Esterification of essential and non-essential fatty acids into distinct lipid classes in ruminant and non-ruminant tissues

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ABSTRACT

Extensive microbial biohydrogenation of polyunsaturated fatty acids (PUFA) in the rumen reduces the essential fatty acids (EFA) available for absorption in ruminant animals but there is no published documentation of ruminants developing EFA deficiency. In ruminants, most circulating PUFA are found in the phospholipid (PL) and cholesteryl ester lipid classes that have slow turn-over compared to other lipid classes. The objective of this experiment was to measure fatty acid esterification patterns of the non-EFA palmitic (16:0) and oleic acid (18:1), and the EFA linoleic (18:2) and linolenic acid (18:3) in small intestine, liver, and muscle tissue of cows and pigs to identify tissues participating in sequestration of these FA in less metabolically active lipid classes in ruminants. Bovine and porcine small intestine, liver, and muscle explants were prepared and incubated in media containing radiolabeled 16:0, 18:1, 18:2, or 18:3 to measure esterification of fatty acids into PL and TG. Neither bovine nor porcine small intestine explants preferentially incorporated non-EFA compared to EFA into PL vs TG. Bovine liver explants esterified a larger proportion of EFA than non-EFA into PL compared to TG, while incorporation was similar among the FA tested in porcine liver explants. Bovine muscle explants showed preferential incorporation of EFA into PL rather than TG. Results show that bovine and porcine liver and muscle esterify EFA and non-EFA differently and that the conservation of EFA in ruminants is a result of preferential incorporation of EFA into PL mediated by bovine liver and muscle, but not the small intestine.

Key words: essential fatty acid, ruminant, liver

Abbreviations:

34 16:0, palmitic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid; CE, cholesteryl
35 ester; EFA, essential fatty acids; FA, fatty acid; HDL, high-density lipoprotein; IDL,
36 intermediate-density lipoprotein; LDL, low-density lipoprotein; LPL, lipoprotein lipase; PL,
37 phospholipid; PUFA, polyunsaturated fatty acid; TG, triglyceride; VLDL, very low-density
38 lipoprotein;

1. Introduction

Essential fatty acids (EFA) are components of dietary fat that are vital for normal animal growth and development. The two essential fatty acids are the omega-6 fatty acid (FA) linoleic acid (18:2) and the omega-3 FA α -linolenic acid (18:3). These are 18-carbon polyunsaturated fatty acids (PUFA) with two or three double bonds, respectively. Their classification into omega-6 vs omega-3 FA is based on the position of the first double bond in the carbon chain in relation to the methyl end. Linoleic and α -linolenic acids can be further desaturated and elongated to produce additional long-chain PUFA. These EFA along with their elongation and desaturation products and downstream metabolites are important components of cell membranes, the lipids of the brain and eye, and are involved in the production of eicosanoids that regulate immunity and inflammation (Uauy et al., 2000). Deficiencies in 18:2 and 18:3 in animals can result in dermatitis, growth retardation, hair loss, immune suppression, and abnormalities in brain and retinal development (Gramlich et al., 2015) due to the lack of EFA, as well as the consequent lack of other PUFAs and their downstream metabolites. Essential fatty acids cannot be synthesized by animals because they lack the Δ 12- and Δ 15-desaturase enzymes capable of inserting double bonds at the 12 and 15 positions of the FA carbon chain. Therefore, animals obtain these FA by consuming plants, which contain abundant EFA, or by consuming herbivorous animals in the case of predators.

Early studies demonstrated that ingestion of EFA-deficient diets resulted in gross dermal lesions in pigs (Sewell and McDowell, 1966; Sewell and Miller, 1966) and increased ratio of trienoic to dienoic and tetraenoic FA in tissues of rats (Rieckehoff et al., 1949), chicks (Bieri et al., 1957), and dogs (Wiese and Hansen, 1951). In ruminants, prior to absorption of dietary fats in the small intestine, the resident bacteria of the foregut biohydrogenate unsaturated FA

(Lourenco et al., 2010) leading to almost complete saturation of the carbon chain and loss of function as EFA (Kalscheur et al., 1997a, b; Looor et al., 2004). Despite the small amounts of 18:2 and 18:3 present in the plasma lipids of newborn ruminants and the low concentration of these FA found in their dam's milk, newborn calves and lambs are able to egress borderline EFA deficiency within days of birth (Jenkins et al., 1988; Jensen, 2002; Leat, 1966). In 3 d-old calves, 6 wk of an EFA-deficient diet did not result in development of external deficiency signs (Jenkins and Kramer, 1986), whereas feeding 3 wk-old pigs an EFA-deficient diet for the same period of time resulted in the development of dermal lesions (Sewell and McDowell, 1966). Because biohydrogenation reduces EFA ingested by ruminants to almost negligible levels before absorption, it is interesting that there is no documentation of ruminants developing EFA deficiency despite the small amounts of absorbed EFA. Conservation of EFA in ruminant animals and not non-ruminants indicates the development of physiological and biochemical mechanisms to cope with rumen bacterial modification of EFA.

Most PUFA, including 18:2 and 18:3, circulate in the phospholipid (PL) and cholesteryl ester (CE) lipid classes in ruminants. These are the two most abundant lipid classes in ruminant plasma (Christie, 1978), making EFA the most abundant FA in ruminant plasma. Additionally, plasma PL and CE have a slow turn-over (Mattos and Palmquist, 1977; Palmquist and Mattos, 1978) compared to other lipid classes such as triglycerides (TG) and non-esterified FA. Conservation of EFA in ruminants might be the result of sequestration of these FA in less metabolically active plasma lipid classes.

We hypothesized that bovine tissues esterify greater proportions of EFA into PL than into TG compared to the pig, a non-ruminant ungulate. The objective of this experiment was to measure lipid esterification patterns of the non-EFA palmitic (16:0) and oleic acid (18:1), and the

EFA 18:2 and 18:3 acids in small intestine, liver, and muscle tissue of cows and pigs to identify tissues participating in sequestration of these FA in less metabolically active lipid classes in ruminants.

2. Materials & methods

2.1. Reagents

Medium 199, fatty acid-free bovine serum albumin, bicarbonate, and insulin (bovine pancreas) were purchased from Sigma Aldrich (St. Louis, MO). Fatty acids were purchased from Nu-Chek Prep, Inc. (Elysian, MN). [1-¹⁴C]-labeled fatty acids were purchased from American Radiolabeled Chemicals Inc. (St. Louis, MO). Hexane, isopropanol, sodium sulfate, ethyl ether, acetic acid, and liquid scintillation cocktail (ScintiSafe 30%) were purchased from Fisher Scientific (Pittsburg, PA).

2.2. Tissue Collection

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Virginia Tech. A portion of the left lobe of the liver, the jejunum of the small intestine, and diaphragm muscle were collected from four cattle (>16 mo of age) and four pigs (>8 months of age) and immediately transported to the lab in 1X DPBS (pH 7.3) on ice.

2.3. In Vitro Cultures

Liver tissue samples were trimmed to remove capsule and nonparenchymal tissue. Small intestine samples were opened longitudinally and pinned epithelial side up on a dissection board and cored using an MD5000 Tissue Coring Press (Alabama Research and Development, Munford, AL) to obtain disks of tissue. Afterwards, the serosa layer of the disk of tissue was removed. Liver and muscle cores were also prepared using the Coring Press, and sliced at 600-micron thickness with a MD4000 Krumdieck automated tissue slicer (Alabama Research and

Development). Tissue slices and small intestine disks were blotted, immediately weighed, and ~150 mg of tissue were placed in 25 mL Erlenmeyer flasks with 3 mL bicarbonate-supplemented Medium 199 with Earle's salts and L-glutamine (pH 7.3), containing 0.15 IU/mL insulin, 0.25 mM 16:0, 0.25 mM 18:1, 0.25 mM 18:2, 0.25 mM 18:3, and 1 μ Ci of [1- 14 C]16:0, [1- 14 C]18:1, [1- 14 C]18:2, or [1- 14 C]18:3. Incubations were prepared in triplicate. Fatty acids were complexed to bovine serum albumin (4:1 molar ratio) to facilitate solubility in the aqueous culture media. Flasks were gassed with O₂:CO₂ (95:5), sealed with rubber stoppers and placed in a 37°C shaking water bath for 2 h. Incubation was terminated by the addition of 0.5 mL 1M H₂SO₄ to the media.

2.4. Measurement of fatty acid incorporation into total lipids

Lipids were extracted according to the method of Hara and Radin (1978) with some modifications. Following the 2 h incubation, tissue explants were removed from the Erlenmeyer flasks using forceps, washed with water from a squirt bottle to remove residual media, blotted on tissue paper, and placed in a 17x100 polypropylene test tube containing 3 mL 3:2 hexane-isopropanol. APRO200 laboratory homogenizer (PRO Scientific, Oxford, CT) was used to homogenize the tissue samples. The homogenate was washed with 2 mL sodium sulfate solution (1 g/15 mL), centrifuged at 2000 x g for 5 min at 4°C to separate the aqueous and organic phases, and the organic phase was transferred to a new 17x100 test tube. The solvent was evaporated in a water bath at 40°C under a stream of N₂. Extracted lipids were dissolved in 200 μ L of hexane. Half of the extracted lipids were transferred to a liquid scintillation vial with liquid scintillation cocktail to quantify 14 C incorporated in total lipids/2 h/150 mg tissue. The remainder of the extracted lipids was separated into lipid classes.

2.5. Measurement of fatty acid incorporation into lipid classes

Triglycerides and PL in extracted lipids were separated from non-esterified FA (substrate) using 250 μ m Silica Gel G thin-layer chromatography plates (Analtech Inc., Newark, DE). Extracted lipids were spotted onto thin-layer chromatography plates and developed with hexane-ethyl ether-acetic acid (85:20:2 v/v). After plate development, plates were allowed to dry for 10 min and were then exposed to a PhosphorImager screen (Bio-Rad, Hercules, CA) for 3 d. Radioactivity in bands corresponding to lipid classes was quantified using a Molecular Imager® FX Pro Plus® MultiImager System (Bio-Rad, Hercules, CA). The densitometry of all bands (complete lane) was measured and the percentage of radioactivity in each lipid class was calculated by dividing the densitometry value of a lipid class by the total of all the bands. Total nmol of FA incorporated into a lipid class/2 h/150 mg tissue was calculated by multiplying the percentage of total radioactivity in a lipid class by total nmol of FA incorporated/2 h/150 mg tissue.

2.6. Statistical Analyses

The data were tested for normality and equality of variances and then analyzed using the Mixed models procedure of SAS (v. 9.2, SAS Institute Inc., Cary, NC). For total FA incorporation, the statistical model included the fixed effects of species, FA, species by FA interaction, and the random effect of animal nested within species. The effect of species was tested using animal within species. Treatment effects were further analyzed using preplanned orthogonal contrasts. Pre-planned orthogonal contrasts were used to compare EFA with non-EFA, 16:0 with 18:1, and 18:2 with 18:3 overall and within species.

3. Results

3.1. Total fatty acid incorporation and PL to TG ratio in bovine and porcine small intestine, liver, and muscle explants

In small intestine, liver, and muscle, explants of bovids and porcids esterified similar amounts of radiolabeled FA during the 2 h incubation period ($P > 0.12$; Fig. 1A). Furthermore, there was no significant difference in the PL:TG ratio of bovids versus porcids ($P > 0.13$; Fig. 1B) in either small intestine, liver, or muscle explants. The PL:TG ratio represents a measure of incorporation of FA into PL vs TG fractions. A ratio ≈ 1 indicates that FA were similarly esterified in the PL and TG fractions; a ratio exceeding 1 indicates that a higher proportion of esterified FA was allocated to the PL fraction, while a ratio less than 1 indicates that a higher proportion was esterified to the TG fraction.

3.2. Fatty acid esterification patterns in bovine and porcine small intestine explants

Porcine small intestine explants esterified greater amounts of non-EFA compared to EFA (17.94 vs 12.39 ± 2.02 nmoles/2 h/150 mg tissue, $P = 0.002$), while no preference for esterification of non-EFA compared to EFA was observed in bovine small intestine explants (Table 1). Additionally, the porcine explants demonstrated a predilection for incorporation of radiolabeled 16:0 compared to 18:1 ($P = 0.015$; Table 1). There was no differential incorporation of 16:0 and 18:1 in bovine small intestine explants, or 18:2 and 18:3 in explants of either species (Table 1).

There was no difference in the incorporation of non-EFA compared to EFA into PL vs TG, as shown by the PL:TG ratio, for bovine or porcine small intestine explants (Table 1). Porcine small intestine cultures, however, esterified more radiolabeled 16:0 than 18:1 into PL compared to TG ($P = 0.008$), as well as more 18:3 compared to 18:2 ($P = 0.004$; Table 1). Incorporation into PL vs TG was similar among FA in bovine small intestine explants (Table 1).

3.3. Fatty acid esterification patterns in bovine and porcine liver explants

Liver explants of both species esterified more non-EFA (16:0 + 18:1) than EFA (18:2 + 18:3) (cow: 17.81 vs 12.94 $\pm$ 2.58 nmoles/2 h/150 mg tissue, $P = 0.003$; pig: 24.04 vs 18.44 $\pm$ 2.58 nmoles/2 h/150 mg tissue, $P < 0.001$; Table 2). There was no preferential incorporation between 16:0 and 18:1 or 18:2 and 18:3 in liver explants of either species (Table 2). Radiolabeled FA were more readily incorporated into TG compared to PL with no significant overall difference in the PL:TG ratio for all FA between bovids and porcids ($P = 0.14$; Fig. 1B). Bovine liver explants esterified a larger proportion of EFA than non-EFA into PL compared to TG ($P = 0.008$; Table 2). This increase in PL:TG ratio in explants incubated with EFA was due to greater incorporation of 18:3 rather than 18:2 into PL ($P = 0.002$; Table 2). Incorporation into PL vs TG was similar among FA in porcine liver explants (Table 2).

3.4. Fatty acid esterification patterns in bovine and porcine muscle explants

Bovine muscle explants esterified a greater EFA compared to non-EFA (10.38 vs 8.18 $\pm$ 1.27 nmoles/2 h/150 mg tissue, $P = 0.003$), specifically through the incorporation of more radiolabeled 18:3 than 18:2 into lipids ($P = 0.008$; Table 3). No differences in incorporation among FA was observed in porcine muscle explants (Table 3).

Bovine muscle explants showed preferential incorporation of EFA into PL rather than TG, compared to non-EFA (2.60 vs 1.11 $\pm$ 0.63 nmoles/2 h/150 mg tissue, $P = 0.002$). Porcine muscle explants demonstrated a tendency towards higher incorporation of EFA compared to non-EFA into PL vs TG (1.42 vs 0.90 $\pm$ 0.60 nmoles/2 h/150 mg tissue, $P = 0.058$; Table 3). In both species, the increased PL:TG in EFA compared to non-EFA was due to increased incorporation of 18:3 compared to 18:2 into PL ($P < 0.002$; Table 3). There was no difference in incorporation of the non-EFA into PL vs TG in either species (Table 3).

4. Discussion

Essential fatty acid deficiency can have serious consequences for animals. Animals that consume plants or prey on animals that consume plants have no difficulty meeting their EFA requirements and avoiding deficiency. Ruminants, as herbivores, are a special case with respect to EFA consumption because they engage in bacterial fermentation of food before nutrient absorption in the small intestine. Polyunsaturated fatty acids, abundantly present in the diets of ruminants, are modified during rumen fermentation through biohydrogenation, reducing the amount absorbed and available for utilization by tissues (Harfoot and Hazlewood, 1988).

Dairy cow consumption of 18:2 and 18:3 ranges from 400-600 g/d (Kalscheur et al., 1997a, b). After these FA reside in the rumen and microbes biohydrogenate them, only 10-30% of consumed 18:2 and 18:3 is available for absorption in the intestine (Kalscheur et al., 1997a, b; Loor et al., 2004). In the pig, consumed 18:2 and 18:3 remains unmodified through digestion and is available for absorption in the small intestine. Although both are ungulates, the impact of biohydrogenation in limiting EFA availability contrasts strongly between the ruminant cow and non-ruminant pig. Despite these losses, ruminants do not experience EFA deficiency. This is the result of EFA conservation mechanisms revealed by the differences in circulating FA and lipid profiles of cows and pigs.

Cows have around 50% more 18:2 in circulation than pigs and 400% more 18:3 (Corl et al., 2001; Seiquer et al., 1994; Tyburczy et al., 2008). This is somewhat unexpected given the considerable reductions in PUFA exiting the rumen that are available for absorption in the small intestine compared to the quantities of 18:2 and 18:3 consumed and absorbed by pigs. The profile of circulating lipid differs in the two species as well. Whereas circulating concentrations of TG are 50% greater in pigs than cows, the circulating concentrations of PL are 290% greater in cows than pigs (Amate et al., 2001; Tyburczy et al., 2008). Triglycerides tend to be more

metabolically available than PL to tissues. Fatty acids esterified to PL are more likely to be conserved through restricted access to peripheral tissues. Additionally, the FA profile of these individual lipid classes differs between cows and pigs. Cows have much greater concentrations of 18:2 and 18:3 in PL than do pigs (Amate et al., 2001; Tyburczy et al., 2008). The inverse is true for TG. Pigs have several-fold greater 18:2 and 18:3 concentrations in TG than do cows. The differences in circulating lipid profiles of the cow and pig led us to examine the esterification patterns of essential and non-EFA by the small intestine, liver, and muscle given their roles in FA absorption, esterification, and use.

The enterocytes of the small intestine absorb FA and re-esterify the absorbed FA into TG and PL. The TG and PL are assembled into chylomicrons, large TG-rich lipoprotein particles, that are the major lipoprotein released into lymph of simple-stomached animals like pigs. In ruminants, the enterocytes more often produce very low-density lipoproteins (VLDL), the predominant lipoprotein secreted by ruminant enterocytes into lymph (Bauchart, 1993). Because chylomicrons have a greater proportion of TG to PL compared to VLDL, the major lipid class found in the lymph of simple-stomached animals is TG, while in ruminants the proportion of PL to TG is greater (Bauchart, 1993).

Small intestine explants did not exhibit a preference for the esterification of non-EFA or EFA into PL or TG in either species. In the pig, small intestine explants incorporated more non-EFA than EFA, a preference not evident in the cow. There was not a clear esterification pattern establishing the small intestine as channeling EFA to PL in the cow compared to the pig.

After assembly in enterocytes, lipoproteins are secreted into the lymphatic system. They exit the lymphatics and enter the circulatory system via the thoracic duct to deliver lipoprotein TG to tissues. Tissues cleave FA from TG via the action of lipoprotein lipase and increase the

density of the lipoprotein by removing TG (Eckel, 1989). The FA obtained by tissues from chylomicrons and VLDL can then be stored as TG, oxidized for energy production, or used to make other molecules, such as the PL found in the cell membrane.

Chylomicrons that escape hydrolysis by peripheral tissues as well as their hydrolyzed remnants reach the liver for absorption and processing. In simple-stomached animals, the liver synthesizes VLDL from chylomicrons and remnants, and releases the newly formed VLDL into circulation to supply FA to peripheral tissues. As the VLDL loses TG it becomes an intermediate-density lipoprotein (IDL) and, later, a low-density lipoprotein (LDL). In ruminants, enterocytes secrete VLDL, and IDL and LDL are found in negligible concentrations in the plasma of lactating cows (Bauchart, 1993). Additionally, the liver in ruminants has limited synthesis and secretion of VLDL in contrast to simple-stomached animals (Bauchart, 1993; Moore and Christie, 1979).

The major plasma lipoprotein in ruminants is the high-density lipoprotein (HDL) synthesized by the liver (Bauchart, 1993). This lipoprotein is utilized to deliver cholesterol from tissues back to the liver for bile secretion, a process known as reverse cholesterol transport. While HDL is the major lipoprotein found in ruminant plasma, it has a relatively slow turn-over rate, especially when compared to VLDL, which can have a metabolic half-life of only 5 min in lactating dairy cows (Raphael et al., 1973). Since the proportion of PL to TG is much higher in HDL compared to VLDL, the high turn-over rate of VLDL suggests that ruminant tissues exhibit a preference for TG hydrolysis. Christie et al. (1986) suggested that liver hydrolysis of TG from VLDL was a mechanism used to protect PUFA from non-essential functions.

The major difference in how these species metabolized EFA vs non-EFA is observed in liver tissue. In both bovine and porcine liver tissue, non-EFA were preferentially esterified

compared to EFA, and there was increased incorporation of EFA and non-EFA into TG compared to PL (PL:TG ratios ranged from 0.37 to 0.76 for both species). An interesting observation is the preference exhibited by bovine liver for EFA, in particular 18:3, for the synthesis of PL while porcine liver used EFA and non-EFA equally for the synthesis of either PL or TG. The liver esterification profile of the pig and cow explants indicate that the liver participates in establishing the circulating FA and lipid profiles that results in EFA conservation in ruminants. Liver re-esterification of EFA from TG to PL is also supported by Stamey Lanier et al. (2013). Despite intravenous infusion of TG enriched in EFA, infused EFA were enriched in circulating PL more than TG.

Skeletal muscle can store FA in the form of TG in lipid droplets (Schrauwen-Hinderling et al., 2006) and oxidize FA to supply energy. Muscle tissue also incorporates FA into PL for membrane synthesis. Skeletal muscle esterified a higher proportion of EFA than non-EFA into PL, with 18:3 being favored over 18:2 in both species. This preferential use of EFA for PL production was more marked in cows than pigs, which can be explained by the increased incorporation of EFA in skeletal muscle by cow but not pig. This leads to conservation of EFA in cow tissues, restricting EFA from TG incorporation and subsequent oxidation.

Results of these *in vitro* studies of FA esterification support our hypothesis that specific bovine tissues esterify greater amounts of EFA in PL compared to TG, thereby conserving the limited amounts of these FA absorbed by the small intestine. Specifically, this preferential esterification of EFA into the lower turnover PL class was observed in bovine liver, as well as skeletal muscle. Because the liver is the organ responsible for production and secretion of HDL, the major lipoprotein in ruminant plasma, the liver in ruminants is conserving EFA by esterifying them into PL which will generate a lipoprotein with a long metabolic half-life. Incorporation of

EFA into PL, compared to TG, in skeletal muscle indicates that these FA are incorporated into membranes rather than lipid droplets that can be subsequently oxidized. The preferential esterification of EFA into PL used for HDL synthesis by the liver and cell membrane production by skeletal muscle results in conservation of ω -3 and ω -6 FA from oxidative loss and non-essential functions. The additive effects of muscle and liver tissues might explain how ruminants avoid EFA deficiency despite the extensive biohydrogenation of these FA in the rumen.

Acknowledgments

The authors wish to thank Mr. Mark Stevenson for his help with the tissue collection. This material is based upon work supported by the National Science Foundation under Grant No. IOS-0920491. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

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Fig. 1. Total FA incorporation (nmoles/150 mg tissue/2 h) (A) and phospholipid to triglyceride ratio (B) in cow and pig small intestine, liver, and muscle explants incubated with radiolabeled 16:0, 18:1, 18:2, and 18:3. Black bars represent small intestine explants; gray bars represent liver explants, and white bars represent muscle explants. There was no significant difference in total FA incorporation or phospholipid to triglyceride ratio between the two species in any tissue.

Table 1

Least squares means for incorporated ^{14}C -labeled fatty acids in cultured bovine and porcine small intestine explants.

	non-EFA		EFA		SE	Contrast <i>P</i>		
	16:0	18:1	18:2	18:3		non-EFA vs. EFA	16:0 vs. 18:1	18:2 vs. 18:3
<u>nmol incorporated^a</u>								
Cow	14.73	12.14	13.65	10.48	2.02	0.38	0.24	0.16
Pig	20.84	15.04	13.90	10.87	2.02	0.002	0.015	0.18
 <u>PL:TG</u>								
Cow	0.81	0.62	0.78	0.93	0.56	0.56	0.56	0.68
Pig	2.57	1.58	1.53	2.63	0.56	0.98	0.008	0.004

^aData for nmols incorporated are expressed in nmoles/2 h/150 mg tissue; n= 4.

Abbreviations: 16:0 = palmitic acid, 18:1 = oleic acid, 18:2 = linoleic acid, 18:3 = α -linolenic acid, EFA = essential fatty acid, SE = standard error, PL:TG = phospholipid to triglyceride ratio

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Table 2

Least squares means for incorporated ^{14}C -labeled fatty acids in cultured bovine and porcine liver explants.

	non-EFA		EFA		SE	<i>P</i>		
	16:0	18:1	18:2	18:3		non-EFA vs. EFA	16:0 vs. 18:1	18:2 vs. 18:3
	<u>nmol incorporated^a</u>							
Cow	19.68	15.94	13.67	12.20	2.58	0.003	0.076	0.47
Pig	22.64	25.43	18.79	18.08	2.58	<0.001	0.178	0.73
<u>PL: TG</u>								
Cow	0.46	0.51	0.51	0.76	0.07	0.008	0.44	0.002
Pig	0.50	0.37	0.38	0.46	0.07	0.78	0.079	0.23

^aData for nmols incorporated are expressed in nmoles/2 h/150 mg tissue; n= 4.

Abbreviations: 16:0 = palmitic acid, 18:1 = oleic acid, 18:2 = linoleic acid, 18:3 = α -linolenic acid, EFA = essential fatty acid, SE = standard error, PL: TG = phospholipid to triglyceride ratio

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Table 3

Least squares means for incorporated ^{14}C -labeled fatty acids in cultured bovine and porcine muscle explants.

	non-EFA		EFA		SE	Contrast <i>P</i>		
	16:0	18:1	18:2	18:3		non-EFA vs. EFA	16:0 vs. 18:1	18:2 vs. 18:3
	<u>nmol incorporated^a</u>							
Cow	8.25	8.11	9.05	11.7	1.27	0.003	0.88	0.008
Pig	7.95	7.54	7.58	7.73	1.14	0.61	0.48	0.75
<u>PL: TG</u>								
Cow	1.11	1.11	1.54	3.66	0.63	0.002	0.99	0.002
Pig	0.97	0.83	0.94	1.90	0.60	0.058	0.68	0.009

^aData for nmols incorporated are expressed in nmoles/2 h/150 mg tissue; n= 4.

Abbreviations: 16:0 = palmitic acid, 18:1 = oleic acid, 18:2 = linoleic acid, 18:3 = α -linolenic acid, EFA = essential fatty acid, SE = standard error, PL: TG = phospholipid to triglyceride ratio

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