufacturer's instructions using bovine serum albumin as the standard. Serum and synovial fluid samples were normalized to 3 mg of protein using 50 mM Tris–HCL buffer (pH 8.0) prior to sample submission.

Protein digestion and tandem mass tag labeling

Samples were submitted to the Iowa State University Protein Facility (Ames, IA), where a pooled control sample was prepared to contain equal amounts of all samples from either serum or synovial fluid. All samples were reduced with dithiothreitol, modified (Cys) with iodoacetamide, and digested with Trypsin/Lys-C (Promega Corporation, Madison, WI) at 37 °C overnight (enzyme:protein = 1:25) with 50 mM Tris–HCl (pH 8.0) as the digestion buffer. Digestion was terminated by adding formic acid before samples were dried using vacuum (SpeedVac; Thermo Fischer Scientific, Waltham, MA). The samples were reconstituted with 5% acetonitrile (ACN), water, and 0.1% trifluoroacetic acid (TFA) and desalted using BioPureSPN Mini columns (The Nest Group, Inc., Southborough, MA). Vacuum centrifugation was used to dry the sample (SpeedVac; Thermo Fischer Scientific, Waltham, MA). Desalted and dried samples were reconstituted with 100 mM triethylammonium bicarbonate buffer (TEAB). Four microliters (1 µg of protein total; 0.25 µg/µl) of sample was added to water to reach a final volume of 20 µl. Protein concentration was determined using the Pierce BCA colorimetric protein assay kit (Thermo Fischer Scientific, Waltham, MA) before tandem mass tag (TMT) labeling.

A total of 25 µg of protein digest for each sample were incubated for 1 h with 10-plex TMT reagents according to the instructions of the TMT 11-plex Isobaric label reagent set kit (Thermo Fischer Scientific, Waltham, MA), with the pooled control labeled with TMT11 (131C). Samples were then quenched with 5% hydroxylamine for 15 min at room temperature. Equal amounts of each sample from the individual TMT label and the control were pooled into a single tube and dried in a SpeedVac (Thermo Fischer Scientific, Waltham, MA). Samples were reconstituted with 5% ACN/water/0.1% formic acid.

LC–MS/MS measurement and proteomics data analysis

Peptides were separated by liquid chromatography (EASY nLC-1200 coupled to a Nanospray FlexIon source; Thermo Fisher Scientific, Waltham, MA) through a pulled glass emitter 75 µm × 20 cm (Agilent, Santa Clara, CA). The emitter tip and column were packed with 5 µm SB-C18 (Aligent, Santa Clara, CA) and UChrom C18 3 µm material (nanoLCMS Solutions, Oroville, CA), respectively. A NanoSpray FlexIon source (Thermo Fisher Scientific, Waltham, MA) coupled to a Thermo Scientific Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer (MS) with a higher energy collision dissociation (HCD) fragmentation cell was used to perform MS/MS. The resulting intact mass- and MS/MS fragmentation pattern was compared to MASCOT (Perkins et al., 1999) and Sequest HT (Tabb et al., 2001) theoretical fragmentation patterns to detect peptides for protein identification using Thermo Scientific's Proteome Discoverer 2.4 software. The MASCOT and Sequest HT search was run against Uniprot *Sus scrofa*.

Statistical analysis

Growth performance data by phase were analyzed as repeated measures using the MIXED procedure of SAS 9.4 (SAS Inst., Cary, NC). Pen was considered the experimental unit, with energy level (2), LA:ALA (2), sex (2), phase (3), and their interactions included as fixed effects in the statistical model. The first-order autoregression covariance structure was selected for the growth performance repeated measures model according to Bayesian information criterion. The spatial power covariance structure was selected for the inflammation parameters repeated measures model according to Bayesian information criterion. Normality and homoscedasticity of the studentized residuals were tested using the UNIVARIATE procedure. Statistical outliers were identified as occurring greater than three standard deviations from the mean and were excluded from the analysis. Outlier removals across all variables and timepoints were as follows: Liver *CD36* [LE × L (1)], liver *CPT1A* [HE × H (2)], liver *FABP1* [LE × L (2); LE × H (1)], liver *PPARα* [HE × L (1); HE × H (1)], adipose PCR all genes [HE × L (1); HE × H (1)], plasma TNF*α* [LE × L (1); HE × L (1)], hock synovial fluid TNF*α* [HE × L (1); HE × H (1)], hock synovial fluid CRP [LE × L (2); LE × H (1); HE × L (1)], gain:feed [LE × L (1); HE × L (1); HE × H (2)], and body weight [LE × L (2)]. Data were reported as least squares means and means separation was done using the PDIFF option. Differences were considered significant if P was ≤ 0.05 and a tendency if P was > 0.05 and ≤ 0.10.

Proteomics data were analyzed using R v.4.2.0 (R Core Team, 2022). The lmFit function of the limma package (v.3.52.1; Ritchie et al., 2015) was used to fit a multilevel linear model on the $\log_2$ transformed normalized protein abundances with pen as a random effect. Coefficient and standard error estimates of pre-planned contrasts were computed using the contrasts.fit and eBayes functions to test the main effects of energy, ratio, and day, and their respective two and three-factor interactions. The $\log_2$ fold changes of significantly different proteins were extracted using the topTable function. P-values were adjusted to control the false discovery rate using the Benjamini and Hochberg method and the resulting P-values were considered significant if $P \leq 0.05$. All plots were constructed using ggplot2 (v.3.3.6) in R 4.2.0 (Wickham, 2016).

Table 5. Fatty acid composition of complete diets[1,2]

Analyzed fatty acids	High energy		Low Energy	
	High ratio	Low eatio	High ratio	Low ratio
Phase 1				
C14:0, %	0.11	0.10	0.00	0.00
C16:0, %	1.90	1.88	0.45	0.45
C16:1, %	0.15	0.14	0.00	0.00
C18:0, %	1.01	1.00	0.06	0.06
C18:1, %	3.15	3.13	1.01	1.02
C18:2, %	1.97	1.97	1.75	1.75
C18:3, %	0.07	0.14	0.06	0.12
LA:ALA	28:1	14:1	29:1	14:1
UFA:SFA	1.8:1	1.8:1	5.5:1	5.6:1
Phase 2				
C14:0, %	0.12	0.11	0.00	0.00
C16:0, %	1.86	1.84	0.41	0.41
C16:1, %	0.14	0.14	0.00	0.00
C18:0, %	1.08	1.04	0.06	0.06
C18:1, %	3.05	3.04	0.92	0.93
C18:2, %	1.80	1.84	1.63	1.63
C18:3, %	0.07	0.14	0.07	0.13
LA:ALA	26:1	13:1	23:1	13:1
UFA:SFA	1.7:1	1.7:1	5.5:1	5.6:1
Phase 3				
C14:0, %	0.13	0.12	0.00	0.00
C16:0, %	1.85	1.84	0.42	0.42
C16:1, %	0.14	0.14	0.00	0.00
C18:0, %	1.13	1.08	0.06	0.06
C18:1, %	3.03	3.04	0.93	0.94
C18:2, %	1.76	1.84	1.62	1.63
C18:3, %	0.07	0.15	0.07	0.13
LA:ALA	25:1	12:1	23:1	12:1
UFA:SFA	1.6:1	1.7:1	5.5:1	5.6:1

[1]Dietary fatty acid ratio as dietary linoleic acid:linolenic acid (LA:ALA): 23:1 and 12:1
[2]SFA, saturated fatty acids; USFA, unsaturated fatty acids.

Results

Mortality for the overall trial period was 1.3%. Pigs did not display any clinical signs of disease or lameness and remained healthy throughout the entire experimental period. Dietary analysis resulted in gross energy and acid hydrolyzed ether extract similar to calculated formulation. (Tables 1–3). Analyzed dietary fatty acid composition was similar to calculated fatty acids from dietary formulation (Table 5).

Growth performance

Initial body weight was not different across dietary treatments (BW = 41.5 ± 6.1 kg; $P \geq 0.318$). Pigs receiving the higher energy diets had increased days 28, 56, and 84 BW ($P = 0.005$; Table 6) compared to low-energy diets. Feed intake was also reduced in the pigs fed the high-energy diets in each of the three phases ($P = 0.014$). While ADG and feed efficiency were not different within each phase ($P \geq 0.522$), pigs receiving the high-energy diets gained more ($P = 0.001$) and had improved feed efficiency ($P < 0.001$) for the overall days 0–84 period (Table 7) compared to pigs receiving the low-energy diets. There was no effect of EFA ratio or energy × ratio interaction on growth performance or feed efficiency within each phase ($P \geq 0.185$) or for the overall period ($P \geq 0.296$).

There was a main effect of sex within each dietary phase and for the overall period ($P < 0.001$; Tables 8 and 9), as well as a phase × sex interaction ($P < 0.001$). Generally, barrows had increased BW, ADG, ADFI, and reduced feed efficiency compared to gilts. There was a significant phase × sex × ratio interaction for BW ($P = 0.024$). Gilts receiving diets formulated with a 12:1 EFA ratio had similar body weights compared to barrows receiving a 12:1 EFA ratio at days 28, 56, and 84. These gilts also had improved overall days 0–84 average daily gain compared to gilts receiving the 23:1 EFA ratio diets ($P = 0.031$; Table 9). High energy diets improved feed efficiency for the overall period in both barrows and gilts ($P = 0.049$). Gilts receiving the high-energy diets had improved feed efficiency compared to barrows receiving high energy diets; however, the feed efficiency of pigs receiving the low-energy diets was not different between barrows and gilts.

Table 6. The effects of energy level and essential fatty acid ratio by phase on growth performance and feed efficiency of grow-finish pigs by weigh period[1]

	Energy[2]		Fatty acid ratio[3]		SEM	*P*-value[4]			
Item[5]	High	Low	High	Low		Phase	Energy × phase	Ratio × phase	Energy × ratio × phase
BW, kg					1.342	<0.001	0.005	0.721	0.269
Day 0	42.18[g]	40.78[g]	41.32	41.65					
Day 28	73.01[e]	69.89[f]	71.19	72.39					
Day 56	105.00[c]	100.68[d]	102.28	103.40					
Day 84	135.27[a]	129.97[b]	131.87	133.37					
ADG, kg					0.020	<0.001	0.547	0.580	0.067
Day 0–28	1.10	1.04	1.07	1.08					
Day 28–56	1.13	1.10	1.12	1.12					
Day 56–84	1.08	1.04	1.04	1.07					
ADFI, kg					0.059	<0.001	0.014	0.185	0.097
Day 0–28	2.22[e]	2.29[e]	2.26	2.26					
Day 28–56	2.82[d]	2.99[c]	2.90	2.91					
Day 56–84	3.14[b]	3.38[a]	3.21	3.30					
G:F					0.010	<0.001	0.522	0.216	0.494
Day 0–28	0.49	0.46	0.47	0.48					
Day 28–56	0.40	0.37	0.39	0.39					
Day 56–84	0.35	0.31	0.33	0.33					

[1]Data are least square means; *n* = 14 pens per treatment with four pigs per pen, totaling 224 pigs; growth calculations included pig days to account for morbidity and mortality.
[2]Calculated energy: high = 3,550 kcal/kg as ME; low = 3,290 kcal/kg as ME.
[3]Calculated fatty acid ratio as linoleic acid:linolenic acid: high = 23:1; low = 12:1.
[4]Within a dependent variable, means without a common superscript differ significantly ($P < 0.05$).
[5]ADG, average daily gain; ADFI, average daily feed intake; BW, body weight; G:F, gain:feed ratio.

Table 7. The effects of energy level and essential fatty acid ratio on overall growth performance and feed efficiency of grow-finish pigs[1]

	Energy[2]		Fatty acid ratio[3]		SEM	*P*-value[4]		
Item[5]	High	Low	High	Low		Energy	Ratio	Energy ×ratio
ADG, kg	1.10[a]	1.06[b]	1.08	1.09	0.009	0.001	0.314	0.296
ADFI, kg	2.72[b]	2.88[a]	2.79	2.82	0.030	0.001	0.448	0.663
G:F	0.41[a]	0.37[b]	0.39	0.39	0.004	<0.001	0.304	0.530

[1]Data are least square means; *n* = 14 pens per treatment with four pigs per pen, totaling 224 pigs; growth calculations included pig days to account for morbidity and mortality.
[2]Calculated energy: high = 3,550 kcal/kg as ME; low = 3,290 kcal/kg as ME.
[3]Calculated fatty acid ratio as linoleic acid:linolenic acid: high = 23:1; low = 12:1.
[4]Within a dependent variable, means without a common superscript differ significantly ($P < 0.05$).
[5]ADG, average daily gain; ADFI, average daily feed intake; BW, body weight; G:F, gain:feed ratio.

Liver and adipose gene transcription

In the liver, there was no effect of dietary EFA ratio on gene mRNA abundance ($P \geq 0.221$). High energy diets decreased the mRNA abundance of *ACACA* ($P = 0.004$; Table 10), *CD36* ($P = 0.034$), and tended to decrease *FASN* ($P = 0.056$). In adipose tissue, high-energy diets decreased gene expression of *ACACA* ($P = 0.001$; Table 11) and *FASN* ($P = 0.017$). A high EFA ratio tended to increase the expression of *ACACA* ($P = 0.069$), *CD36* ($P = 0.072$), and lipoprotein lipase (*LPL*; $P = 0.072$).

Plasma and synovial fluid cytokines

Energy level, EFA ratio, or their interaction did not impact plasma CRP, TNFα, and IL-6 ($P \geq 0.123$; Table 12). There was an effect of day for CRP and TNFα, where both cytokines were reduced on day 84 compared to day 0 ($P \leq 0.014$). In the hock and carpus synovial fluid, low-energy diets tended to reduce CRP ($P \leq 0.095$) and TNFα ($P \leq 0.096$; Table 13) in the hock and carpus synovial fluid; however, no impact of dietary LA:ALA was observed ($P \geq 0.559$). C-reactive protein was also reduced on day 84 compared to day 0 in both the hock and carpus synovial fluid ($P = 0.001$; Table 13). There was no effect of energy level, LA:ALA, day, or their interactions for IL-6 in either the hock or carpus synovial fluid ($P \geq 0.216$).

Proteomics

Given the similarities in carpus and hock synovial fluid inflammatory markers, only the hock was selected for proteomic

Table 8. The effects of energy level, essential fatty acid ratio, and sex on overall growth performance and feed efficiency of grow-finish pigs[1]

Item[5,6]	Energy[2]				Fatty acid ratio[3]				SEM	*P*-value[4]			
	High		Low		High		Low			Sex	Phase ×sex × energy	Phase ×sex × ratio
	M	F	M	F	M	F	M	F					
BW, kg									1.990	0.005	0.885	0.024
Day 0	42.49	41.86	40.78	40.79	41.90^{i}	40.73^{i}	41.37^{i}	41.92^{i}				
Day 28	74.46	71.56	71.44	69.71	73.27^{g}	69.12^{h}	72.63^{gh}	70.79^{gh}				
Day 56	107.70	102.31	103.66	97.70	106.25^{d}	98.32^{f}	105.11^{de}	101.69^{ef}				
Day 84	138.05	132.48	133.53	126.67	136.87^{a}	126.87^{c}	134.45^{ab}	132.29^{b}				
ADG, kg									0.030	< 0.001	0.868	0.603
Day 0–28	1.14	1.06	1.10	0.99	1.12	1.01	1.12	1.04				
Day 28–56	1.19	1.08	1.16	1.04	1.19	1.04	1.16	1.07				
Day 56–84	1.08	1.08	1.04	1.03	1.07	1.02	1.06	1.09				
ADFI, kg									0.081	<0.001	0.714	0.554
Day 0–28	2.39	2.06	2.43	2.16	2.42	2.09	2.40	2.12				
Day 28–56	3.02	2.62	3.18	2.80	3.13	2.67	3.07	2.75				
Day 56–84	3.26	3.01	3.52	3.23	3.38	3.04	3.40	3.20				
G:F									0.013	<0.001	0.283	0.788
Day 0–28	0.47	0.51	0.45	0.46	0.46	0.48	0.47	0.48				
Day 28–56	0.39	0.41	0.37	0.37	0.38	0.39	0.38	0.39				
Day 56–84	0.33	0.36	0.30	0.32	0.32	0.34	0.32	0.34				

[1]Data are least square means; *n* = 14 pens per treatment with four pigs per pen, totaling 224 pigs; growth calculations included pig days to account for morbidity and mortality.
[2]Calculated energy: high = 3,550 kcal/kg as ME; low = 3,290 kcal/kg as ME.
[3]Calculated fatty acid ratio as linoleic acid:linolenic acid: high = 23:1; low = 12:1
[4]Within a dependent variable, means without a common superscript differ significantly ($P < 0.05$).
[5]ADG, average daily gain; ADFI, average daily feed intake; BW, body weight; F, female; G:F, gain:feed ratio; M, male.
[6]For all variables, $P < 0.01$ for the phase × sex interaction. There were no differences across variables for the interaction of phase × sex × energy × ratio ($P > 0.10$).

Table 9. The effects of energy level, essential fatty acid ratio, and sex on overall growth performance and feed efficiency of grow-finish pigs[1]

Item[5]	Energy[2]				Fatty acid ratio[3]				SEM	*P*-value[4]			
	High		Low		High		Low			Sex	Sex × energy	Sex ×ratio	Sex × energy ×ratio
	M	F	M	F	M	F	M	F					
ADG, kg	1.14	1.07	1.10	1.02	1.13^{a}	1.03^{c}	1.11^{a}	1.07^{b}	0.018	<0.001	0.569	0.032	0.643
ADFI, kg	2.89	2.56	3.04	2.73	2.97	2.60	2.96	2.68	0.061	<0.001	0.891	0.253	0.993
G:F	0.39^{b}	0.42^{a}	0.36^{c}	0.37^{c}	0.38	0.40	0.38	0.40	0.006	<0.001	0.049	0.885	0.566

[1]Data are least square means; *n* = 14 pens per treatment with four pigs per pen, totaling 224 pigs; growth calculations included pig days to account for morbidity and mortality.
[2]Calculated energy: high = 3,550 kcal/kg as ME; low = 3,290 kcal/kg as ME.
[3]Calculated fatty acid ratio as linoleic acid:linolenic acid: high = 23:1; low = 12:1.
[4]Within a dependent variable, means without a common superscript differ significantly ($P < 0.05$).
[5]ADG, average daily gain; ADFI, average daily feed intake; BW, body weight; F, female; G:F, gain:feed ratio; M, male.

evaluation. There were no significantly different proteins in the serum or hock synovial fluid for the effects of energy, ratio, or their interactions; however, there was an effect of time for 41 proteins in the serum ($P < 0.05$; Figure 1). and 31 proteins in the hock synovial fluid ($P < 0.05$; Figure 2). In the serum, expression of 27 proteins was increased and 14 proteins was decreased on day 84 compared today 0. In the hock synovial fluid, 15 proteins had increased abundance on day 84 vs day 0 whereas 16 proteins had decreased abundance.

Discussion

Supplemental fats and oils represent one of the most expensive ingredients in swine diets. Dietary lipid sources can be highly variable in their fatty acid composition, especially LA and ALA content (NRC, 2012). Limited research has investigated the impact of LA:ALA from commonly available, plant-based lipid sources in growing pigs. The presented study evaluated the effects of dietary LA:ALA and metabolizable energy level based on corn oil, flaxseed oil, choice white grease, and beef

Table 10. Effect of energy level and essential fatty acid ratio on liver gene mRNA abundance of grow-finish pigs

Gene[1]	Energy[2]		Fatty acid ratio[3]		SEM	*P*—values[4]		
	High	Low	High	Low		Energy	Ratio	Energy × ratio
ACACA	0.588[b]	1.505[a]	0.869	1.223	0.198	0.004	0.221	0.053
CD36	0.327[b]	0.556[a]	0.383	0.500	0.071	0.034	0.256	0.889
CPT1A	0.753	0.675	0.729	0.698	0.478	0.817	0.927	0.164
FABP1	0.572	0.838	0.618	0.775	0.257	0.266	0.505	0.102
FASN	0.177	0.404	0.237	0.302	0.160	0.056	0.557	0.110
PPARα	0.671	0.946	0.730	0.870	0.297	0.293	0.585	0.144

[1]*ACACA*, acetyl co-A carboxylase; *CD36*, cluster of differentiation 36; *CPT1A*, carnitine palmitoyltransferase 1A; *FABP1*, fatty acid binding protein 1; *FASN*, fatty acid synthase; *PPARα*, peroxisome proliferator-activated receptor alpha.
[2]Calculated energy: high = 3,550 kcal/kg as ME; low = 3,290 kcal/kg as ME.
[3]Calculated fatty acid ratio as linoleic acid:linolenic acid: high = 23:1; low = 12:1.
[4]Within a dependent variable, means without a common superscript differ significantly ($P < 0.05$).

Table 11. Effect of energy level and essential fatty acid ratio on adipose gene mRNA abundance of grow-finish pigs

Gene[1]	Energy[2]		Fatty acid ratio[3]		SEM	*P*-values[4]		
	High	Low	High	Low		Energy	Ratio	Energy × ratio
ACACA	0.729[b]	1.409[a]	1.190	0.864	0.256	0.001	0.069	0.147
CD36	1.348	1.608	1.853	1.170	0.253	0.474	0.072	0.062
FABP4	1.077	1.229	1.212	1.092	0.273	0.619	0.700	0.096
FASN	1.048[b]	1.641[a]	1.504	1.144	0.285	0.017	0.126	0.618
HSL	1.151	1.182	1.289	1.056	0.435	0.926	0.490	0.766
LPL	0.902	1.376	1.415	0.877	0.262	0.109	0.072	0.166
PPARϒ	1.776	1.964	2.080	1.677	0.264	0.696	0.403	0.121
PPARα	1.317	1.685	1.543	1.438	0.190	0.192	0.697	0.679

[1]*ACACA*, acetyl co-A carboxylase; *CD36*, cluster of differentiation 36; *FABP4*, fatty acid binding protein 4; *FASN*, fatty acid synthase; *HSL*, hormone sensitive lipase; *LPL*, lipoprotein lipase; *PPARϒ*, peroxisome proliferator-activated receptor gamma; *PPARα*, peroxisome proliferator-activated receptor alpha.
[2]Calculated energy: high = 3,550 kcal/kg as ME; low = 3,290 kcal/kg as ME.
[3]Calculated fatty acid ratio as linoleic acid:linolenic acid: high = 23:1; low = 12:1.
[4]Within a dependent variable, means without a common superscript differ significantly ($P < 0.05$).

tallow on the growth performance, lipid metabolism, and inflammation in grow-finish pigs. The 25:1 dietary LA:ALA ratios was selected in this study to replicate typical commercial diets. It is recognized in human and pet literature that a 4:1 LA:ALA ratio is suggested to improve health (Calder, 2008); however, due to limitations in diet formulation with keeping dietary ME low, a 12:1 dietary LA:ALA was selected. This study was conducted during the months of February through April in the Midwest when the ambient temperatures were cool. Choice white grease and beef tallow were sourced directly from harvesting facilities and fully refined, feed grad corn and flaxseed oils were sourced specifically for use in this trial (Double S Liquid Feed Services, Inc., Danville, IL). No antioxidants were used. All dietary fat sources were stored in sealed, opaque containers in a cool indoor environment and away from sunlight for the duration of the study. Vaccinations were implemented in the study to a subset of pigs to mimic additional immune activation as typically experienced by developing replacement gilts during the grow-finish period to further increase the inflammatory response of the immune system.

As expected, increasing dietary ME by 260 kcal/kg of diet increased final BW, overall ADG, and feed efficiency. Improved overall BW gain and feed efficiency that resulted from increased dietary energy agree with previous research in growing pigs (De le Llata et al., 2001; Hinson et al., 2011; Liu et al., 2018). It has been reported that growing pigs can adjust voluntary feed consumption in response to dietary energy density (Henry, 1985; Jasper et al., 2020). Feed consumption can be affected by pig occupancy rate, with a lower number of pigs per feeder space having increased ADFI, and thus increased ADG (Li et al., 2017). As dietary energy level is decreased, pigs will consume more feed to maintain daily energy intake until feed intake is restrained by physical gut capacity or other environmental factors (Beaulieu et al., 2009). This agrees with the feed intake data presented in the current study, as pigs receiving low-energy diets had increased ADFI.

It has been well documented that barrows grow faster than gilts, while gilts are more feed efficient compared to barrows (Cromwell et al., 1993; Cornelison et al., 2018). Results of this study agree with previous findings, as the effect of sex was significant across all growth performance parameters. However, contrary to previously published literature, gilts in this study had similar final BW as barrows when fed the 12:1 LA:ALA diets, regardless of energy level. This increase in final BW was a result of increased overall ADG. There is a paucity of data evaluating dietary fat source and sex interactions on

Table 12. Effect of energy level and essential fatty acid ratio on plasma inflammatory marker concentrations of grow-finish pigs

	Energy[1]		Fatty acid ratio[2]		SEM	*P*-value[3]					
Item[4]	High	Low	High	Low		Energy	Ratio	Day	Energy ×ratio	Energy × day	Ratio × day
CRP, µg/mL					12.499	0.377	0.839	0.003	0.911	0.137	0.098
Day 0	65.90	49.64	45.27	70.26							
Day 21	53.41	72.08	57.92	67.57							
Day 42	52.11	83.88	78.70	57.29							
Day 84	28.92	27.35	38.89	17.38							
TNFα, pg/mL					14.571	0.247	0.646	0.014	0.332	0.894	0.534
Day 0	60.54	45.78	63.36	43.74							
Day 21	23.74	22.80	18.36	29.48							
Day 42	42.65	24.79	33.25	31.79							
Day 84	21.66	17.06	15.95	23.18							
IL-6, pg/mL					36.832	0.123	0.520	0.116	0.541	0.084	0.464
Day 0	68.05	91.99	61.99	101.00							
Day 21	39.33	40.71	29.25	54.74							
Day 42	116.41	25.81	54.75	54.88							
Day 84	58.43	28.86	34.29	31.06							

[1]Calculated energy: high = 3,550 kcal/kg as ME; low = 3,290 kcal/kg as ME.
[2]Calculated fatty acid ratio as linoleic acid:linolenic acid: high = 23:1; low = 12:1.
[3]Within a dependent variable, means without a common superscript differ significantly ($P < 0.05$).
[4]CRP, C-reactive protein; TNFα, tumor necrosis factor-alpha; IL-6, interleukin-6.

Table 13. Effect of energy level and essential fatty acid ratio on synovial fluid inflammatory marker concentrations of grow-finish pigs

	Energy[1]		Fatty acid ratio[2]		SEM	*P*-value[3]					
Item[4]	High	Low	High	Low		Energy	Ratio	Day	Energy × ratio	Energy × day	Ratio × day
Hock											
CRP, ng/mL					162.65	0.081	0.986	0.001	0.962	0.386	0.810
day 0	2637.08	2419.17	2497.91	2558.34							
day 84	1917.53	1289.48	1629.59	1577.41							
TNFα, pg/mL					6.892	0.096	0.617	0.198	0.923	0.119	0.341
Day 0	25.59	29.44	28.56	26.38							
Day 84	27.24	16.04	17.83	24.51							
IL-6, pg/mL					9.124	0.247	0.782	0.266	0.874	0.663	0.548
Day 0	31.22	34.87	30.40	29.82							
Day 84	32.45	28.68	28.23	31.51							
Carpus											
CRP, ng/mL					156.03	0.095	0.845	0.001	0.951	0.412	0.742
Day 0	2545.32	2422.71	2463.03	2601.14							
Day 84	1815.86	1378.45	1539.19	1602.57							
TNFα, pg/mL					7.542	0.088	0.559	0.325	0.898	0.215	0.482
Day 0	28.14	26.35	26.58	27.29							
Day 84	25.03	17.12	20.59	23.67							
IL-6, pg/mL					8.412	0.321	0.781	0.512	0.945	0.189	0.216
Day 0	27.40	26.55	30.07	28.24							
Day 84	25.12	22.52	25.89	24.78							

[1]Calculated energy: high = 3,550 kcal/kg as ME; low = 3,290 kcal/kg as ME.
[2]Calculated fatty acid ratio as linoleic acid:linolenic acid: high = 23:1; low = 12:1.
[3]Within a dependent variable, means without a common superscript differ significantly ($P < 0.05$).
[4]CRP, C-reactive protein; TNFα, tumor necrosis factor-alpha; IL-6, interleukin-6.

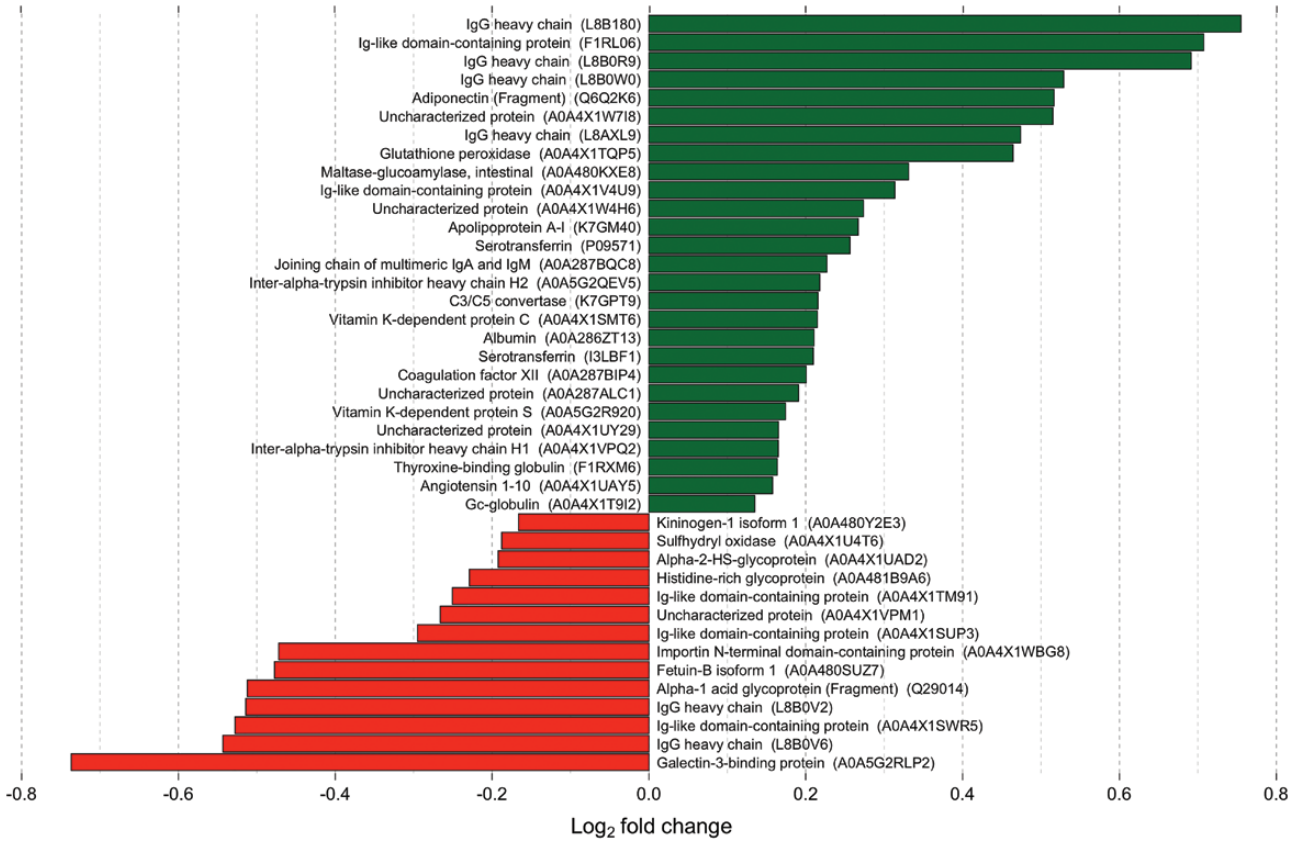

Figure 1. Log2 fold change of significantly different proteins ($P < 0.05$) in porcine serum on day 84 compared to day 0.

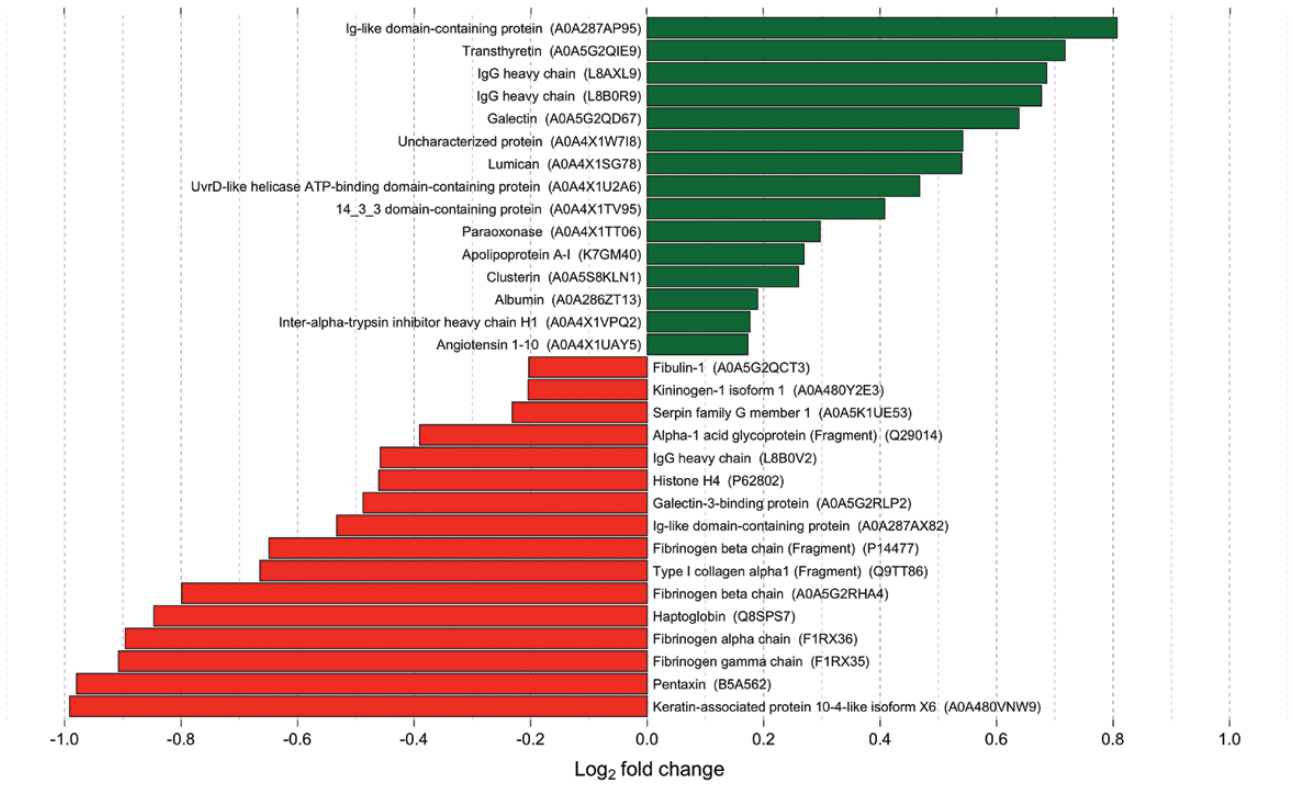

Figure 2. Log2 fold change of significantly different proteins ($P < 0.05$) in porcine hock synovial fluid on day 84 compared to day 0.

pig growth performance. Dugan et al. (2004) reported similar ADG between gilts and barrows fed 2% canola oil or 5% tallow in the grow-finish phase, though this was not evident in pigs fed 5% canola oil or 2% tallow. The 2% canola oil diets negatively impacted the barrows' feed efficiency in this study conducted by Dugan et al., (2004); however, this difference was not observed in tallow diets. Canola oil is a lipid source rich in oleic acid (C18:1; 56.1%) and LA (20.3%), with moderate ALA content (9.3%; NRC, 2012). Tallow consists of 36% oleic acid, 3.1% LA, and 0.6% ALA, and also has 24.9% palmitic acid (C16:0). Analyzed fatty acid composition varied from NRC (2012) values in the study conducted by Dugan et al. (2004), resulting in a LA:ALA ratio of 5.6:1 and 7.6:1 for the 2% canola oil and 5% tallow diets, respectively. Evaluation of dietary LA:ALA ratios lower than those utilized in the current study are needed to confirm the results of Dugan et al. (2004). In contrast, Benz et al. (2011) did not report an interaction between dietary fat source and sex when feeding soybean oil or choice of white grease to growing pigs. It is hypothesized that these changes in gilt growth performance result from altering the eicosanoids production and delaying the onset of estrus. Eicosanoids are end products of essential fatty acid metabolism and have hormone-like activities (Bhathena, 2000). Previous research in gilts demonstrated that dietary supplementation of flaxseed oil reduced prostaglandin concentrations in post-pubertal gilts, both pre- and post-mating (Chartrand et al., 2003).

Lipogenesis is unique in the pig, as it primarily occurs in the adipose tissue, whereas in humans, birds, and rodents, this process primarily occurs in the liver (O'Hea and Leveille, 1969; Pearce, 1980; Dodson, 2010). The *ACACA* gene encodes for acetyl-CoA carboxylase, which is the enzyme responsible for the irreversible conversion of acetyl-CoA to malonyl-CoA as the first step for fatty acid synthesis. The increase in liver *ACACA* relative mRNA abundance in lower-energy diets compared to high-energy diets is supported by previous work (Duran-Montgé et al., 2009; Kellner et al., 2017). Duran-Montgé et al. (2009) observed an increase in liver *ACACA* only in pigs fed 10% high oleic sunflower oil compared to a no-fat diet, but no differences when fed 10% tallow, sunflower oil, linseed oil, a fish oil blend, or a tallow, sunflower oil, and linseed oil blend. It has been observed in pigs and other species that monounsaturated fatty acids, such as oleic acid, have the inability to suppress transcription of lipogenic genes relative to polyunsaturated fatty acids (Klingenberg et al., 1995; Xu et al., 1999). In the current study, *ACACA* abundance increased in adipose tissue, which agrees with Duran-Montgé et al. (2009), who reported reduced adipose *ACACA* abundance with added dietary fat, regardless of source. In adipose tissue, Kellner et al. (2017) did not report a significant change in *ACACA* abundance with 5% added fat. Differences in adipose *ACACA* abundance between Kellner et al. (2017) and the data reported herein could be a result of the longer 84-d feeding duration of high-energy diets in the current study, compared to the 10-d experimental period used by Kellner et al. (2017).

The *FASN* gene encodes for fatty acid synthase, which is an enzyme complex responsible for the synthesis of palmitate from acetyl-CoA and malonyl-CoA in pig adipocytes during de novo lipogenesis. Pigs receiving low-energy diets had increased *FASN* abundance in both the liver and adipose tissues compared to the high energy diets. The response in the adipose tissue is consistent with previously reported literature (Bee et al., 2002; Liu et al., 2007; Duran-Montgé et al., 2009; Kellner et al., 2017). Increasing dietary fat inclusion reduces fatty acid synthase activity; therefore, reducing the rate of de novo lipogenesis in the pig. Changes in *FASN* abundance in the liver due to added fat have been variable because the liver is not the primary site of lipogenesis in pigs, though it is important for lipid transport, phospholipid and cholesterol synthesis, and fatty acid β-oxidation.

Linoleic acid and ALA are precursors for downstream production of inflammatory metabolites. The dietary LA:ALA did not influence markers of inflammation either systemically or locally in the joint. Pigs remained healthy during the entirety of the study and did not exhibit any signs of lameness; therefore, inflammatory marker concentrations remained relatively low across all time points. C-reactive protein and TNFα were reduced on day 84 compared to day 0. This could be a result of reduced growth rate and protein gain as pigs mature during late-finishing. High energy diets also tended to increase CRP and TNFα in both the carpus and hock synovial fluid. While this same effect was not observed in systemic circulation, data from human literature would support the increase in inflammation as a result of high-fat diets (Duan et al., 2018). The same-day effect was observed in the synovial fluid for CRP. There was a general decrease in the total inflammatory marker concentrations between plasma and synovial fluid in the present study. Synovial fluid is an ultrafiltrate of blood plasma that provides lubrication and reduces friction in joints (Ogston and Stanier, 1953). Due to its plasma origin, the protein composition of synovial fluid often reflects that of blood plasma, though large proteins are selectively excluded from the synovial joint space (Bennike et al., 2014); however, very limited data exist reporting the basal cytokine levels in synovial fluid of healthy, growing pigs with modern genetics.

Immunoglobulin G (IgG) is the most abundant, in serum, of the five immunoglobulin isotypes and plays a significant role in secondary immune responses by providing the majority of antibody-based immunity against foreign antigens (Vidarsson et al., 2014). Immunoglobulin G heavy chain abundance was increased on day 84 compared to day 0 in both serum and synovial fluid (Figures 1 and 2). This agrees with Piñeiro et al. (2019), who reported general increases in serum IgG during the grow-finish phase. Serum IgG has been reported to be higher in healthy adults compared to children (Stoop et al., 1969). Apolipoprotein A-I was also increased in both the serum and synovial fluid with time. This is a major structural protein of high-density lipoprotein (HDL) and is important in cholesterol transport from peripheral tissues to the liver- or steroid-producing tissues (Mangaraj et al., 2016). Serum glutathione peroxidase increased with age. This enzyme is critical for catalyzing the reduction of detrimental peroxide radicals to water and oxygen. This increase with age agrees with previously published literature on pigs (Jorgensen et al., 1977; Lei et al., 1997). Increased adiponectin abundance was also observed on day 84. Adiponectin is a hormone derived from adipocytes and is found in high concentrations in circulation (Kershaw and Flier, 2004). Adiponectin functions to increase fatty acid oxidation, increase insulin release from the pancreas, and promote glucose uptake into tissues, while preventing gluconeogenesis, and has anti-inflammatory properties (Nguyen, 2020; Khoramipour et al., 2021). The positive association between adiponectin and age has been reported in human (Isobe et al., 2005; Obata et al., 2013)

and pig studies (Jacobi et al., 2004). In the hock synovial fluid, paraoxonase increased with age of the pig. Paraoxonase has roles in preventing lipid peroxidation and oxidation of HDL and low-density lipoproteins (LDL; Aviram et al., 1998). In human literature, paraoxonase is associated with reducing oxidative stress and protecting against cardiovascular disease (Kowalska, 2015). In dogs with osteoarthritis, increased synovial fluid paraoxonase has been observed (Garner et al., 2013). Similarly, in humans with rheumatoid arthritis and osteoarthritis, increases in serum paraoxonase have been observed (Baskol et al., 2005; Soran et al., 2008). Despite a lack of differences based on dietary treatment, these protein changes in serum and synovial fluid contribute to the limited, but growing knowledge concerning how the proteome changes with age in the pig.

In conclusion, a high energy diet improved growth performance and tended to increase markers of inflammation in the synovial fluid, regardless of LA:ALA. Low energy diets increased feed intake and expression of genes involved in fatty acid synthesis. Barrows and gilts receiving a 12:1 LA:ALA had similar final BW, which was a result of increased gain of low LA:ALA gilts. Irrespective of diet, some pro-inflammatory markers were reduced with time, both systemically and locally in the joint. Overall, differing LA:ALA ratios can be fed at varying energy levels without impacting growth, as there were no energy × ratio interactions observed. Furthermore, LA:ALA ratios can differentially impact the growth of barrows and gilts. Research evaluating the hormone production and reproductive characteristics in growing gilts fed varying LA:ALA is warranted to understand the physiological mechanisms behind the improved gilt growth performance observed in the present study.

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Conflict of interest statement

The authors declare no conflict of interest.

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