Feeding steam-pelleted rapeseed affects expression of genes involved in hepatic lipid metabolism and fatty acid composition of chicken meat

S. Li,[*,†,1] A. Schiller Vestergren,[†] H. Wall,[‡] S. Trattner,[†] J. Pickova,[†] and E. Ivarsson[‡]

[*]*School of Food Science and Technology, Dalian Polytechnic University; National Engineering Research Center of Seafood; Ganjingzi 116304, Dalian, Liaoning, China;* [†]*Department of Molecular Sciences, Swedish University of Agricultural Sciences, P.O. Box 7015, 75007 Uppsala, Sweden; and* [‡]*Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, P.O. Box 7024, 75007 Uppsala, Sweden*

ABSTRACT This study investigated the dietary effect of steam-pelleted rapeseed (RS) diets with different inclusion levels on the fatty acid composition of chicken meat and the expression of lipid metabolism-related genes in the liver. Experimental diets included 6 different wheat-soybean meal based diets either in nonpelleted or steam-pelleted form supplemented with 80, 160, and 240 g RS/kg feed and one nonpelleted wheat-soybean meal based diet without RS supplementation as the control. These diets were fed to newly hatched broiler chickens (Ross 308) for 34 days. Compared to the control diet, steam-pelleted diets containing 160 or 240 g/kg RS significantly increased the content of omega-3 long chain polyunsaturated fatty acids (n-3 LC-PUFA) in the breast and drumstick, while their meat yields were not affected. Moreover, the mRNA levels of fatty acid desaturase 1 (FADS1) and acyl-coenzyme A oxidase 1 (ACOX1) in their livers increased. Therefore, steam-pelleted diets with 160 or 240 g/kg RS can be used to increase the n-3 LC-PUFA content in chicken meat without compromising meat yield.

Key words: rapeseed, fatty acid, n-3 LC-PUFA, FADS1, ACOX1

2017 Poultry Science 96:2965–2974
http://dx.doi.org/10.3382/ps/pex047

INTRODUCTION

The long chain omega-3 polyunsaturated fatty acids (**n-3 LC-PUFA**), e.g. eicosapentaenoic acid (**EPA**, 20:5n-3) and docosahexaenoic acid (**DHA**, 22:6n-3), provide well-known beneficial effects on human health. These effects mainly include a reduced risk of cardiovascular disease (**CVD**), a role in human brain development, and anti-inflammatory properties (Howe et al., 2006; Calder, 2014). Because the biosynthesis of the n-3 LC-PUFA in the human body is limited, n-3 LC-PUFA are regarded as dietary essentials (Givens and Gibbs, 2008). Hence fatty fish, whose n-3 LC-PUFA content is high, is recommended for regular consumption. Because the intake level of EPA and DHA in healthy populations is negatively related to the risk of developing CVD in a dose-dependent way up to 250 mg/d (one to 2 servings/week of fatty fish), the European Food Safety Agency (**EFSA**) has proposed a recommended daily intake of 250 mg/d EPA and DHA for adults. However, in European countries, the daily intake of EPA and DHA is below 100 mg/d, particularly in young people, because of an aversion to eating oily fish (Givens and Gibbs, 2008). Moreover, concerns about the possible presence of harmful environmental pollutants (e.g., dioxins, polychlorinated biphenyls, methylmercury, and pesticides) in some fish species also restrict fatty fish consumption (Tur et al., 2012; Bhalerao et al., 2014).

In developed countries, besides fish and seafood, important sources of n-3 LC-PUFA are meat and meat products, including poultry products. Poultry products account for over 40% of the total meat consumption (Jankowski et al., 2012). Lipid composition of edible tissues of monogastric animals, such as poultry and pig, can be relatively easily manipulated by altering their dietary lipid composition (Rymer and Givens, 2005). Poultry meat enriched with n-3 PUFA has thus a considerable potential to facilitate our dietary intake of EPA and DHA (Givens and Gibbs, 2008; Bhalerao et al., 2014). Traditionally, EPA and DHA are enriched in poultry meat by including fish oil in poultry diets. However, this inclusion is costly and may introduce undesirable flavor and odor to the meat. Moreover, the continued and increased use of fish oils is not sustainable, and alternatives are required.

Full-fat rapeseed (**RS**), which contains about 41% oil and 21 to 23% protein, has long been recognized as a valuable source of protein and energy for animal feed (Ajuyah et al., 1993). Moreover, RS also has been known as a good source of α-linolenic acid (**ALA**, C18:3n-3), which can be readily converted to

Received July 27, 2016.
Accepted February 25, 2017.
[1]Corresponding author: shengjie.li2016@outlook.com

n-3 LC-PUFA in poultry (Ajuyah et al., 1991b). As a result, there is a growing interest in the feed industry in using full-fat RS in poultry diets (Assadi et al., 2011). However, the use of RS in poultry diets has previously been limited because of a concern about its antinutritional content, mainly the degradation products of glucosinolates, shown to adversely affect poultry growth performance (Khajali and Slominski, 2012). However, the glucosinolate content in modern rapeseed varieties, e.g., double-zero RS, has been widely improved through plant breeding and is generally not considered problematic today. The degradation process of glucosinolates is catalyzed by the enzyme myrosinase present in the seeds. However, heat treatment, such as the steam-pelleting process, can efficiently inactivate this enzyme, thus reducing or eliminating the adverse effects (Campbell and Slominski, 1990). The pelleting process has been shown to improve lipid availability in RS, and might enhance the absorbance and utilization of dietary lipid (Barekatain et al., 2015). However, this process is energy demanding, and farmers who prefer to use home-mixed diets may not have the possibility to pellet the feed. To date, most studies on the effect of a steam-pelleted RS diet have focused on bird growth performance, and few have examined its effect on the nutritional values of chicken meat, particularly its fatty acid composition.

In chicken, the liver is the main site of fatty acid synthesis, accounting for 95% of the de novo FA synthesis, and it is generally assumed almost all fat accumulating in broiler adipose tissue is synthesized in the liver or derived from the diet (Zhang et al., 2013). Several enzymes are involved in the lipid homeostasis, and changes in dietary fatty acid composition are known to influence the expression of gene coding for these enzymes (Jordal et al., 2005; Torstensen et al., 2009; Mirshekar et al., 2015), such as lipoprotein lipase (**LPL**; Goldberg et al., 2008), the sterol regulatory element binding transcription factor 2 (**SREBF2**; Goldberg et al., 2008), fat mass and obesity-associated (**FTO**; Wang et al., 2012), and fatty acid-binding proteins (**FABP**; Murai et al., 2009). Moreover, conversion of ALA to n-3 LC-PUFA involves a series of elongation and desaturation steps, catalyzed by enzymes, such as elongase of very long chain fatty acids and fatty acid desaturase 1 and 2 (**FADS1** and **FADS2**), as well as acyl-coenzyme A oxidase 1 (**ACOX1**), and liver carnitine palmitoyl transferease 1 (**L-CPT1**) involved in oxidation of fatty acids.

This study aimed to examine the dietary effects of different RS inclusion levels in combination with the steam-pelleting process of the feed on the expression of genes involved in hepatic lipid metabolism and the fatty acid composition of chicken breast and drumstick meat. Moreover, the effects of these dietary treatments on meat yield were considered.

MATERIALS AND METHODS

Animals and Housing

A total of 280 unsexed one-day-old broiler chickens (Ross 308) was randomly divided into 35 pens (1.50 × 0.75 m) with 8 chickens per pen. Pens were randomly assigned to one of 7 dietary treatments, giving 5 replicates per treatment. Pens were raised from the floor, equipped with solid floors covered with fresh wood shavings. Room temperature was gradually decreased from 33°C on d zero to 23°C on d 24 and kept at 23°C until chickens were sacrificed. Continuous artificial light was set to 24 h for the first 2 d, and then the dark period was gradually increased to 6 h from d 8 of age to slaughter at 34 days. Chickens had free access to water and feed throughout the experiment. The experiment was approved by the ethical committee of the Uppsala region, Sweden (approval number C 334/12).

Experimental Design and Diets

Chickens were fed one of 7 different experimental diets (Table 1) for 34 days. The control diet was a nonpelleted wheat-soybean meal based diet with RS inclusion level at zero g/kg. In the experimental diets, 3 RS inclusion levels of 80 g/kg, 160 g/kg, and 240 g/kg were tested as nonpelleted diets (RS8N, RS16N, and RS24N, respectively) and steam-pelleted diets (RS8P, RS16P, and RS24P, respectively). RS was finely ground before being mixed into the wheat-soybean meal based diets and either not processed or steam-pelleted to reach 75°C. These diets were formulated to be isoenergetic and isonitrogenous with a calculated content of 200 g crude protein and 12.3 MJ of metabolizable energy/kg feed. The ingredient and chemical composition of the 7 experimental diets are described in Table 1. The fatty acid compositions of the 7 experimental diets are shown in Table 2.

Sampling

On d 34, birds were weighed group-wise and an average mean value per pen was calculated. Feed intake also was recorded on pen basis. One average sized chicken per pen was selected and sent to an experimental slaughter facility. Chickens were electrically stunned and bled by cutting the carotid artery and the external jugular vein. After bleeding, chickens were placed in a scalding tank with an average temperature of 60°C for 2 min, and then immediately defeathered using an automatic turning defeathering machine. Next, carcasses were eviscerated. Liver samples were collected, snap-frozen in liquid nitrogen, and stored at −80°C. Carcass parts, i.e., breast with ribs and drumstick, were removed and weighed, and then muscle samples for fatty acid composition analysis were taken and stored at −80°C until analysis.

Table 1. Ingredient and analyzed composition, as-is basis, of the 7 experimental diets.

	Control	RS8N	RS8P	RS16N	RS16P	RS24N	RS24P
Ingredient %							
Wheat	64	62	62	57	57	43	43
Soy bean meal	26	24	24	21	21	28	28
Rape seed	0	8.0	8.0	16	16	24	24
AK-standard[1]	5.7	3.0	3.0	1.0	1.0	1.0	1.0
Limestone	1.6	1.6	1.6	2.4	2.4	2.2	2.2
Mono calcium phosphate	0.7	0.8	0.8	0.8	0.8	0.9	0.9
L-Lysine- HCl	0.36	0.32	0.32	0.31	0.31	0.03	0.03
DL- Methionine	0.24	0.23	0.23	0.22	0.22	0.19	0.19
L-Threonine	0.06	0.04	0.04	0.02	0.02	0.00	0.00
Premix	0.25	0.20	0.20	0.20	0.20	0.20	0.20
Sodium chloride	0.21	0.21	0.21	0.21	0.21	0.31	0.31
Mono sodium phosphate	0.17	0.16	0.16	0.17	0.17	0.24	0.24
Chemical composition (Analyzed) g/kg							
DM	870	870	870	880	870	880	870
Ash	41	42	51	60	55	71	68
Crude protein	210	200	190	200	190	190	200
Crude fiber	26	28	38	31	35	32	37
Ether extract	71	74	50	100	89	116	129
Glucosinolates (μmol/g)	0	0.87	0.87	2.64	1.74	3.54	2.64

[1]Liquid vegetable fat comprising a mixture of fatty acids (AkoFeed Standard, AkoFeed, Karlshamn, Sweden).

Abbreviations: nonpelleted rapeseed (RS) diets with RS inclusion levels at 80 g/kg (RS8N), 160 g/kg (RS16N), and 240 g/kg (RS24N); steam-pelleted diets with RS inclusion levels at 80 g/kg (RS8P), 160 g/kg (RS16P), and 240 g/kg (RS24P).

Table 2. Fatty acid composition of 7 experimental diets (mg/100 g as-is basis, least square means).

	Control	RS8N	RS8P	RS16N	RS16P	RS24N	RS24P
C12:0	50	27	39	9	18	9	14
C14:0	44	26	22	11	15	14	13
C16:0	1350	942	454	592	561	728	613
C16:1n-7	7.0	11	8.7	15	12	22	18
C17:0	4.7	2.7	2.1	2.5	2.6	1.8	2.8
C18:0	456	306	160	188	174	247	204
C18:1n-9	1680	2650	1670	3470	2870	5190	4160
C18:1n-7	55	130	99	200	170	290	250
C18:2n-6	1300	1500	1200	1700	1500	2300	1900
C18:3n-3	120	330	220	510	410	770	620
C20:0	24	30	22	38	32	56	46
C20:1n-9	15	42	32	65	52	98	78
C22:0	11	16	12	22	18	30	25
C22:4n-6	7.0	8.4	5.7	10	7.9	13	11
C24:1	0.0	0.0	2.4	8.2	6.3	12	9.9
Total	5186.0	6090.0	3772.9	6853.5	5851.5	9764.2	7961.8
LA/ALA	10.6	4.6	4.7	3.4	3.7	2.9	3.1
SAFA	1959.5	1353.4	712.0	862.9	820.8	1086.8	917.6
MUFA	1754.4	2828.7	1814.3	3759.2	3109.1	5618.2	4510.6
PUFA	1428.8	1884.2	1240.7	2231.4	1921.6	3059.2	2533.6

SAFA, saturated fatty acids (C12:0, C14:0, C16:0, C17:0, C18:0, C20:0, C22:0); MUFA, monounsaturated fatty acids (C16:1n-7, C18:1n-9, C18:1n-7, C20:1n-9, C24:1); PUFA, polyunsaturated fatty acids (C18:2n-6, C18:3n-3, C22:4n-6). Abbreviations, see Table 1.

Feed and Fatty Acid Analysis

Dry matter, ash, crude protein, crude fiber, and ether extract for each diet were determined as previously described (Kalmendal and Tauson, 2012). The glucosinolates were analyzed according to ISO method-106331 (ISO, 1995). The total lipids from feed, breast muscle, and drumstick muscle were extracted according to the method of Hara and Radin (1978). Fatty acid methyl esters (**FAME**) from the feed and muscle tissues were prepared according to Appelqvist (1968), and FAME were analyzed by gas-liquid chromatography (**GC**) using a CP-3800 GC instrument (Varian AB, Stockholm, Sweden) equipped with a fused silica capillary column BPX 70 (50 m, 0.25 mm inner diameter, 0.25 μm film thickness, SGE, Austin, TX; Fredriksson Eriksson and Pickova, 2007). Peaks were identified by comparing their retention times with those of corresponding fatty acids in the standard mixture GLC-68 A (Nu-chek Prep, Elysian, MN). Peak areas were integrated using Star chromatography workstation software version 5.5 (Varian AB, Stockholm, Sweden), and fatty acids were quantified using the internal standard methyl-15-methylheptadecanoate (Larodan Fine Chemicals AB, Malmö, Sweden).

Gene Expression Analysis

Gene expression in the liver was investigated by quantitative real-time PCR using an array of target

Table 3. Primers used for gene expression analysis.

Gene	Accession no.	Forward (5′-3′)	Reverse (5′-3′)	Amplicon (bp)
FADS1	XM_420557	GAGCCATCGGTGAGGGTTTC	CTCCAGTCCTTTCCTTGCGT	186
FADS2	NM_001160428	ACTGGTGGAACCATCGTCAC	GCAGAGGTGGGAAGATGAGG	189
L-CPT1	AY675193	GCCCTGATGCCTTCATTCAA	ATTTTCCCATGTCTCGGTGA	60
ACOX1	NM_001006205	CCAGTCAGCTTGGTAGAGGC	AGTGACAGTGTGCCTCAGATG	160
GAPDH	NM_204305	GCTAAGGCTGTGGGGAAAGT	TCAGCAGCAGCCTTCACTAC	161
β-actin	NM_205518	AAATCAAGATCATTGCCCCACCT	AGGGGTGTGGGTGTTGGTAA	181
SREBP2	AJ310769	AGCCTCAGATCATCAAGACG	TTCCATTGCTCCCAACAAGG	153
FTO	EU751607.1	TAGTGATTGGAACCTGAAGG	CATCAAGCATCAAGTAGAGG	128
L-FABP	NM_204192.3	GAAGGGTAAGGACATCAA	TCGGTCACGGATTTCAGC	219
LPL	NM_205282	TTGGTGACCTGCTTATGCTA	ATTGCTGCCTCTTCTCCTTT	187

gene (Table 3) coding for enzymes involved in lipid homeostasis. Total RNA was isolated from the liver using the SV Total RNA Isolation System according to the manufacturer's instructions (Z3105, Promega, Madison, WI). The extracted total RNA was quantified using a GeneQuant^{TM} 1300 spectrophotometer (GE Healthcare Life Sciences, Uppsala, Sweden). The first strand cDNA was synthesized using a High-Capacity cDNA Archive kit (Applied Biosystems, part of Thermo Fisher Scientific, Waltham, MA). The reverse transcription (**RT**) reaction was performed on a Veriti® 96-well Fast Thermal Cycler (Applied Biosystems). The RT condition was as follows: one cycle at 37°C for 60 min and one cycle at 95°C for 5 minutes. cDNA samples were diluted 1:10 before use. Real-time PCR analysis was done using a Fast SYBR® Green Master Mix (Applied Biosystems) with the StepOnePlus^{TM} Real-time PCR System (Applied Biosystems). PCR reaction mixtures (20 μL) consisted of one μL of each forward and reverse primer (final concentration 0.5 μM), 2 μL cDNA, and 10 μL master mix. The reaction was incubated at 95°C for 3 min, 40 cycles of 95°C for 10 s, and 60°C for 30 s, followed by a melt curve analysis to ensure only a single product was amplified. NADPH and β-actin were evaluated for their stability across all experimental samples, and NADPH was chosen as the most stable reference gene. On each plate, samples from all treatments were analyzed in triplicate for one target gene, the reference gene, NADPH, and the nontemplate control. The ΔC_T for each sample was calculated per plate by subtracting the C_T value for the reference gene (NADPH) from the C_T for the target gene. The relative expression ($\Delta\Delta C_T$) was calculated by subtracting the ΔC_T values for group fed the control diet from the ΔC_T values for each experimental diet and presented using the term $2^{-\Delta\Delta CT}$ and reported as arbitrary fold change units (Livak and Schmittgen, 2001).

Statistical Analysis

A completely randomized design with 7 dietary treatments (Control, RS8N, RS8P, RS16N, RS16P, RS24N, and RS24P) with 5 replicates was used. The statistical analysis was carried out using the one-way ANOVA of GLM procedure of SAS. Least squared means (**LSM**) were calculated, and the option probability of differences (**PDIFF**) was used for multiple comparisons.

RESULTS

Meat Yield

The body weight (d 34), the breast and drumstick weight, and feed intake (d 34) of birds fed with different diets are shown in Table 4. Compared with the control diet, the nonpelleted RS diets decreased ($P < 0.05$) body weight and breast and drumstick weight with increasing inclusion levels of RS (RS24N<RS8N/RS16N<Control). In contrast, there was no difference ($P > 0.05$) between chickens fed the steam-pelleted RS diets and chickens fed the control diet in body weight or breast and drumstick weight. The feed intake (d 34) was reduced ($P < 0.05$) in chickens fed the nonpelleted diets RS8N and RS24N compared with chickens fed the control. However, chickens fed steam-pelleted RS diets had similar feed intake to chickens fed the control diet. At the same RS inclusion level, chickens fed the steam-pelleted diets had higher body weight ($P < 0.05$) and lower feed intake ($P < 0.05$) than those fed nonpelleted diets. However, only chickens in the steam-pelleted RS24P group showed significantly higher weight values of breast and drumstick compared with the nonpelleted group (RS24N).

Fatty Acid Composition in Breast and Drumstick Muscles

The fatty acid composition (mg/100 g fresh meat) in breast and drumstick muscles is shown in Tables 5 and 6, respectively. Dietary treatments did not affect ($P > 0.05$) the contents of total FA in these muscles, but significantly affected their fatty acid composition.

The saturated fatty acids (**SAFA**) content in breast muscle was not affected by dietary treatments ($P = 0.2129$), whereas the RS24P diet significantly decreased the SAFA content in the drumstick compared with other diets. Similarly, like the major component of SAFA, palmitic acid (C16:0) was lower in drumsticks from chickens fed the RS24P diet than that in chickens fed other diets. In both the breast and drumstick, no significant dietary effects were observed for the content

Table 4. Body weight (g), feed intake (g) at d 34, breast and drumstick weight (g) in chickens fed 7 different diets (n = 5, least square means).

	Control	RS8N	RS8P	RS16N	RS16P	RS24N	RS24P	Pooled SEM	*P*-value
Body weight (d 34)	2072^{a}	1824^{b,c}	1993^{a}	1692^{c}	2072^{a}	1114^{d}	1947^{a,b}	50.9	<0.0001
Feed intake (d 34)	3157^{a,b}	2857^{c}	3330^{a}	2915^{b,c}	3292^{a}	2686^{c}	3380^{a}	88.0	<0.0001
Breast weight	578^{a}	449^{b}	524^{a,b}	469^{b}	520^{a,b}	336^{c}	530^{a,b}	31.1	0.0003
Drumstick weight	229^{a}	176^{b,c}	198^{a,b}	199^{a,b}	200^{a,b}	158^{c}	211^{a}	11.7	0.0058

$^{a-c}$Different letters within the same row indicate significant difference at the $P < 0.05$ level.
Pooled SEM, pooled standard error of the mean.
Abbreviations, see Table 1.

Table 5. Fatty acid composition of breast muscle in chickens fed 7 different diets (mg/100 g fresh weight, n = 5; least square means).

	Control	RS8N	RS8P	RS16N	RS16P	RS24N	RS24P	Pooled SEM	*P*-value
C14:0	4.3^{a}	2.5^{b,c}	2.8^{a,b}	3.0^{a,b}	2.6^{b,c}	3.3^{a,b}	1.7^{c}	0.48	0.0112
C15:0	0.6	0.4	0.5	0.7	0.5	0.8	0.7	0.10	0.0951
C16:0	153.5	104.6	101.1	124.8	99.6	150.9	90.5	20.53	0.1573
C16:1n-9	3.2	2.8	2.6	3.9	3.5	4.7	3.0	0.49	0.0781
C16:1n-7	21.1^{a}	15.2^{a,b}	10.0^{b}	20.2^{a}	9.4^{b}	22.1^{a}	5.7^{b}	3.36	0.0067
C17:0	0.4^{b,c}	0.2^{c}	0.4^{b,c}	0.5^{b,c}	0.5^{b,c}	0.8^{a,b}	1.0^{a}	0.12	0.0021
C18:0	51.5	41.7	39.8	46.1	44.3	55.8	50.2	4.91	0.2657
C18:1n-9	210.9	144.2	145.8	216.8	205.3	245.9	192.5	30.40	0.0858
C18:1n-7	21.0	21.1	17.7	23.6	21.0	25.8	20.8	1.83	0.1054
C18:2n-6	105.2	78.3	79.5	92.2	107.4	110.3	88.6	11.17	0.2449
C18:3n-3	6.1^{c,d}	4.9^{d}	7.0^{b,c,d}	10.1^{a-c}	17.3^{a}	11.2^{a,b}	12.5^{a,b}	2.19	0.0021
C20:1n-9	2.7	2.8	2.7	3.3	3.1	3.2	2.6	0.33	0.5793
C20:2n-6	3.1	2.7	3.0	2.7	3.3	2.6	3.0	0.28	0.6657
C20:3n-6	4.0^{b,c}	5.5^{a}	4.6^{a,b}	4.5^{b,c}	3.6^{c,d}	4.4^{b,c}	2.9^{d}	0.35	0.0007
C20:4n-6	19.4^{c}	18.7^{c}	21.1^{b,c}	18.4^{c}	24.8^{a-c}	27.3^{a,b}	28.1^{a}	2.37	0.0206
C20:5n-3	1.8^{c}	3.9^{a,b}	3.6^{a,b}	3.9^{a}	4.4^{a}	4.1^{a}	3.0^{b}	0.31	<0.0001
C22:4n-6	4.3	3.4	3.9	3.3	4.3	4.1	4.7	0.41	0.2517
C22:5n-3	3.5^{f}	4.7^{e,f}	6.1^{d,e}	6.5^{c,d}	10.9^{a,b}	8.5^{b,c}	12.7^{a}	0.94	<0.0001
C22:6n-3	3.5^{e}	4.1^{d,e}	5.5^{c,d}	5.9^{b,c}	8.1^{b}	7.0^{b,c}	13.5^{a}	0.88	<0.0001
SAFA	212.6	150.5	147.0	174.4	149.4	212.7	143.0	25.86	0.2129
MUFA	261.3	190.8	184.1	272.2	248.7	306.2	234.6	35.46	0.0973
PUFA	153.2	127.6	136.7	150.4	187.0	182.0	173.7	16.32	0.1072
n-3 LCPUFA	8.9^{e}	12.7^{d}	15.2^{c,d}	16.3^{c,d}	23.5^{a,b}	19.6^{b,c}	29.2^{a}	1.86	<0.0001
n-3	15.5^{e}	18.3^{d,e}	23.4^{c,d}	28.0c	42.5^{a,b}	31.7^{b,c}	44.9^{a}	3.51	<0.0001
n-6	137.6	109.1	113.1	122.2	144.1	150.1	127.8	13.21	0.2589
n-6/n-3	8.8^{a}	5.9^{b}	4.8^{c}	4.3^{d}	3.4^{e}	4.7^{c,d}	2.8^{f}	0.21	<0.0001
Total FA	656.5	492.5	478.9	617.8	595.2	731.9	586.2	70.52	0.1906

$^{a-f}$Different letters within the same row indicate significant difference at the $P < 0.05$ level.
SAFA, saturated fatty acids (C14:0, C15:0, C16:0, C17:0, C18:0); MUFA, monounsaturated fatty acids (C16:1n-9, C16:1n-7, C18:1n-9, C18:1n-7, C20:1n-9); PUFA, polyunsaturated fatty acids (C18:2n-6, C18:3n-3, C20:2n-6, C20:3n-6, C20:4n-6, C20:5n-3, C22:4n-6, C22:5n-3, C22:6n-3); n-3, n-3 polyunsaturated fatty acids (C18:3n-3, C20:5n-3, C22:5n-3, C22:6n-3); n-6, n-6 polyunsaturated fatty acid (C18:2n-6, C20:2n-6, C20:3n-6, C20:4n-6, C22:4n-6); n-3 LC-PUFA, EPA (C20:5n-3)+DPA(C22:5n-3)+DHA (C22:6n-3); total FA, total fatty acid. C18:2 n-6, LA; C18:3 n-3, ALA; C20:4 n-6, AA; C20:5 n-3, EPA; C22:5 n-3, DPA; C22:6 n-3, DHA.
Abbreviations, see Table 1.

of monounsaturated fatty acid (**MUFA**) and its major proportion — oleic acid (C18:1 n-9). Diets RS16P and RS24P increased ($P < 0.05$) the content of PUFA in drumsticks compared with the control diet, whereas no significant dietary effects were observed for the PUFA content in breasts. The content of n-3 PUFA in both muscles was highest in chickens fed diet RS24P or RS16P, followed by those fed diets RS24N, RS16N, and RS8P, with the lowest content in chickens fed the RS8N or control diet. In drumsticks, the content of ALA, the main member of n-3 PUFA, followed the same change as n-3 PUFA, whereas, for breasts only, chickens fed the RS16P, RS24N, and RS24P diets had higher ($P < 0.05$) levels of ALA content than chickens fed the control diet. In both breasts and drumsticks, the contents of n-3 LC-PUFA, docosapentaenoic acid (**DPA**; C22:5 n-3) and DHA increased significantly in a graded manner with increasing inclusion levels of RS, with the highest in groups RS16P and RS24P, followed by RS24N, RS16N, RS8P, and RS8N, and the lowest in the control group. At the RS inclusion levels of 16 and 24%, steam-pelleted diets significantly increased the contents of n-3 PUFA, n-3 LC-PUFA, DPA, and DHA in both breast and drumstick muscles compared with nonpelleted diets with the same level of RS inclusion. Compared to chickens fed the control diet, those fed RS diets either in steam-pelleted form or in nonpelleted form had higher levels of EPA in breasts and drumsticks. In addition, chicken drumstick meat had higher n-3 LC-PUFA and ALA content than did the breast meat, and chickens

Table 6. Fatty acid composition of drumstick muscle in chickens fed 7 different diets (mg/100 g fresh weight, n = 5, least square means).

	Control	RS8N	RS8P	RS16N	RS16P	RS24N	RS24P	Pooled SEM	*P* value
C14:0	12.7^{a}	10.1^{a,b}	11.2^{a,b}	10.1^{a,b}	8.9^{b}	11.3^{a,b}	5.7^{c}	1.55	0.0015
C15:0	1.6^{b}	1.5^{b}	1.6^{b}	1.9^{a,b}	1.8^{b}	2.3^{a}	1.7^{b}	0.16	0.0173
C16:0	394.7^{a}	406.1^{a}	349.5^{a}	420.7^{a}	352.2^{a}	411.8^{a}	229.8^{b}	32.28	0.0037
C16:1n-9	8.7^{c}	10.7^{b,c}	10.4^{b,c}	13.1^{a,b}	13.4^{a,b}	15.7^{a}	13.7^{a,b}	1.24	0.0069
C16:1n-7	64.5$^{a–c}$	82.3^{a,b}	58.1^{b,c}	90.0^{a}	52.6^{c}	76.9$^{a–c}$	18.5^{d}	9.68	0.0004
C17:0	1.6^{b}	1.5^{b}	1.4^{b}	1.7^{b}	2.2^{a,b}	2.8^{a}	2.9^{a}	0.29	0.0029
C18:0	130.0	130.9	122.9	132.8	128.5	148.8	116.7	9.71	0.4252
C18:1n-9	568.3	612.7	588.6	753.5	809.8	759.5	714.4	64.30	0.0731
C18:1n-7	42.0	51.9	44.5	56.0	55.3	55.7	53.0	4.24	0.1382
C18:2n-6	259.8^{b}	235.3^{b}	252.9^{b}	270.6^{b}	370.3^{a}	288.8^{b}	367.0^{a}	23.90	0.0010
C18:3n-3	17.4^{d}	23.5^{c,d}	31.5^{b,c}	38.9^{b}	70.6^{a}	40.0^{b}	77.9^{a}	4.71	<0.0001
C20:1n-9	6.5^{c}	7.7^{b,c}	7.6^{b,c}	9.3^{a,b}	10.9^{a}	9.0^{a,b}	10.5^{a}	0.77	0.0033
C20:2n-6	4.1^{a,b}	3.4^{b}	3.6^{b}	3.7^{b}	4.9^{a}	3.9^{a,b}	4.9^{a}	0.36	0.0251
C20:3n-6	6.3	7.3	6.5	7.5	6.5	7.5	5.7	0.54	0.0935
C20:4n-6	38.0^{c}	39.4^{b,c}	41.2^{b,c}	37.2^{c}	47.0$^{a–c}$	50.7^{a,b}	53.5^{a}	3.89	0.0299
C20:5n-3	2.0^{c}	4.8^{a,b}	4.2^{b}	5.4^{a,b}	5.8^{a}	5.4^{a,b}	4.8^{a,b}	0.41	<0.0001
C22:4n-6	9.1	7.8	8.1	7.2	8.0	8.5	7.5	0.68	0.5774
C22:5n-3	6.8^{d}	9.9^{c,d}	11.1^{c}	11.1^{c}	18.7^{a,b}	15.2^{b}	19.5^{a}	1.24	<0.0001
C22:6n-3	4.9^{d}	6.3^{c,d}	7.8^{c}	7.5^{c}	11.7^{b}	11.3^{b}	17.8^{a}	1.04	<0.0001
SAFA	545.0^{a}	556.2^{a}	493.9^{a}	570.4^{a}	499.7^{a}	581.2^{a}	361.3^{b}	42.86	0.0182
MUFA	693.4	768.2	711.4	924.6	947.0	923.7	817.3	78.43	0.1278
PUFA	352.1^{b}	341.8^{b}	369.7^{b}	392.4^{b}	546.9^{a}	435.1^{b}	561.5^{a}	34.09	0.0001
n-3 LCPUFA	13.6^{d}	21.0^{c}	23.1^{c}	24.0^{c}	36.2^{a,b}	31.9^{b}	42.1^{a}	2.35	<0.0001
n-3	30.7^{d}	43.8^{c,d}	54.9^{b,c}	63.1^{b,c}	106.3^{a}	71.5^{b}	120.1^{a}	6.43	<0.0001
n-6	320.4^{b}	296.4^{b}	314.1^{b}	328.8^{b}	439.0^{a}	362.3^{a,b}	440.6^{a}	27.97	0.0024
n-6/n-3	10.3^{a}	6.5^{b}	5.7^{c}	5.2^{d}	4.1^{e}	5.0^{d}	3.6^{f}	0.23	<0.0001
Total FA	1607.2	1658.7	1579.7	1894.4	1993.4	1952.3	1741.4	148.00	0.2892

$^{a-f}$Different letters within the same row indicate significant difference at the $P < 0.05$ level.

SAFA, saturated fatty acids (C14:0, C15:0, C16:0, C17:0, C18:0); MUFA, monounsaturated fatty acids (C16:1n-9, C16:1n-7, C18:1n-9, C18:1n-7, C20:1n-9); PUFA, polyunsaturated fatty acids (C18:2n-6, C18:3n-3, C20:2n-6, C20:3n-6, C20:4n-6, C20:5n-3, C22:4n-6, C22:5n-3, C22:6n-3); n-3, n-3 polyunsaturated fatty acids (C18:3n-3, C20:5n-3, C22:5n-3, C22:6n-3); n-6, n-6 polyunsaturated fatty acid (C18:2n-6, C20:2n-6, C20:3n-6, C20:4n-6, C22:4n-6); n-3 LC-PUFA, EPA (C20:5n-3)+DPA(C22:5n-3)+DHA (C22:6n-3); total FA, total fatty acid. C18:2 n-6, LA; C18:3 n-3, ALA; C20:4 n-6, AA; C20:5 n-3, EPA; C22:5 n-3, DPA; C22:6 n-3, DHA.

Abbreviations, see Table 1.

fed the steam-pelleted RS24P diet contained the highest level of n-3 LC-PUFA in breasts (29.2 mg/100 g) and drumsticks (42.1 mg/100 g).

Compared to chickens fed the control diet, chickens consuming different RS diets had similar amounts of n-6 PUFA and LA in the breast. In the drumstick, higher levels of n-6 PUFA and LA were found only in chickens fed the RS16P and RS24P diets compared with chickens fed the other diets. In both muscles, the contents of arachidonic acid (**AA**; C20:4 n-6) from chickens fed the RS24N or RS24P diet were higher than the control diet fed chickens. Regarding the ratio of n-6 to n-3, when diets were subjected to nonpelleted treatment, the n-6/n-3 ratio in both muscles decreased significantly with increasing RS inclusion levels from zero to 16% with no further decrease from 16 to 24%; however, when diets were subjected to steam-pelleted treatment, this ratio decreased significantly with increasing RS inclusion levels from zero to 24%. Furthermore, steam-pelleted diets fed chickens had a lower n-6/n-3 ratio than chickens fed nonpelleted diets with the same RS inclusion level.

Gene Expression

The hepatic expression of ACOX1, L-CPT1, L-FABP1, FADS1, FADS2, FTO, LPL, and SREBP2 are shown in Table 7. Compared with the control group, the ACOX1 mRNA level was significantly higher in chickens fed the RS24P diet. RS16P and RS24P diets fed chickens had higher ($P < 0.05$) levels of FADS1 than chickens fed the control diet. For other examined genes, no significant differences were observed in RS diets compared with the control diet.

DISCUSSION

Effect of Dietary Treatments on Fatty Acid Composition in Meat

In this study, dietary treatments significantly affected the fatty acid composition in breast and drumstick muscles, but did not affect the total fatty acid content. In monogastric animals, such as chickens, dietary FA are to a large extent incorporated unchanged into body fat (Mourot et al., 2001). As a result, the fatty acid composition of chicken tissue lipids is easily affected by the fatty acid composition of dietary lipid (Haug et al., 2007; Nyquist et al., 2012), which is amply confirmed in the present study.

As the RS inclusion level increased in our experimental diets, increase in ALA content was found to be the main change among different diets. These differences

Table 7. Dietary effect on the relative expression of lipid related genes in liver from chickens fed 7 different diets (n = 5, fold change, least square means).

	Control	RS8N	RS8P	R16N	R16P	R24N	R24P	Pooled SEM
ACOX1	1.00	1.10	1.40	1.02	1.55	1.12	1.93*	0.280
L-CPT1	1.00	0.43	0.64	1.38	0.87	0.48	1.13	0.560
L-FABP	1.00	0.75	1.92	0.75	0.94	1.08	1.50	0.328
FADS1	1.00	1.58	1.71	1.34	1.97*	1.70	2.05*	0.332
FADS2	1.00	0.93	0.88	0.84	0.81	0.70	0.91	0.145
FTO	1.00	1.33	1.37	1.07	1.20	1.62	1.35	0.321
LPL	1.00	0.69	0.85	0.87	0.77	0.97	1.07	0.145
SREBP2	1.00	1.34	1.54	1.23	2.22	1.81	1.83	0.534

*Indicates significant difference compared with the control group at the level of $P < 0.05$. A value less than 1 down-regulation and more than 1 up-regulation. Abbreviations, see Table 1.

can be attributed to the inclusion of RS in these diets because RS contains about 40% oil, which is an excellent source of ALA (Ajuyah et al., 1991a). The de novo biosynthesis pathway to produce ALA and LA in higher animals does not exist; ALA and LA are essential fatty acids and must therefore be provided in their diets. The ALA content in the edible tissues of poultry is readily increased by increasing concentration of ALA in their diets, particularly meat with skin, and dark meat to a greater extent than white meat (Rymer and Givens, 2005). In agreement with this, this study showed that the ALA contents in breasts and drumsticks from chickens increased when high levels of RS (160 or 240 g/kg) were included in the diets, with the highest content in chickens fed the RS24P diet. Similarly, an increase in ALA content also was observed in a study on turkey breast when fed a diet containing 10% RS (Ajuyah et al., 1993). Furthermore, our study also showed an increase in EPA, DPA, and DHA contents in both muscles with increasing levels of ALA in chicken diets, leading to highest concentration of n-3 LC-PUFA in chickens fed the RS24P diet. This is explained by the existing pathway in chickens to produce DHA from ALA through a series of elongation and desaturation steps. Thus, as RS inclusion level increased, more ALA would be readily ingested and absorbed by birds and subsequently converted to its longer chain metabolites (EPA, DPA, and DHA). This agrees well with a previous study, which showed that broiler chickens fed a diet containing 5% RS oil increased the levels of EPA and DPA in the breast, compared with chickens fed a diet with 5% soybean oil (Nyquist et al., 2012). In contrast, a significant increase in AA contents in chicken meat was observed only in chickens fed the highest level of RS (24%), and this could be explained by the increase in the content of its precursor — LA in these diets. The presence of a considerable amount of ALA in RS, despite an excess of LA, could account for the lack of a significant conversion of LA to AA and be due to the competition of ALA with LA for the same desaturase and elongase enzymes. In addition, although inclusion of RS decreased the SAFA and increased PUFA contents in chicken diets, a significant decrease in SAFA content was observed only in drumsticks from chickens fed the RS24P diet, and no significant dietary effects were observed for the MUFA content in both muscles. Therefore, this study showed that feeding a chicken diet containing a high level of RS could increase the content of n-3 PUFA in breast and drumstick meat, in particular, DPA and DHA, but barely affect that of n-6, MUFA, or SAFA.

In this study, at the RS inclusion levels of 160 and 240 g/kg, steam-pelleted diets contained relatively less ALA and LA compared with nonpelleted diets with the same RS level. However, chickens fed those steam-pelleted diets had similar levels of ALA and LA in the breast, higher levels of ALA and LA in the drumstick, and higher levels of n-3 LC-PUFA, particularly DPA and DHA, in both muscles, compared with chickens fed nonpelleted diets with the same RS inclusion levels. Moreover, at the same RS inclusion level, n-6/n-3 ratio was found to be lower in both muscles from chickens fed steam-pelleted diets compared with chickens fed nonpelleted diets, with the lowest in chickens fed the RS24P diet. These differences could be explained by the increase in feed intake and possible improvement of fat availability for steam-pelleted diets. The feed intake in chickens consuming the nonpelleted RS diets was significantly lower than those fed the steam-pelleted diets at the same inclusion level (Table 4). The steam-pelleting process was shown to enhance oil digestibility (Shen et al., 1983). As a result, in this study it could be deduced that chickens fed those steam-pelleted diets had more ALA digested and absorbed, more of which were then metabolized to LC-PUFA through chain elongation and desaturation. To our knowledge, this is the first study to show that at high inclusion levels of RS, the steam-pelleting process increased chicken meat nutritional value in terms of n-3 fatty acid content and n-6/n-3 ratio. In addition, the n-3 LC-PUFA and ALA content in drumstick meat was much higher than that in breast meat from chickens with the same treatment. This finding is probably attributed to the higher lipid content of dark meat compared with white meat, although white meat has higher enrichment efficiency than dark meat for DHA (Givens and Gibbs, 2008). A similar observation was made for turkey dark (leg) and white (breast) meat (Ratnayake, 1989).

The western diet has been estimated to be deficient in n-3 FA with a high ratio of n-6 to n-3 of 15 to 20/1

(Simopoulos, 2006). Excessive amounts of n-6 PUFA and a very high n-6/n-3 ratio, promote pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas increased levels of n-3 FA, i.e., a lower n-6/n-3 ratio, exerts suppressive effects (Simopoulos, 2008). In particular, there is mounting evidence supporting the beneficial effects of an increased intake of EPA and DHA (Howe et al., 2006; Calder, 2014). Moreover, the bioactive effects of DPA have been gaining recognition in the literature (Kaur et al., 2011). A level of 250 mg/d was proposed by the EFSA as the labeling reference intake value of EPA and DHA for adults to prevent cardiovascular disease. As shown in this study, chickens fed the steam-pelleted RS24 diet provided the most nutritious meat in terms of n-3 PUFA content, particularly n-3 LC-PUFA content and n-6/n-3 ratio. These chickens had the highest content of n-3 PUFA in their meat, with 44.9 mg/100 g fresh meat in the breast and 120.1 mg/ 100 g in the drumstick, and these levels were about 3 times as much as in chickens fed the control nonpelleted diet. Furthermore, a 100-g proportion of these breast or drumstick meats would provide consumers with 29.2 mg or 42.1 mg n-3 LC-PUFA, respectively, compared with 8.9 or 13.6 mg from chickens fed the control nonpelleted diet. Besides, the n-6/n-3 ratios in these breast and drumstick meats were 2.8 and 3.6, respectively, which were also superior to meat from chickens fed the control nonpelleted diet (8.8 for breasts and 10.3 for drumsticks). In comparison, chickens fed diets containing 40 g/kg fishmeal were reported to contain 58.6 and 67.3 mg/100 g n-3 LC-PUFA in the breast and in thigh, respectively (Ratnayake, 1989). Therefore, feeding chickens a steam-pelleted, RS-included diet can be a great strategy to increase human intake of n-3 PUFA, in particular n-3 LC-PUFA, while maintaining sustainability and reducing feeding cost.

Effect of Dietary Treatments on Lipid-related Gene Expression In Liver

Dietary lipids are digested and absorbed from the intestine and transported in the blood to the liver and other tissues in the form of portomicrons (Hermier and Suppl, 1997). In this study, the effects of dietary treatments on the abovementioned genes were examined. The hepatic expression of the FADS1 gene was upregulated in chickens fed the RS16P and RS24P diets compared with that in the control diet fed chickens. This finding agrees with a previous study in which an ALA-enriched diet containing 75% RS oil increased the expression of hepatic FADS1 in salmon compared with control fed 100% fish oil (Jordal et al., 2005). The biosynthesis of n-3 LC-PUFA from ALA includes a series of desaturation, elongation, and, ultimately β-oxidation, reactions (Dyall, 2015). One of the 2 rate-limiting steps in this pathway is the desaturation of eicosatetraenoic acid (20:4n-3) to EPA (20:5n-3), which is determined by the activity of Δ5 desaturase encoded by the FADS1 gene (Cho et al., 1999). The formation of n-3 LC-PUFA depends on the amount of substrate and removal of the subsequent products, and increased desaturase gene expression could be influenced by either increased dietary concentration substrates or removal or absence of the products (Mirshekar et al., 2015). Therefore, the increase of hepatic FADS1 gene expression observed in our study could be due to the increased dietary ALA content in the RS diets. Moreover, the higher expression of the FADS1 gene in RS diet fed chickens could, at least in part, account for the higher EPA content in meat observed in chickens fed RS16P and RS24P diets compared with chickens fed the control diet. In addition, Δ6 desaturase is regarded as another time-limiting step in the n-3 LC-PUFA biosynthesis pathway (Zheng et al., 2004); however, the mRNA level of the FADS2 gene was not affected by any dietary treatments in this study.

Compared to the control diet, the RS24P diet up-regulated the expression of the ACOX1 gene in chicken liver. The final step in the pathway of DHA synthesis occurs in peroxisomes through a single round β-oxidation of tetracosahexaenoic acid (24:6n-3) (Wanders, 2004; Dyall, 2015). Acyl-coenzyme-A oxidase (ACOX1 gene) is the rate-limiting enzyme for this peroxisomal fatty acid β-oxidation (Ding et al., 2003). The increased hepatic expression of the ACOX1 gene in chickens fed the RS24P diet may be attributed to the increase in feed intake and possible improvement of fat availability for steam-pelleted diets, which led to the increased levels of ALA. Consequently, the higher levels of ALA might trigger a higher expression of the ACOX1 gene, leading to the increased formation of DHA in chickens fed the steam-pelleted diets, compared with those fed the control diet.

Effect of Dietary Treatments on Meat Yield

The breast and drumstick are the 2 most important parts of a chicken carcass. The breast consists mainly of white muscle and the drumstick of dark muscles. In this study, breasts with ribs and drumsticks were weighed to reflect meat yield of chicken carcasses. Feeding steam-pelleted diets did not affect breast and drumstick weights of broiler chickens with increasing RS inclusion levels. This is in agreement with the changes in chicken body weight, showing that increasing inclusion levels of RS did not affect live weight at d 34 for chickens fed steam-pelleted diets, but did reduce live weight for chickens fed nonpelleted diets. Overall, our study showed that steam pelleting enables high inclusion levels (160 or 240 g/kg) of RS in chicken feed without adversely affecting chicken meat yield as reflected by the weights of breasts and drumsticks. The compromise in meat yield and live weight from chickens receiving the nonpelleted feed may be caused by the decrease in feed intake for those chickens observed in this study.

Similarly, Summers et al.(1982) reported that feeding 17.5% full-fat canola seed to broiler chickens led to depressed fat utilization and body weight gain. When fed high levels of RS, growth performance of broilers can be affected by degradation products of glucosinolate in seeds. The glucosinolate degradation products can interfere with the function of the thyroid gland and decrease feed palatability leading to undesirable effects on growth performance (McCurdy, 1990; Khajali and Slominski, 2012). However, the total content of glucosinolates was below 4 μmol/g in all diets (Table 1), which has been considered as a safe limit for chickens (Khajali and Slominski, 2012). Another explanation could be attributed to the reduced energy use from rapeseed in nonpelleted form due to lower oil availability. The lower availability has been suggested to result from the oil-encapsulating effect of the cell wall polysaccharides (Lee et al., 1991). On the other hand, the steam-pelleting process can improve the fat availability in RS (Shen et al., 1983; Salmon et al., 1988; Barekatain et al., 2015). Additionally, in comparison to nonpelleted diets, steam-pelleting has been shown to enhance oil digestibility, possibly due to the efficient pulverization effect of the steam-pelleting process (Shen et al., 1983).

In conclusion, this study showed that the steam-pelleting process enables high inclusion levels of RS (160 or 240 g/kg) in chicken diets and leads to significant increases in n-3 PUFA content in the meat, particularly n-3 LC-PUFA, without compromising the meat yield or total fatty acid content. Further, genes responsible for fatty acid desaturation and peroxisomal β-oxidation were affected when chickens were fed steam-pelleted diets with high inclusion levels of RS. In conclusion, feeding chickens steam-pelleted high RS-included diets can be a feasible strategy to increase human intake of n-3 LC-PUFA without compromising meat yield of the chickens.

ACKNOWLEDGMENTS

We would like to thank Galia Zamaratskaia (University of Agricultural Sciences, Uppsala, Sweden) for her great suggestions in the statistical analysis, and also Monika Johansson, Janak K. Vidanarachchi, and Pedro Gómez Requeni (University of Agricultural Sciences, Uppsala, Sweden) for their assistance in sample collection.

REFERENCES

Ajuyah, A. O., K. H. Lee, R. T. Hardin, and J. S. Sim. 1991a. Changes in the yield and in the fatty acid composition of whole carcass and selected meat portions of broiler chickens fed full-fat oil seeds. Poult. Sci. 70:2304–2314.

Ajuyah, A. O., K. H. Lee, R. T. Hardin, and J. S. Sim. 1991b. Influence of dietary full-fat seeds and oils on total lipid, cholesterol and fatty acid composition of broiler meats. Can. J. Anim. Sci. 71:1011–1019.

Ajuyah, A. O., R. T. Hardin, and J. S. Sim. 1993. Studies on canola seed in turkey grower diet: Effects on ω-3 fatty acid composition of breast meat, breast skin and selected organs. Can. J. Anim. Sci. 73:177–181.

Appelqvist, L. Å. 1968. Rapid methods of lipid extraction and fatty acid methyl ester preparation for seed and leaf tissue with special remarks on preventing the accumulation of lipid contaminants. Arkiv För Kemi. 28:551–570.

Assadi, E., H. Janmohammadi, A. Taghizadeh, and S. Alijani. 2011. Nutrient composition of different varieties of full-fat canola seed and nitrogen-corrected true metabolizable energy of full-fat canola seed with or without enzyme addition and thermal processing. J. Appl. Poult. Res. 20:95–101.

Barekatain, M. R., S. B. Wu, M. Toghyani, and R. A. Swick. 2015. Effects of grinding and pelleting condition on efficiency of full-fat canola seed for replacing supplemental oil in broiler chicken diets. Anim. Feed Sci. Technol. 207:140–149.

Bhalerao, S., M. Hegde, S. Katyare, and S. Kadam. 2014. Promotion of omega-3 chicken meat production: An Indian perspective. Worlds Poult. Sci. J. 70:365–374.

Calder, P. C. 2014. Very long chain omega-3 (n-3) fatty acids and human health. Eur. J. Lipid Sci. Technol. 116:1280–1300.

Campbell, L. D., and B. A. Slominski. 1990. Extent of thermal decomposition of indole glucosinolates during the processing of canola seed. J. Am. Oil Chem. Soc. 67:73–75.

Cho, H. P., M. Nakamura, and S. D. Clarke. 1999. Cloning, expression, and fatty acid regulation of the human delta-5 desaturase. J. Biol. Chem. 274:37335–37339.

Ding, S. T., Y. C. Li, K. E. Nestor, S. G. Velleman, and H. J. Mersmann. 2003. Expression of turkey transcription factors and acyl-coenzyme oxidase in different tissues and genetic populations. Poult. Sci. 82:17–24.

Dyall, S. C. 2015. Long-chain omega-3 fatty acids and the brain: A review of the independent and shared effects of EPA, DPA and DHA. Front. Aging Neurosci. 7:1–15.

Fredriksson Eriksson, S., and J. Pickova. 2007. Fatty acids and tocopherol levels in M. Longissimus dorsi of beef cattle in Sweden - A comparison between seasonal diets. Meat Sci. 76:746–754.

Givens, D. I., and R. A. Gibbs. 2008. Current intakes of EPA and DHA in European populations and the potential of animal-derived foods to increase them. Proc. Nutr. Soc. 67:273–280.

Goldberg, I. J., R. H. Eckel, and N. A. Abumrad. 2008. Regulation of fatty acid uptake into tissues: Lipoprotein lipase- and CD36-mediated pathways. J. Lipid Res. 50:S86–S90.

Hara, A., and N. S. Radin. 1978. Lipid extraction of tissues with a low-toxicity solvent. Anal. Biochem. 90:420–426.

Haug, A., S. Eich-Greatorex, A. Bernhoft, J. P. Wold, H. Hetland, O. A. Christophersen, and T. Sogn. 2007. Effect of dietary selenium and omega-3 fatty acids on muscle composition and quality in broilers. Lipids Health Dis. 6:29–33.

Hermier, D., and M. Suppl. 1997. Lipoprotein metabolism and fattening in poultry. J. Nutr. 127:8055–8085.

Howe, P., B. Meyer, S. Record, and K. Baghurst. 2006. Dietary intake of long-chain omega-3 polyunsaturated fatty acids: contribution of meat sources. Nutr. 22:47–53.

ISO 10633-1. 1995. Oilseed Residues−Determination of Glucosinolates Content. Part 1: Method Using High-performance Liquid Chromatography. International Organization for Standardization, Geneva, Switzerland.

Jankowski, J., Z. Zdunczyk, D. Mikulski, J. Juskiewicz, J. Naczmanski, J. F. Pomianowski, and P. Zdunczyk. 2012. Fatty acid profile, oxidative stability, and sensory properties of breast meat from turkeys fed diets with a different n -6/ n -3 PUFA ratio. Eur. J. Lipid Sci. Technol. 114:1025–1035.

Jordal, A.-E. O., B. E. Torstensen, S. Tsoi, D. R. Tocher, S. P. Lall, and S. E. Douglas. 2005. Dietary rapeseed oil affects the expression of genes involved in hepatic lipid metabolism in Atlantic salmon (Salmo salar L.). J. Nutr. 135:2355–2361.

Kalmendal, R., and R. Tauson. 2012. Effects of a xylanase and protease, individually or in combination, and an ionophore coccidiostat on performance, nutrient utilization, and intestinal morphology in broiler chickens fed a wheat-soybean meal-based diet. Poult. Sci. 91:1387–1393.

Kaur, G., D. Cameron-Smith, M. Garg, and A. J. Sinclair. 2011. Docosapentaenoic acid (22:5n-3): A review of its biological effects. Prog. Lipid Res. 50:28–34.

Khajali, F., and B. A. Slominski. 2012. Factors that affect the nutritive value of canola meal for poultry. Poult. Sci. 91:2564–2575.

Lee, K. H., J. M. Olomu, and J. S. Sim. 1991. Live performance, carcass yield, protein and energy retention of broiler chickens fed canola and flax full-fat seeds and the restored mixtures of meal and oil. Can. J. Anim. Sci. 71:897–903.

Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 25:402–408.

McCurdy, S. M. 1990. Effects of processing on the functional properties of canola/rapeseed protein. J. Am. Oil Chem. Soc. 67:281–284.

Mirshekar, R., F. Boldaji, B. Dastar, A. Yamchi, and S. Pashaei. 2015. Longer consumption of flaxseed oil enhances n-3 fatty acid content of chicken meat and expression of FADS2 gene. Eur. J. Lipid Sci. Technol. 117:810–819.

Mourot, J., D. Hermier, J. M. Ourota, and D. H. Ermierb. 2001. Lipids in monogastric animal meat. Reprod. Nutr. 41:109–118.

Murai, A., M. Furuse, K. Kitaguchi, K. Kusumoto, Y. Nakanishi, M. Kobayashi, and F. Horio. 2009. Characterization of critical factors influencing gene expression of two types of fatty acid-binding proteins (L-FABP and Lb-FABP) in the liver of birds. Comp. Biochem. Physiol. A: Mol. Integr. Physiol. 154:216–223.

Nyquist, N. F., L. M. Biltvedt, R. Rødbotten, M. Mielnik, M. Thomassen, B. Svihus, and A. Haug. 2012. Effect of varying ratios of n-6 and n-3 on selenium content in broiler breast muscle. Acta Agri. Scan. A: Anim. Sci. 62:81–92.

Ratnayake, W. 1989. Effect of redfish meal enriched diets on the taste and n-3 pufa of 42-day-old broiler chickens. J. Sci. Food Agri. 2012:59–74.

Rymer, C., and D. I. Givens. 2005. n-3 fatty acid enrichment of edible tissue of poultry: A review. Lipids 40:121–130.

Salmon, R. E., V. I. Stevens, and B. D. Ladbrooke. 1988. Full-fat canola seed as a feedstuff for turkeys. Poult. Sci. 67:1731–1742.

Shen, H., J. D. Summers, and S. Leeson. 1983. The influence of steam pelleting and grinding on the nutritive value of Canola rapeseed for poultry. Anim. Feed Sci. Technol. 8:303–311.

Simopoulos, A. P. 2006. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. Biomed. Pharm. 60:502–507.

Simopoulos, A. P. 2008. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp. Biol. Med. 233:674–688.

Summers, J. D., H. Shen, and S. Leeson. 1982. The value of canola seed in poultry diets. Can. J. Anim. Sci. 62:861–868.

Torstensen, B. E., D. A. Nanton, P. A. Olsvik, H. Sundvold, and I. Stubhaug. 2009. Gene expression of fatty acid-binding proteins, fatty acid transport proteins (cd36 and FATP) and β-oxidation-related genes in Atlantic salmon (Salmo salar L.) fed fish oil or vegetable oil. Aquacult. Nutr. 15:440–451.

Tur, J. A., M. M. Bibiloni, A. Sureda, and A. Pons. 2012. Dietary sources of omega 3 fatty acids: Public health risks and benefits. Br. J. Nutr. 107:S23–S52.

Wanders, R. J. A. 2004. Peroxisomes, lipid metabolism, and peroxisomal disorders. Mol. Genet. Metab. 83:16–27.

Wang, Y., K. Rao, L. Yuan, N. Everaert, J. Buyse, R. Grossmann, and R. Zhao. 2012. Chicken FTO gene: Tissue-specific expression, brain distribution, breed difference and effect of fasting. Comp. Biochem. Physiol. A: Mol. Integr. Physiol. 163: 246–252.

Zhang, Q., H. Shi, W. Liu, Y. Wang, Q. Wang, and H. Li. 2013. Differential expression of L-FABP and L-BABP between fat and lean chickens. Genet. Mol. Res. 12:4192–4206.

Zheng, X., D. R. Tocher, C. A. Dickson, J. G. Bell, and A. J. Teale. 2004. Effects of diets containing vegetable oil on expression of genes involved in highly unsaturated fatty acid biosynthesis in liver of Atlantic salmon (Salmo salar). Aquac. 236: 467–483.