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Institute of Genetics and Animal Biotechnology
of the Polish Academy of Sciences, Jastrzębiec, Poland

Effects of dietary supplementation with algae, sunflower oil, or soybean oil, and age on fat content, fatty acid profile and the expression of related genes in rabbits

Dorota Maj*, Łukasz Migdał, Piotr Zapletal

[1] Department of Genetics, Animal Breeding and Ethology,
University of Agriculture in Krakow, al. Mickiewicza 21, 31-120 Krakow, Poland

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The aim of this study was determine the effect of different dietary supplements (algae, sunflower oil, or soybean oil) and age (12 and 18 weeks) on the fat content, fatty acid (FA) profile, and expression of the fat mass and obesity associated (*FTO*) and fatty acid binding protein 4 (*FABP4*) genes in rabbit muscle. Rabbits (n = 160) were randomly divided into four groups. The control group (C) received non-supplemented pellets, while in the other groups the pellet contained 1% algae (A), 3% sunflower oil (OS), or 3% soybean (SO) oil. Soybean and sunflower oil (3%) in the diet increased the linoleic acid (LA) content in meat of 12-week-old rabbits. The use of algae (1%) in the diet increased the n-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) compared with the vegetable oil and control groups, and decreased the n-6/n-3 ratio. The effect of the diet on *FTO* and *FABP4* gene expression depended on the age of the rabbits. In older animals (18 weeks of age) the expression of these genes was highest in the group with 1% algae. Furthermore, the FA profile and *FTO* and *FABP4* gene expression were affected by the age of rabbits, but not by sex. The results showed that diet is an important tool to modulate the FA profile in rabbit meat by changing the expression of fat metabolism–related genes.

KEY WORDS: PUFAs / feeding / meat / PCR / rabbit

The rise in chronic diseases associated with food and lifestyle such as diabetes, cancer, or cardiovascular disease affects both developed and developing countries.

*Corresponding author: dorota.maj@urk.edu.pl

Due to the close relationship between food and health, consumers increasingly choose products that meet their nutritional preferences [Petrescu *et al*. 2020]. In recent years, consumers' interest in functional food has grown. Nutritional factors have a powerful influence on the chemical composition of meat, and in particular on its lipid composition [Pereira and Vicente 2013]. In the prevention of lifestyle diseases, n-3 and n-6 unsaturated fatty acids (FAs) are particularly desirable. Increasing the content of n-3 and n-6 FAs in animal feed can improve the nutritional value of meat [Costa *et al*. 2020] without forcing consumers to change their eating habits. This approach aims to produce high-quality products with a FA profile that will meet nutritionist recommendations [Teixeira and Rodrigues 2021]. With regard to nutrition and human health, rabbit meat fits in well with the current consumer preferences, being lean meat low in fat and cholesterol, rich in protein, and with high biological value [Petrescu and Petrescu-Mag 2018]. However, the n-6/n-3 ratio of rabbit meat is >4 [Perna *et al*. 2019] and this meat contains only small amounts of eicosapentaenoic acid **(**EPA) and docosahexaenoic acid **(**DHA) [Ramírez *et al*. 2005]. Therefore, to improve the nutritional value of rabbit meat the content of n-3 FAs needs to be increased.

In the nutrition of rabbits various raw materials (linseed oil, fish oil, white lupine seeds, and silkworm pupae) have been used to increase the level of unsaturated fatty acids (UFAs) and to decrease the n-6/n-3 ratio [Dal Bosco *et al*. 2018, Volek *et al.* 2018, Rodríguez *et al*. 2019, Mattioli *et al*. 2020, Jaworska *et al*. 2020, Kowalska *et al*. 2020, Czauderna *et al*. 2021]. Unfortunately, there is a hazard of polyunsaturated fatty acid (PUFA) accumulation in animal tissues. They stimulate oxidative stress, producing a rancid odor and taste, both of which decrease consumer acceptance. Moreover, high levels of PUFAs have toxic effects on rumen microorganisms and inhibit their activity [Mattioli *et al*. 2020].

Vegetable oils, such as sunflower and soybean oils, are good sources of omega-6 FA, *mainly* linoleic acid (LA), which is a precursor of vaccenic acid, the substrate for conjugated linoleic acid (CLA) synthesis [González *et al*. 2014]. The use of sunflower oil in diets is economically important in many countries. The high levels of naturally occurring antioxidants (tocopherols) and phytosterols in conventional sunflower oil [Sanchez *et al*. 2000] could maintain a better protective effect than other monounsaturated oils. Soybean oil is one of the most commonly used energy source ingredient in diets thanks to its high metabolizable energy content, as well as digestibility [Kierończyk *et al*. 2022]. Another rich source of FA is marine algae, characterized by a high content of long-chain n-3 PUFAs, mainly DHA and EPA [Mordenti *et al*. 2010]. Macro- and micronutrients as well as antioxidants present in algae could provide opportunities to save on other feed ingredients [Bature *et al.* 2022]. Adding algae to the diet is economical, as studies have shown that even exceptionally low levels of algal supplementation produce positive results [Kotrbáček *et al*. 2015].

Many studies have demonstrated that the fat content and FA levels are influenced by animal age [Bednárová *et al*., 2012] and the fat content increases with age [Polak *et al*. 2006].

Among the factors affecting body weight and fat tissue distribution, many genes play an important role, including the fat mass and obesity associated (*FTO*) gene responsible for obesity and its metabolic consequences [da Fonseca *et al*. 2019]. In rats a diet high in fat significantly increases *FTO* expression in the hypothalamus [Tung *et al*. 2010]. However, the mechanism, by which the *FTO* gene influences obesity development is still unexplained. Little is known about the *FTO* gene function in rabbits. Zhang *et al*. [2013] researched the relationship between the *FTO* gene and the growth and meat quality in rabbits. The authors found a relationship between the *FTO* gene and rabbit body weight. The intramuscular fat content in meat also depends on genes that encode proteins involved in the transport and metabolism of FAs in the cells. FA transport occurs via fatty acid-binding protein 4 (*FABP4*), among other proteins [Hong *et al*. 2015]. In addition, *FABP4* messenger RNA (mRNA) and protein levels may be important metabolic indicators of an animal's ability to deposit intramuscular fat, depending on the muscle type and breed [Albrecht *et al*. 2011]. However, little is known about dietary influence on the expression of lipid metabolism–related genes in rabbits. There have been several studies on the relationship between diet and *FABP* expression, but they are limited to sheep [Dervishi *et al*. 2011, Fan *et al*. 2019] and cattle [da Costa *et al*. 2013, Oliveira *et al*. 2014].

We hypothesized that dietary supplementation with algae, soybean oil, or sunflower oil positively affects rabbit meat by improving its FA profile. We also hypothesized that these ingredients in diets alter *FTO* and *FABP4* gene expression in rabbit muscle and these changes depend on the rabbit's age. Thus, we aimed to determine the effect of the inclusion of different ingredients in diets (algae, soybean oil, and sunflower oil) and rabbit age (12 and 18 weeks) on the fat content, FA profile (especially the n-6/n-3 ratio), and *FTO* and *FABP4* gene expression in rabbit muscle (*M. longissimus lumborum*).

Material and methods

Animals and diets

We used Blanc de Termonde rabbits (n = 160, 80 females and 80 males). Rabbits were weaned on day 35 of life and randomly sorted into four groups balanced for sex (40 rabbits/group, 2 rabbits/cage, 20 cages/group) according to diet. Animals were housed in the same environmental conditions (temperature 15-20°C, humidity 65%-70%, lighting 14L:10D) in a closed rabbitry, in wire net cages (0.45 × 0.80 × 0.55 m) arranged in batteries. From weaning to 12 or 18 weeks of age, the rabbits were fed pellets *ad libitum* and fresh water was available at all times from automatic nipple drinkers. Animals in the control group (C) received non-supplemented pellets throughout the experiment. In the other three groups, the pellets contained 1% marine algae (A) (DHA Gold®) - *Schizochytrium* sp., 3% sunflower oil (OS), or 3% soybean oil (SO). Marine algae (DHA Gold®) used as an additive contained 42.3% DHA, 17.7% docosapentaenoic acid (DPA),and 1.9% EPA of total FA (according to

Table 1. Ingredients of the experimental diets (%)

Component	Diet			
	C	A	OS	SO
Oat	25.00	24.10	23.09	23.09
Alfalfa	22.00	22.00	22.00	22.00
Wheat bran	19.88	19.00	19.00	19.00
Sunflower meal	19.22	19.00	19.00	19.00
Barley	5.00	10.00	5.00	5.00
Beet pulp	4.07	-	5.00	5.00
Rapeseed oil	1.28	1.28	-	-
Algae (DHA-Gold®)	-	1.00	-	-
Sunflower oil	-	-	3.00	-
Soybean oil	-	-	-	3.00
Beet molasses	1.00	1.00	1.00	1.00
Chalk fodder	0.9	0.9	0.9	0.9
Min.-vit. premix, Rovimix 0.5%	0.50	0.50	0.50	0.50
Binder	0.50	0.50	0.50	0.50
Sodium chloride	0.39	0.39	0.39	0.39
Lysine	-	0.07	0.36	0.36
Dicalcium phosphate	0.14	0.14	0.14	0.14
Herbal mixture -Bell Gold	0.05	0.05	0.05	0.05
Sodium butyrate encapsulated	0.03	0.03	0.03	0.03
Herbal mixture - Bell Premium	0.03	0.03	0.03	0.03
Vitamin E 50%	0.01	0.01	0.01	0.01

Diet – control (C), algae (A), sunflower oil (OS), soybean oil (SO).

Table 2. Chemical composition and fatty acid profile of the experimental diets

Component	Diet			
	C	A	OS	SO
Chemical composition (g/kg DM)	882	882	885	885
Dry matter (g/kg)	75.22	72.71	74.71	74.71
Crude ash	170	170	170	170
Total protein	33.37	42.28	49.61	49.61
Crude fat	158	158	156	156
Crude fibre	7.01	7.42	8.85	8.85
Lysine	3.45	3.42	3.38	3.38
Methionine	3.27	3.27	3.18	3.18
Cysteine	6.50	6.39	6.39	6.39
Threonine	2.36	2.34	2.31	2.31
Tryptophan	9.42	9.71	9.91	9.91
Metabolizable energy[1] (MJ/kg DM)	0.12	0.45	0.11	0.43
Fatty acid (% of total FA)				
Myristic C14:0	0.12	0.45	0.11	0.43
Palmitic C16:0	4.55	12.5	6.40	11.00
Stearic C18:0	3.14	4.32	4.10	4.20
Oleic C18:1 c9	59.60	54.75	22.60	24.70
Linoleic C18:2 n-6	23.84	24.83	65.40	53.60
Linolenic C18:3 n-3	8.55	2.82	0.78	5.70

Diet – control (C), algae (A), sunflower oil (OS), soybean oil (SO); DM – dry matter.
[1]Metabolizable energy of the diets was calculated according to NRC [1977].

the producer: Novus Company, Poland). The diets were balanced by lowering the proportion of other feed components. All diets were isoprotein, but differed in their crude fat content. Pellets were prepared and supplied by the feed company Farmer Sp. z o.o. (Biskupice Ołoboczne, Poland). The metabolizable energy of the diets was calculated according to NRC [1977]. The ingredients and chemical composition of experimental diets are presented in Tables 1 and 2. The experiment consisted of two stages: stage I, slaughter of rabbits at 12 weeks of age, n = 80 (40 males and 40 females) with an average body weight of 2.5 kg; and stage II, slaughter of rabbits at 18 weeks of age, n = 80 (40 males and 40 females) with an average body weight of 3.4 kg.

Slaughter procedures

Rabbits were handled following the guidelines for animal experiments, indicated in the EU Directive 2010/63/EU regarding the protection of animals used for experiments and Council Regulation (EC) no. 1099/2009 on the protection of animals at the time of killing. The animals were slaughtered under permission from the Local Ethics Committee (No. 2/2018). The animals were slaughtered after fasting for 24 h. The rabbits were stunned (using a captive bolt pistol for stunning), slaughtered by cutting the carotid artery and jugular vein, immediately bled, pelted, and eviscerated. After slaughter, samples (5g) for RNA analysis from the right loin (*M. longissimus lumborum*) were collected rapidly, immediately snap-frozen using liquid nitrogen, and stored at -80°C. Hot carcasses were suspended in a ventilated area for 45 min and then were chilled at 4°C until 24 h *post mortem*. The right loin (*M. longissimus lumborum*) was dissected and trimmed of allexternal fat and epimysial connective tissue. All the muscle samples (about 50 g) were vacuum-packed and transported on ice in thermal boxes to the laboratory and immediately frozen at -20°C until analysis (n = 20 in each group for fat content and n = 8 in each group for fatty acids and gene expression).

Chemical analyses

The chemical composition of the experimental diets and the intramuscular fat content (*M. longissimus lumborum*) were analyzed according to the procedures of the Association of Official Analytical Chemists [AOAC 2012]. Dry matter (procedure 934.01), crude protein (procedure 2001.11), crude fiber (procedure 978.10), and ash (procedure 967.05) were determined. Lipids of diets and muscle were extracted using a mixture of chloroform and methanol (2/1,v/v) [Folch *et al*. 1957]. Fatty acid methyl esters (FAME) were prepared and analyzed using the same procedure described below for rabbit meat samples. Amino acids in the diets were determined on an automatic Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc., Pleasanton, CA, USA).

Fatty acid composition

The FA composition of muscle lipids (*M. longissimus lumborum*) was determined according to Folch *et al*. [1957], following extraction with a mixture of chloroform

and methanol (2/1, v/v). Aliquots of muscle lipids (10 mg) were methylated (0.5 N NaOH in methanol) and esterified (12% BF3 in methanol). The FAMEs produced were determined in hexane extracts using a gas chromatograph (TRACE GC Ultra, Thermo Scientific, Italy) with a flame ionization detector (FID) and a SUPELCOWA column (30 m × 0.25 mm × 0.25 m) by injecting 1 µL of the esterified extract. The injector temperature was 220°C and the detector temperature was 250°C. The carrier gas was helium (1 mL min^{-1}). Individual FAMEs were identified by comparison with the standard mixture (Supelco 37 component FAME Mix, Sigma-Aldrich Co., Germany). Each sample was analyzed in two replicates. The FA content results are presented as the percentage of total FA detected; they were calculated with the ChromQuest 4.1 software (Thermo Electron, Milan, Italy).

Total RNA extraction and reverse transcription

Total RNA from muscle samples (100 mg) was extracted using the TriPure Isolation Reagent (RocheDiagnostics GmbH, Germany) according to the manufacturer's protocol. The RNA concentration was determined at 260 nm using a spectrophotometer (BioPhotometer, Eppendorf, Germany). The 260nm/280 nm ratio of all preparations was between 1.6 and 1.9. To determine the degree of degradation of the isolated RNA, electrophoretic separation was performed in a 1% agarose gel with the addition of ethidium bromide in 0.5 Tris-Borate-EDTA (TBE) buffer. Reverse transcription reactions were performed using the Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics GmbH) according to the manufacturer's protocol. A detailed description of the concentrations of the components in the complementary DNA (cDNA) synthesis mixture, which total volume was 20 µL, is given in Table 3. Then samples were stored at -20°C.

Table 3. Composition of the reaction mixture (Roche Diagnostics GmbH, Germany) for the synthesis of complementary DNA (cDNA)

Component	Volume	Final concentration
Total RNA	1 µg/10 µl H_20	
Random Hexamer Primer 600 pmol/ µl	2 µl	60 µM
Mixture dNTP, 10mM each	2 µl	1 mM
RNase Inhibitor, 40 U/ µl	0.5 µl	20 U
Reverse transcription buffer 5x (8mM $MgCl_2$)	4 µl	1x
Reverse Transcriptase 20U/ µl	0,5 µl	10 U
RNase free water	1 µl	
Total volume	20 µl	

Real-time polymerase chain reaction

The real-time polymerase chain reaction (PCR) was performed in a thermal cycler (LightCycler® 96, Roche Diagnostics GmbH) on 96-well plates using the FastStart Essential DNA Probes Master and Universal Probe Library (FAM) fluorescent probes (Roche Diagnostics GmbH) and primers for the *FTO* gene with the reference gene

glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) and the *FABP4* gene with the reference geneβ-actin (*ACTB*). As there is very little information concerning reference genes for expression in rabbit muscles, we examined the expression of the five most common candidate housekeeping genes in the analyzed muscle. We performed the analysis with the most stable genes, namely GAPDH and ACTB. We designed primers to span exon-exon junctionsusing Primer3 Input (version 0.4.0) software (http://bioinfo.ut.ee/primer3-0.4.0). The real-time PCR reaction was performed according to the manufacturer's protocol (Roche Diagnostics GmbH). The reaction mixture contained 10 µL of FastStart DNA Master Mix, 2 µL of probe mix and primers specific for the tested genes (primer-probe mix), 3 µL of water for PCR, and 5 µL of cDNA. The characteristics of the primers and probes used for the qPCR reaction are shown in Table 4. The reactions were carried out under the following conditions: preincubation at 95°C for 10 min, followed by two-step amplification at 95°C for 15 s and 60°C for 45 s with total 55 cycles. The data were collected and analyzed using the LightCycler Software 4.05 program. Expression of the examined genes was normalized to the reference index obtained by calculating the arithmetic mean of the expression of the reference genes: *GAPDH* for *FTO* and *ACTB* for *FABP4*. The relative expression levels were calculated according to the method described by Pfaffl [2001], which is based on Ct values that are corrected for the amplification efficiency for each primer pair.

Table 4. Characteristics of primers and probes used for qPCR reactions (Roche Diagnostics Universal Probe Library UPL)

Gene	The sequence of the primers (5'-3')	Accession number	Ta	Probe, Cat.No.
FTO	F- AAGGCAGAGATCCTGACATTTG R- GCGTCTCTATGTCCCACGAG	ENSOCUG00000001741	60	#139, 04694236001
FABP4	F -TGCAGATGACAGGAAAGTCAA R-TTCCATCCCACTTCTGCAC	ENSOCUT00000015479	60	#37, 04687957001
GAPDH	F-GTCTACATGTTCCAGTATGATTCC R- CCCGTTGATGACCAGCTT	NC_013676.1	60	#111, 04693442001
ACTB	F-ATCACCATCGGCAACGAG R-ATCACCATCGGCAACGAG	NW_003159504.1	60	#134, 04694180001

FTO – Fat mass and obesity associated; *FABP4* – Fatty acid binding protein 4; *GAPDH* – Glyceraldehyde 3-phosphate dehydrogenase; *ACTB* – Beta-actin; F – forward primer and R – reverse primer; Ta – annealing temperature.

Statistical analyses

We analyzed the results using the least squares method using the general linear model (GLM) procedure. We used the following linear model:

$$y_{ijkl} = \mu + D_i + S_j + A_k + (DS)_{ij} + (DA)_{ik} + (SA)_{jk} + (DSA)_{ijk} + e_{ijkl}$$

where:

y_{ijkl} – the value of the trait; μ is the overall mean;

D_i – the effect of the i-th diet (i = 1,…, 4);

S_j – the effect of the j-th sex (j = 1, 2);

A_k – the effect of the k-th age (k = 1, 2);

$(DS)_{ij}$ – the effect of the interaction between diet and sex;

$(DA)_{ik}$ – the effect of the interaction between diet and age;

$(SA)_{jk}$ – the effect of the interaction between sex and age;

$(DSA)_{ijk}$ – the effect of the interaction between diet, sex and age;

e_{ijkl} – random error.

Interactions between fixed effects were removed from the model if they were not significant.

We compared the least squares means for the studied groups using the Tukey-Kramer multiple comparisons post hoc test. We considered $p<0.05$ to indicate significant differences. These statistical analyses were carried out in the SAS package [SAS 2014].

Because the analysis of variance and statistical tests showed no significant differences between male and female rabbits in the examined traits; therefore, the results summarized in Tables 5-8 and in Figure 1 are the averages for the examined rabbits of both sexes.

Results and discussion

Fat content and fatty acid profile

Fat content and the FA profile in rabbit tissues depend on breed, age, sex, type of tissue, and diet. The FA profile determines the meat quality parameters, such as softness, hardness, oxidative stability, color and taste, affecting the overall acceptance of meat by consumers [Wood *et al*. 2008].

In this study the algae, soybean oil or sunflower oil used in the diet did not affect the intramuscular fat (IMF) percentage (Tab. 5). The average fat content was 2.4%, indicating a low fat percentage in rabbit meat. This intramuscular fat percentage is similar to that found by Costa *et al*. [2020] after inclusion of soybean and sunflower oils in the diet. We observed that the fat percentage decreased with age. Metzger *et al*. [2011] also found that the fat content in meat decreased in older rabbits and it increased in rabbits of higher body weight.

Saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids, along with fatty acid types and ratios are shown in Tables 5-8. In the present

Table 5. Fat content and saturated fatty acids in meat depending on the diet and age (% of total fatty acids)

Trait	Age-12 week				Age-18 week				SEM	P-value		
	C	A	OS	SO	C	A	OS	SO		Diet	Age	Diet x Age
Fat (%)	2.60	2.22	2.66	2.77	1.98	2.24	2.49	2.02	0.17	0.247	0.006	0.130
Fatty acids												
C10:0	0.082^{a}	0.057^{ab}	0.051^{ab}	0.028^{a}	0.032^{b}	0.025^{b}	0.038^{b}	0.046^{ab}	0.004	0.420	0.032	0.054
C12:0	0.101^{a}	0.070^{ab}	0.083^{a}	0.052^{b}	0.056^{b}	0.064^{ab}	0.082^{a}	0.037^{b}	0.004	<0.001	0.013	0.091
C14:0	1.304	1.547	1.241	1.256	1.351	1.747	1.769	1.283	0.065	0.163	0.120	0.442
C15:0	0.339^{ab}	0.356^{ab}	0.327^{ab}	0.311^{ab}	0.357^{ab}	0.376^{a}	0.272^{b}	0.318^{ab}	0.008	0.009	0.871	0.193
C16:0	24.33	24.61	24.15	23.43	23.78	24.68	24.23	23.56	0.248	0.507	0.897	0.964
C17:0	0.41^{a}	0.44^{a}	0.40^{a}	0.39^{ab}	0.42^{a}	0.42^{a}	0.32^{b}	0.38^{ab}	0.009	0.042	0.161	0.266
C18:0	6.57	5.90	6.06	6.39	6.10	6.13	5.80	5.93	0.108	0.610	0.303	0.689
C20:0	0.072	0.063	0.076	0.071	0.072	0.071	0.074	0.066	0.001	0.235	0.921	0.501

Diet – control (C), algae (A), sunflower oil (OS), soybean oil (SO), (n = 8 in each group for fatty acids, and n=20 in each group for fat content); SEM – standard error of mean.
[abcd]In row means bearing different superscripts differ significantly at $p<0.05$.

Table 6. Monounsaturated fatty acids depending on the diet and age (% of total fatty acids)

Trait	Age-12 week				Age-18 week				SEM	P-value		
	C	A	OS	SO	C	A	OS	SO		Diet	Age	Diet x Age
n	8	8	8	8	8	8	8	8				
C14:1	0.05	0.10	0.07	0.10	0.08	0.21	0.18	0.14	0.01	0.140	0.009	0.544
C16:1 n-9	0.64^{a}	0.47^{b}	0.50^{b}	0.47^{b}	0.62^{a}	0.44^{b}	0.49^{b}	0.48^{b}	0.01	<0.001	0.301	0.874
C16:1 n-7	1.61	2.10	1.94	2.16	2.19	3.18	3.69	2.75	0.19	0.281	0.006	0.559
C17:1	0.16	0.13	0.14	0.15	0.17	0.15	0.18	0.17	0.005	0.223	0.009	0.565
C18:1 n-9	25.45^{acd}	23.56^{ab}	21.08^{b}	21.20^{b}	27.64^{c}	26.12^{ad}	24.46^{bd}	22.61^{ac}	0.48	<0.001	0.002	0.772
C18:1 n-7	2.11^{ab}	1.89^{ab}	1.85^{ab}	1.71^{b}	2.33^{a}	1.69^{b}	1.89^{ab}	2.03^{ab}	0.05	0.005	0.249	0.162
C20:1	0.29^{a}	0.21^{b}	0.18^{b}	0.16^{b}	0.29^{a}	0.21^{b}	0.20^{b}	0.16^{b}	0.009	<0.001	0.602	0.967

Diet – control (C), algae (A), sunflower oil (OS), soybean oil (SO). SEM – standard error of mean.
[abcd]In row means bearing different superscripts differ significantly at $p<0.05$.

study among the FAs the percentages of palmitic acid (C16:0), oleic acid (C18:1 n-9), and linoleic acid (C18:2 n-6) were highest. The use of 3% vegetable oil (sunflower or soy) in the feed increased the PUFA content, including linoleic acid and the n-6/n-3

Table 7. Polyunsaturated fatty acids depending on the diet and age (% of total fatty acids)

Fatty acids	Age-12 week				Age-18 week				SEM	P-value		
	C	A	OS	SO	C	A	OS	SO		Diet	Age	Diet x Age
n	8	8	8	8	8	8	8	8				
C18:2 n-6 (LA)	25.39^{a}	19.29^{c}	29.83^{bd}	32.46^{b}	24.62^{a}	17.92^{c}	26.95^{ad}	28.35^{ad}	0.885	<0.001	0.034	0.665
C18:3 n-6	0.14^{a}	0.13^{ac}	0.15^{a}	0.14^{ac}	0.13^{ac}	0.12^{c}	0.10^{b}	0.11^{bc}	0.004	0.403	0.002	0.187
C18:3 n-3 (ALA)	1.97	1.97	2.45	2.44	2.26	1.90	2.06	1.99	0.078	0.492	0.334	0.338
C20:3 n-6	0.46	0.33	0.37	0.33	0.30	0.28	0.274	0.32	0.018	0.527	0.038	0.469
C20:4 n-6 (AA)	4.81	4.94	4.65	3.99	4.32	5.36	4.26	5.69	0.258	0.806	0.574	0.454
C20:5 n-3 (EPA)	0.16^{a}	0.35^{b}	0.13^{a}	0.09^{a}	0.09^{a}	0.35^{b}	0.06^{a}	0.09^{a}	0.018	<0.001	0.053	0.155
C22:4 n-6	1.19^{ac}	0.31^{b}	1.16^{ac}	1.09^{ac}	0.96^{a}	0.18^{b}	1.07^{a}	1.51^{c}	0.093	<0.001	0.960	0.394
C22:5 n-6	0.52^{a}	1.59^{b}	0.67^{a}	0.45^{a}	0.42^{a}	1.10^{b}	0.42^{a}	0.57^{a}	0.068	<0.001	0.024	0.052
C22:5 n-3 (DPA)	0.90	0.64	0.67	0.55	0.77	0.38	0.58	0.79	0.044	0.084	0.480	0.191
C22:6 n-3 (DHA)	0.27^{a}	8.58^{b}	1.32^{a}	0.12^{a}	0.21^{a}	6.57^{b}	0.12^{a}	0.12^{a}	0.533	<0.001	0.078	0.349
CLA	0.03	0.04	0.03	0.04	0.03	0.05	0.04	0.05	0.002	0.093	0.655	0.769

Diet – control (C), algae (A), sunflower oil (OS), soybean oil (SO). LA – linoleic acid; ALA – α-linolenic acid; AA – arachidonic acid; EPA – eicosapentaenoic acid; DPA – docosapentaenoic acid; DHA – docosahexaenoic acid; CLA – conjugated linoleic acid; SEM – standard error of mean.
^{abcd}In row means bearing different superscripts differ significantly at $p<0.05$.

Table 8. Fatty acid types and ratios depending on the diet and age (% of total fatty acids)

Fatty acids	Age-12 week				Age-18 week				SEM	P-value		
	C	A	OS	SO	C	A	OS	SO		Diet	Age	Diet x Age
n	8	8	8	8	8	8	8	8				
SFA	31.92	31.50	31.14	30.66	30.81	31.76	30.81	30.34	0.74	0.463	0.482	0.849
MUFA	30.31^{ac}	28.46^{abd}	25.76^{b}	25.94^{b}	33.30^{c}	32.01^{ad}	31.09^{ac}	28.35^{ab}	1.28	0.007	<0.001	0.670
PUFA	35.81^{ac}	38.14^{abc}	41.39^{b}	41.65^{b}	34.10^{a}	34.16^{a}	35.90^{ac}	39.55^{bc}	1.60	0.007	0.007	0.608
PUFA n-3	3.30^{a}	11.54^{b}	4.57^{a}	3.20^{a}	3.34^{a}	9.20^{b}	2.81^{a}	3.00^{a}	0.39	<0.001	0.023	0.191
PUFA n-6	32.51^{ad}	26.60^{c}	36.83^{ab}	38.46^{b}	30.76^{d}	24.96^{c}	33.08^{ad}	36.55^{ab}	1.52	<0.001	0.074	0.910
n-6/n-3	9.85^{a}	2.33^{b}	10.10^{a}	12.05^{a}	9.25^{a}	2.73^{b}	11.80^{a}	12.17^{a}	0.51	<0.001	0.491	0.556
PUFA/SFA	1.12	1,21	1.33	1.36	1.11	1.08	1.17	1.30	0.10	0.085	0.366	0.512
UFA	66.12	66.59	67.15	67.60	67.40	66.17	66.99	67.89	0.73	0.379	0.659	0.732
UFA/SFA	2.07	2.13	2.16	2.21	2.19	2.09	2.19	2.24	0.07	0.471	0.488	0.808

Diet – control (C), algae (A), sunflower oil (OS), soybean oil (SO). SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids; UFA – unsaturated fatty acids; SEM – standard error of mean.
^{abcd}In row means bearing different superscripts differ significantly at $p<0.05$.

ratio. Costa *et al.* [2020] similarly showed that sunflower and soy oil treatments resulted in a higher n-6/n-3 ratio. The n-6/n-3 ratio is very important for human health; it should be < 4 [Perna *et al.* 2019]. Higher ratios can be due to the large amounts of

linoleic acid (18:2 n-6) in these oils. In contrast to our results, a significant reduction in the n-6/n-3 ratio has also been reported in the meat of rabbits supplemented with linseed oil [Mattioli *et al*. 2020], fish oil [Rodríguez *et al*. 2019], and lupine seed oil [Volek *et al*. 2018].

The n-6/n-3 and PUFA/SFA ratios are health indicators of FAs in foods. In this study the PUFA/SFA ratio was between 1.08 and 1.36, which is much higher than the recommended minimum of 0.40 [WHO 2008]. By increasing the proportion of PUFAs relative to SFAs, high dietary linoleic acid intake may improve the plasma lipid profile, glycemic control, and insulin resistance [Marangoni *et al.* 2020]. In the present study supplementation with algae (1%) led to an increase in the n-3 FAs (EPA and DHA) and a decrease in the n-6/n-3 ratio (about 2.5). Lowering the *n*-6/n-3 ratio can have beneficial health implications. Similarly to our results, Fan *et al*. [2019] observed in sheep that a diet with algae significantly increased EPA and DHA in meat and decreased the n-6/n-3 ratio. In contrast to our results, Peiretti and Meineri [2011] found an increase in n-6 FAs in the meat of rabbits fed with feed with the addition of algae (0%, 5%, 10%, or 15%). According to these authors, the n-6/n-3 ratio ranged from 9.4 and 21.4 in meat from the control group and the group fed with algae (150 g/kg). The increase in n-6 PUFAs in the meat of rabbits fed with fodder with the addition of algae was also confirmed by Dal Bosco *et al*. [2014]. These authors reported a higher n-6/n-3 ratio compared with our results.

The FA composition of a tissue is determined by dietary lipid composition, *de novo* lipogenesis, desaturation, and the difference in the utilization of various FAs by the animal's body [Fan *et al*. 2019]. FA in tissues may also originate from *de novo* synthesis or bioconversion of C18:3 n-3 and C18:2 n-6 into appropriate long-chain PUFAs [Masek *et al*. 2014]. Parveen *et al*. [2016] found that the levels of PUFAs in tissues can be influenced by their dietary concentration. In our study the reduction in the n-6/n-3 ratio and the increase in the n-3 FA (EPA and DHA) by algae (1%) supplementation implied an improvement in the nutritional quality of rabbit meat. A balanced n-6/n-3 ratio is important for health, as well as prevention and management of obesity [Simopoulos 2016].

We observed that the FA profile was affected by the age, but not the sex (data not shown) of the rabbits. According to Gašperlin *et al*. [2006], the FA composition varied slightly with slaughter age, whereas the n-6/n-3 ratio decreased as age increased. Trocino *et al*. [2018] found that SFAs and MUFAs increased and PUFAs decreased with age of farmed brown hares; similarly to our study, sex did not affect the FA content. Also Jaworska *et. al*. [2020] showed that the sex of rabbits did not have a statistically significant effect on the concentrations of SFA, MUFA and PUFA in muscle. Our results could be considered in the production of better-quality rabbit meat.

***FTO* and *FABP4* gene expression**

Animal feeding is an important tool to modify the FA profile in meat by changing the expression of genes related to fat metabolism [Dervishi *et al*. 2011]. Little

information is available in literature about gene expression levels in rabbit muscle and its regulation by dietary factors. Our study is the first to demonstrate that diet modulates the expression of the *FTO* gene in rabbit muscle. We found that in older rabbits (at 18 weeks) supplementation with algae and sunflower or soybean oil increased the *FTO* mRNA level compared with the control diet (Fig. 1). The study by Zhang *et al*. [2013] is the only other one that examined the *FTO* gene in rabbits,

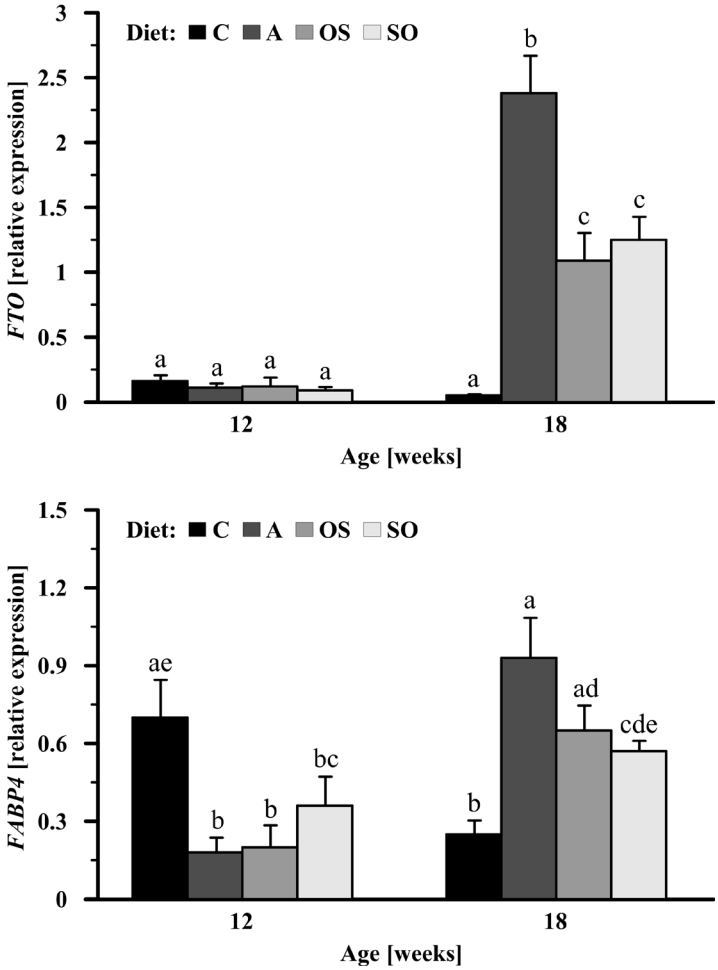

Fig. 1. Relative expression levels of FTO and FABP4 genes in the muscle (M. longissimus lumborum) of rabbits depending on diet and age. Diet – control (C), algae (A), sunflower oil (OS), soybean oil (SO); FTO – Fat mass and obesity associated; FABP4 – Fatty acid binding protein 4; bars present least square means (LSM) ± standard error of mean (SEM), for 8 rabbits per group; a, b, c, d – bars with different letters differ significantly (P<0.05). P-value: FTO – Diet <0.001, Age <0.001, Diet x Age <0.001; FABP4 – Diet 0.612, Age 0.001, Diet x Age <0.001.

but those authors analyzed polymorphisms of this gene. In rats restrictive feeding reduced the *FTO* mRNA level in the arcuate nucleus of the hypothalamus, in which gene expression is particularly high, suggesting that *FTO* is involved in the regulation of food intake [Tung *et al*. 2010]. Similarly to our results, another study showed that a high-fat diet increases *FTO* mRNA in white adipose cells in rats [Nowacka-Woszuk *et al*. 2017]. In sheep *FTO* mRNA was detected in the hypothalamus, pancreas, kidneys, heart, adipose tissue, liver, and skeletal muscle. These studies showed that in obese sheep there was a significant increase in *FTO* mRNA [Sebert *et al.* 2010]. *FTO* mRNA and protein expression in Suzhong pig tissues with a high fat content was significantly higher than in tissues with a low fat content. Based on the results obtained so far we suggest that the *FTO* gene may be involved in fat storage mechanisms and it is one of the major genes affecting meat quality traits.

Research suggests that the *FTO* gene plays a role in white adipose tissue, modifying its response to a high-fat diet. In one study researchers fed wild-type and *FTO*-deficient mice for 16 weeks with a standard or high-fat diet. In *FTO*-deficient mice a greater expression of adipogenesis-related genes was observed, preventing the proliferation of adipocytes in mice fed a high-fat diet [Ronkainen *et al*. 2015]. Zhong *et al*. [2017] demonstrated that a high-fat diet did not affect *FTO* expression in mouse fat tissue. Madsen *et al*. [2010] showed that *FTO* expression in the cortex and in the cerebellum of Gottingen minipigs varied depending on the stage of brain development and diet (high cholesterol and normal mix). Yuzbashian *et al*. [2021] found a significant positive association between MUFA and PUFA intake and *FTO* gene expression in subcutaneous and visceral adipose tissues in humans. Our results also showed that *FTO* mRNA expression was affected by age and increased with rabbit age (Fig. 1). Similarly, Tao *et al*. [2013] demonstrated that *FTO* mRNA increased with the age of the pigs and with increasing body weight. There was also a significant interaction between the diet and rabbit age. The *FTO* mRNA expression was not affected by sex (data not show). Wang *et al*. [2018] showed in humans that the FTO protein level was not correlated with age or sex.

In our study *FABP4* expression was not affected by diet, whereas we observed an interaction between the diet and age (Fig. 1); this finding suggests that the diet effect might be modified by rabbit age. In older rabbits (at 18 weeks) the use of algae or vegetable oils (sunflower or soy) in the feed increased *FABP4* gene expression in muscle. The opposite occurred in younger rabbits (at 12 weeks). To our knowledge, there are no available data on the effect of diet on *FABP4* gene expression in rabbits. In our previous study [Migdał *et al*. 2018] we showed that single nucleotide polymorphisms within the rabbit *FABP4* gene were associated with fatness traits. Fan *et al.* [2019] found that dietary treatment (algae) had no significant effect on the expression of *FABP* genes in sheep muscle. On the other hand, *FABP4* expressionin muscle of cattle fed with fodder with the addition of soy oil as a source of lipids was significantly higher than in the control group [Oliveira *et al*. 2014]. Higher *FABP4* expression (3.83 times) was found in the muscle of Barrosa cattle fed high-fat silage and low-fiber

content compared with low-fat silage and high-fiber silage [da Costa *et al*. 2013]. Teixeira *et al.* [2017] reported that Nellore bulls fed ground corn had greater *FABP4* expression. Our results also indicated that *FABP4* gene expression depended on the age of the rabbits and increased with rabbit age (Fig. 1). Similarly, Li *et al*. [2018] observed that *FABP4* gene expression increased with cattle age. Expression of this gene was also significantly higher in sheep at 160 and 200 days of age than at 90 days of age [Xu *et al*. 2011]. Albrecht *et al*. [2011] found a significant age influence on *FABP4* expression in muscle of cattle at 10, 14, 18, 22 and 26 months of age: *FABP4* gene expression was highest at 22 months of age. Furthermore, we observed that the *FABP4* mRNA expression was unaffected by sex (data not shown). To our knowledge, no study has examined the effects of sex on *FABP4* expression. Our results provide new data to better understand the mechanisms underlying FA regulation in rabbit meat and will help nutritionists to use feed additives to modulate the expression of genes and increase meat quality, which could be valuable in human nutrition.

Conclusion

Sunflower or soybean oil (3%) inclusion in the diet resulted in a higher linoleic acid content in meat of 12-week-old rabbits. The use of 1% *algae* in the diet increased n-3 FAs (EPA and DHA) and provided meat with health-promoting properties with a low n-6/n-3 acid ratio (2.5). *FTO* and *FABP4* gene expression in rabbit muscle was regulated by dietary factors. The expression of these genes increased with age and was highest in the rabbits fed with 1% algae supplementation in the diet. The FA profile and *FTO* and *FABP4* gene expression were affected by the age of rabbits, but not by sex. Our results indicate that dietary supplementation modifies *FTO* and *FABP4* expression in muscle and changes the FA profile in rabbits. Further studies are required to better understand nutrigenomic regulation of lipid metabolism in rabbits.

REFERENCES

1. ALBRECHT E., GOTOH T., EBARA F., WEGNER J. MAAK S., 2011 - Technical note: Determination of cell specific gene expression in bovine skeletal muscle using laser microdissection and RT-qPCR. ***Journal of Animal Science*** 89, 4339-4343.
2. AOAC, 2012 - Official methods of analysis. Arlington, VA, USA: Association of Official Analytical Chemists, 19th ed.
3. BATURE, A. MELVILLE L., RAHMAN K.M. AULAK P., 2022 - Microalgae as feed ingredients and a potential source of competitive advantage in livestock production: A review. ***Livestock Science*** 259, 104907.
4. BEDNÁROVÁ A., MOCÁK J., GÖSSLER W., VELIK M., KAUFMANN J. STARUCH L., 2012 - Effect of animal age and gender on fatty acid and elemental composition in Austrian beef applicable for authentication purposes. ***Chemical Papers*** 67, 274-283.
5. CZAUDERNA M., BIAŁEK M., BIAŁEK A. KARPINSKA M., 2021 - Diet supplemented with lycopene and selenized yeast change contents of fatty acids in the liver and femoral muscles of rabbits. ***Livestock Science*** 250, 104598.

6. COSTA F.S., CABRAL A.R., SILVA S.L., SILVA M.A.I., HENRIQUE W., MAZALLI M.R., BALDI F.S., MUELLER L.F., FERRINHO A.M., CORTE R.R.P.S., PEREIRA A.S.C., 2020 - Effects of n-3 and n-6 feeding sources on the quality and lipid oxidation of meat from feedlot-finished *Bos indicus* steers. ***Meat Science*** 161, 107966.
7. DA COSTA A.S.H., PIRES V.M.R., FONTES C.M.G.A. PRATES J.A.M., 2013 - Expression of genes controlling fat deposition in two genetically diverse beef cattle breeds fed high or low silage diets. ***BMC Veterinary Reserch*** 9, 118.
8. DA FONSECA A.C.P., ABREU G.M., ZEMBRZUSKI V.M., CAMPOS JUNIOR, M., CARNEIRO J.R.I., NOGUEIRA NETO J.F., CABELLO G.M.K. CABELLO P.H., 2019 - The association of the fat mass and obesity-associated gene (FTO) rs9939609 polymorphism and the severe obesity in a Brazilian population. ***Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*** 12, 667-684.
9. DAL BOSCO A., GERENCSÉR Z.S., SZENDRÕ Z.S., MUGNAI C., CULLERE M., KOVÀCS M., RUGGERI S., MATTIOLI S., CASTELLINI C. DALLE ZOTTE A., 2014 - Effect of dietary supplementation of Spirulina (*Arthrospira platensis*) and Thyme (*Thymus vulgaris*) on rabbit meat appearance, oxidative stability and fatty acid profile during retail display. ***Meat Science*** 96, 114-119.
10. DAL BOSCO A, MATTIOLI S., CULLERE M., SZENDRŐ Z, GERENCSÉR Z., MATICS Z., CASTELLINI C., SZIN M., DALLE ZOTTE A., 2018 - Effect of diet and packaging system on the oxidative status and polyunsaturated fatty acid content of rabbit meat during retail display. ***Meat Science*** 143, 46-51.
11. DERVISHI E., SERRANO C., JOY M., RODELLAR C. CALVO J.H., 2011 - The effect of feeding system in the expression of genes related with fat metabolism in semitendinous muscle in sheep. ***Meat Science*** 89(1), 91-97.
12. FAN Y., REN C., MENG F., DENG K., ZHANG G. WANG F., 2019 - Effects of algae supplementation in high-energy dietary on fatty acid composition and the expression of genes involved in lipid metabolism in Hu sheep managed under intensive finishing system. ***Meat Science*** 157, 107872.
13. FOLCH J., LEE M. STANLEY G.H.S., 1957 - A simple method for the isolation and purification of total lipids from animal tissues. ***Journal of Biological Chemistry*** 226, 497.
14. GAŠPERLIN L, POLAK T., RAJAR A., SKVARÈA M. LENDER B., 2006 - Effect of genotype, age at slaughter and sex on chemical composition and sensory profile of rabbit meat. ***World Rabbit Science*** 14, 157-166.
15. GONZÁLEZ L., MORENO T., BISPO E., DUGAN M.E.R., FRANCO D., 2014 - Effect of supplementing different oils: linseed, sunflower and soybean, on animal performance, carcass characteristics, meat quality and fatty acid profile of veal from "Rubia Gallega" calves. ***Meat Science*** 96, 829-836.
16. HONG J., KIM D., CHO K., SA S., CHOI S., KIM Y., PARK J., SCHMIDT G. S., DAVIS M.E. CHUNG H., 2015 - Effects of genetic variants for the swine FABP3, HMGA1, MC4R, IGF2, and FABP4 genes on fatty acid composition. ***Meat Science*** 110, 46-51.
17. JAWORSKA D., CZAUDERNA M., PRZYBYLSKI W., RANACHOWSKA A., 2020 - The effect of fish oil, lycopene and organic selenium as feed additives on rabbit meat quality. ***Journal of Applied Animal*** **Research** 48, 476-483.
18. KIEROŃCZYK B., SYPNIEWSKI J., RAWSKI M., PRUSZYŃSKA-OSZMAŁEK E., SASSEK M., KOŁODZIEJSKI P., JÓZEFIAK D., 2022 - Replacement of soybean oil with cold-extracted fat from *Hermetia illucens* in young turkey diets: Effects on performance, nutrient digestibility, selected organ measurements, meat and liver tissue traits, intestinal microbiota modulation, and physiological and immunological status. ***Animal Feed Science and Technology*** 286,115210.
19. KOTRBÁČEK V., DOUBEK J., DOUCHA J., 2015 - The chlorococcalean alga *Chlorella* in animals nutrition, a review. ***Journal of Applied Phycology*** 27, 2173-2180.

20. KOWALSKA D., GUGOŁEK A., STRYCHALSKI J., 2020 - Evaluation of slaughter parameters and meat quality of rabbits fed diets with silkworm pupae and mealworm larvae meals. ***Annals of Animal Science*** 20, 551-564.
21. LI X.Z., YAN C.G., GAO Q.S., YAN Y., CHOI S.H., SMITH S.B., 2018 - Adipogenic/lipogenic gene expression and fatty acid composition in chuck, loin, and round muscles in response to grain feeding of Yanbian Yellow cattle. ***Journal of Animal Science*** 96, 2698-2709.
22. MADSEN M.B., BIRCK M.M., FREDHOLM M., CIRERA S., 2010 - Expression studies of the obesity candidate gene FTO in pig. ***Animal Biotechnology*** 21, 51-63.
23. MARANGONI F., AGOSTONI C., BORGHI C., CATAPANO A. L., CENA H., GHISELLI A. POLI, A., 2020 - Dietary linoleic acid and human health: Focus on cardiovascular and cardiometabolic effects. ***Atherosclerosis*** 292, 90-98.
24. MASEK T., STARCEVIC K., FILIPOVIC N., STOJEVIC Z., BROZIC D., GOTTSTEIN Z. SEVERIN K., 2014 - Tissue fatty acid composition and estimated D desaturase activity after castration in chicken broilers fed with linseed or sunflower oil. ***Journal of Animal Physiology and Animal Nutrition*** 98, 384-392.
25. MATTIOLI S., CASTELLINI C., MANCINI S., ROSCINI V., MANCINELLI A.C., COTOZZOLO E., PAUSELLI M., DAL BOSCO A., 2020 - Effect of trub and/or linseed dietary supplementation on in vivo oxidative status and some quality traits of rabbit meat. ***Meat Science*** 163, 108061.
26. METZGER S., ODERMATT M., SZABÓ A., RADNAI I., BIRÓ-NÉMETH E., NAGY I. SZENDRÖ Z., 2011 - Effect of age and body weight on carcass traits and meat composition of rabbits. ***Archives Animal Breeding*** 54, 406-418.
27. MIGDAŁ Ł., KOZIOŁ K., PAŁKA S., MIGDAŁ W., OTWINOWSKA-MINDUR A., KMIECIK M., MIGDAŁ A., MAJ D., BIENIEK J., 2018 - Single nucleotide polymorphisms within rabbits (Oryctolagus cuniculus) fatty acids binding protein 4 (FABP4) are associated with meat quality traits. ***Livestock Science*** 210, 21-24.
28. MORDENTI A.L., SARDI L., BONALDO A., PIZZAMIGLIO V., BROGNA N., CIPOLLINI I., TASSINARI M., ZAGHINI G., 2010 - Influence of marine algae (*Schizochytrium spp.*) dietary supplementation on doe performance and progeny meat quality. ***Livestock Science*** 128, 179-184.
29. NOWACKA-WOSZUK J., PRUSZYNSKA-OSZMALEK E., SZYDLOWSKI M., SZCZERBAL I., 2017 - Nutrition modulates Fto and Irx3 gene transcript levels, but does not alter their DNA methylation profiles in rat white adipose tissues. ***Gene*** 610, 44-48.
30. NRC, 1977 - *Nutrient Requirements of Rabbits.* Washington, D.C: The National Academies Press, 2nd rev. ed.
31. OLIVEIRA D.M., CHALFUN A. – JUNIOR, CHIZZOTTI M.L., BARRETO H.G., COELHO T.C., PAIVA L.V., COELHO, C.P., TEIXEIRA P D., SCHOONMAKER J.P. LADEIRA M.M., 2014 - Expression of genes involved in lipid metabolism in the muscle of beef cattle fed soybean or rumen-protected fat, with or without monensin supplementation. ***Journal of Animal Science*** 92, 5426-5436.
32. PARVEEN R., KHAN M.I., ANJUM F.M. SHEIKH M.A., 2016 - Investigating potential roles of extruded flaxseed and a-tocopherol acetate supplementation for production of healthier broiler meat. ***British Poultry Science*** 57, 566-575.
33. PEIRETTI P.G., MEINERI, G., 2011 - Effects of diets with increasing levels of Spirulina platensis on the carcass characteristics, meat quality and fatty acid composition of growing rabbits. ***Livestock Science*** 140, 218-224.
34. PEREIRA P.M., VICENTE A.F., 2013 - Meat nutritional composition and nutritive role in the human diet. ***Meat Science*** 93, 586-592.

35. PERNA A, SIMONETTI A., GRASSI G. GAMBACORTA E., 2019 - Effect of a cauliflower (Brassica oleraceae var. Botrytis) leaf powder-enriched diet on performance, carcass and meat characteristics of growing rabbit. ***Meat Science*** 149, 134-140.
36. PETRESCU D.C., PETRESCU-MAG R.M., 2018 - Consumer behaviour related to rabbit meat as functional food. ***World Rabbit Sciene*** 26, 321–333.
37. PETRESCU, D.C., VERMEIR I., PETRESCU-MAG R.M,. 2020 - Consumer Understanding of Food Quality, Healthiness, and Environmental Impact: A Cross-National Perspective. ***International Journal of Environmental Research and Public Health*** 17, 169.
38. PFAFFL M.W., 2001 - A new mathematical model for relative quantification in real-time RT-PCR. ***Nucleic Acids Research*** 29, 2002-2007.
39. POLAK T., GAŠPERLIN L., RAJAR A. ŽLENDER B., 2006 - Influence of genotype lines, age at slaughter and sexes on the composition of rabbit meat. ***Food Technology and Biotechnology*** 44, 65-73.
40. RAMÍREZ J.A., DÍAZ I., PLA M., GIL M., BLASCO A. OLIVER M.À., 2005 - Fatty acid composition of leg meat and perirenal fat of rabbits selected by growth rate. ***Food Chemistry*** 90, 251-256.
41. RODRÍGUEZ M., CARRO M.D., VALIENTE V., FORMOSO-RAFFERTY N., REBOLLAR P.G., 2019 - Supplementation with fish oil improves meat fatty acid profile although impairs growth performance of early weaned rabbits. ***Animals*** 9, 437.
42. RONKAINEN J., HUUSKO T. J., SOININEN R., MONDINI E., CINTI F., MAKELA K. A., KOVALAINEN M., HERZIG K.H., JARVELIN M.R., SEBERT S., SAVOLAINEN M.J. SALONURMI T., 2015 - Fat mass- and obesity-associated gene Fto affects the dietary response in mouse white adipose tissue. ***Scientific Reports*** 5, 9233.
43. SANCHES A., DELGADO M.M., BUSTAMENTE M. FERNANDEZ J., 2000 - Automated analysis of vitamin E isomers in vegetable oils by continuous membrane extraction and liquid chromatography electrochemical detection. ***Journal of Chromatography A*** 881, 229-241.
44. SAS, Institute Inc.,2014 - The SAS System for Windows. Version 9.4. Cary, NC, USA.
45. SÉBERT S., HYATT M., CHAN L., YIALLOURIDES M., FAINBERG H., PATEL N., SHARKEY D., STEPHENSON T, RHIND S., BELL,R., 2010 - Influence of prenatal nutrition and obesity on tissue specific fat mass and obesity-associated (FTO) gene expression. ***Reproduction*** 139, 265-274.
46. SIMOPOULOS A. P., 2016 - An increase in the omega-6/omega-3 fatty acids ratio increases the risk for obesity. ***Nutrients*** 8, 128.
47. TAO X., MEN X.M., DENG B. XU Z.W., 2013 - Effects of breed, postnatal development, and nutrition on mRNA expression of the *FTO* gene in porcine muscle and its relationship with intramuscular fat deposition. ***Czech Journal of Animal Science*** 58, 381–388.
48. TEIXEIRA P.D., OLIVEIRA D.M., CHIZZOTTI M.L., CHALFUN A.-JUNIOR, COELHO T.C., GIONBELLI M.P., PAIVA L.V., CARVALHO J.R.R., LADEIRA M.M., 2017 - Subspecies and diet affect the expression of genes involved in lipid metabolism and chemical composition of muscle in beef cattle. ***Meat Science*** 133, 110-118.
49. TEIXEIRA A., RODRIGUES S., 2021 - Consumer perceptions towards healthier meat products. ***Current Opinion*** **in** ***Food Science*** 38, 147-154.
50. TROCINO A., BIROLO M., DABBOU S., GRATTA F., RIGO N. XICCATO G., 2018 - Effect of age and gender on carcass traits and meat quality of farmed brown hares. ***Animal*** 12, 864-871.
51. TUNG Y.C., AYUSO E., SHAN X., BOSCH F., O'RAHILLY S., COLL A.P. YEO G.S., 2010 - Hypothalamic-specific manipulation of Fto, the ortholog of the human obesity gene FTO, affects food intake in rats. ***PLoS One*** 5, e8771.

52. VOLEK Z., BUREŠ D. UHLÍŘOVÁ L., 2018 - Effect of dietary dehulled white lupine seed supplementation on the growth, carcass traits and chemical, physical and sensory meat quality parameters of growing-fattening rabbits. ***Meat Science*** 141, 50-56.
53. WANG Q., WANG L., LIN H., HUO X., ZHU Q., ZHANG M., 2018 - Relationship between fat mass and obesity-associated gene expression and type 2 diabetes mellitus severity. ***Experimental and Therapeutic Medicine*** 15), 2917-2921.
54. WOOD J.D., ENSER M.B., FISHER A.V., NUTE G.R., SHEARD P.R., RICHARDSON R.I. HUGHES S.I., 2008 - Fat deposition, fatty acid composition and meat quality: a review. ***Meat Science*** 78, 343-358.
55. WORLD HEALTH ORGANIZATION, 2008 - *Interim summary of conclusions and dietary recommendations on total fat and fatty acids.* Joint FAO/WHO expert consultation on fats and fatty acids in human nutrition. WHO, Geneva.
56. XU Q.L. ZHANG Q.L., CHEN Y.L., 2011 - Cloning, expression and structure modeling analysis of adipocyte fatty acid binding protein gene (*FABP4*) of sheep. ***Journal of Agricultural Biotechnology*** 19, 483-489.
57. YUZBASHIAN E., ASGHARI G., CHAN C.B., HEDAYATI M., SAFARIAN M., ZARKESH M., MIRMIRAN P., KHALAJ A., 2021 - The association of dietary and plasma fatty acid composition with FTO gene expression in human visceral and subcutaneous adipose tissues. ***European Journal of Nutrition*** 60, 2485-2494.
58. ZHANG G.W., GAO L., CHEN S.Y., ZHAO X.B., TIAN Y.F., WANG X., SONG D.X. SONG L.J., 2013 - Single nucleotide polymorphisms in the FTO gene and their association with growth and meat quality traits in rabbits. ***Gene*** 527, 553-557.
59. ZHONG T., DUAN X.Y., ZHANG H., LI L., ZHANG H.P., NIU L., 2017 - Angelica sinensis suppresses body weight gain and alters expression of the FTO gene in high-fat-diet induced obese mice. ***BioMed Research International***, 6280972.