



Changes in rabbit meat texture and calpain system genes expression following dietary supplementation with algae or vegetable oils*

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Meat texture is one of the most important parameters of meat quality and it could be improved by lipid sourced from vegetable oils. This study was undertaken to analyze the changes in meat texture, and in the mRNA expression of calpains (*CAPN1* and *CAPN3*) and calpastatin 2 (*CAST2*) following diet supplemented with 1% algae (A), 3% sunflower (OS) oil, or 3% soybean oil (SO), using Blanc de Termonde rabbits at different age (12 and 18 weeks). Animals in the control group (C) received feed without additives. Animals were randomly sorted (40 rabbits/each group, balanced for sex). The General Linear Model (GLM) procedure was used to analyze the data and the Tukey–Kramer test was used for multiple comparisons. Our results indicate that the texture of rabbit meat depends on the age and algae, sunflower, and soybean oil supplementation, and diet-age interaction. The influence of the diet on *CAPN1*, *CAPN3*, and *CAST2* expression depends on the rabbit age. In 12-week-old animals, meat shear force was greater following algae and soybean oil supplementation. Moreover, soybean oil decreased *CAPN1*, *CAPN3*, and *CAST2*, and algae supplement decreased *CAST2* genes expression in rabbit muscle. In 18-week-old rabbits, supplementation with sunflower or soybean oil improved meat hardness, chewiness, and shear force. Additionally, sunflower oil increased *CAPN1* and decreased *CAST2* gene expression in the muscle tissue, providing new information about the molecular mechanism of meat texture in rabbits.

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Rabbit meat is recommended for children and patients with cardiovascular diseases such as hypertension and hyperlipidemia due to its nutritional and dietary properties (low fat and cholesterol content) [Cullere and Dalle Zotte 2018, Chen *et al.* 2021]. Yet, rabbit meat consumption is decreasing because of many reasons from welfare concerns to individual taste preferences [Cullere and Dalle Zotte 2018]. Therefore, except for complying with other requirements, it is essential for producers to ensure their product is high quality and palatable.

Texture (hardness, springiness, cohesiveness, and chewiness) is one of the most important parameters of rabbit meat quality. Consumers consider the parameter to influence the acceptability of the meat [Lian *et al.* 2013] and drive the contentment, repeat purchase, and readiness-to-pay high prices [Warner *et al.* 2022]. Meat texture is influenced by a range of factors, including dietary, environmental and genetic ones such as sex, breed, age, diet, intramuscular fat content, structure of the muscle fibres, and pre-slaughter stress [Ruiz De Huidobro *et al.* 2005, Chen *et al.* 2021, Kumar *et al.* 2023].

Literature links the hardness or tenderness of distinct types of meat to lipids in diet and highlights far-reaching benefits of lipid supplementation. Meat hardness is related to lower fat and greater collagen contents [Łapa *et al.* 2008]. Since rabbit meat has a low intramuscular fat [Daszkiewicz and Gugolek 2020] it may be tough. However, Kim *et al.* [2012] observed that the hardness, chewiness, and cohesiveness of the fish dorsal muscle were markedly affected by dietary lipid source (soybean and linseed oils). It has also been reported that dietary supplementation with macadamia oil improved meat's tenderness in lambs [Dias *et al.* 2020]. Consequently, vegetable oils, including sunflower and soybean oils, and algae are a rich source of lipids that may be used as a simple strategy to improve meat texture. Moreover, for humans, these oils are useful sources of essential fatty acids (FA), mainly of linoleic acid (LA) [González *et al.* 2014]. In many countries the use of sunflower oil in an everyday diet is important, due to its abundance in phytosterols and antioxidants beneficial for human health [Adeleke and Babalola 2020]. Due to high metabolizable energy content and digestibility, soybean oil is used as an energy source in a diet [Kierończyk *et al.* 2021]. Additionally, marine algae are a good source of long-chain n-3 polyunsaturated fatty acids (PUFAs), predominantly docosahexaenoic (DHA) and eicosapentaenoic (EPA) ones [Barta *et al.* 2021]. Among the factors affecting meat texture, the calpain complex genes also play a vital role [Coria *et al.* 2018]. Three types of calpains are expressed in skeletal muscles: calpain 1 (*CAPN1*; μ -calpain), calpain 2 (*CAPN2*; m-calpain), and calpain 3 (*CAPN3*; asp94), as well as *CAST* being a *CAPN* inhibitor [Pinto *et al.* 2010, Calvo *et al.* 2014, Shu *et al.* 2015]. Calpains are calcium-ion-activated neutral proteinases, belonging to a group of cysteine endopeptidases, responsible for the breakdown of myofibrillar proteins [Goll *et al.* 2003, Calvo *et al.* 2014]. Calpastatin and its calcium-controlled isoforms (*CAST*, *CAST1*, *CAST2*, *CAST3*, and *CAST4*), act as endogenous inhibitors of the proteolytic activity of calpains [Cònsolo *et al.*

2016]. In cattle, *CAPN3* and *CAPN1* genes polymorphisms were associated with meat tenderness [Barendse et al. 2008, Sun et al. 2018]. It was reported that selection based on *CAST* gene markers may enhance meat tenderness in Brahman cattle [Café et al. 2010]. The analysis of porcine *CAPN3* gene expression showed that its higher transcript abundance correlated with lower tenderness in muscles [Gandolfi et al. 2011]. Malheirosa et al. [2018] demonstrated the association between the expression of *CAST2* and meat tenderness in cattle. According to another study, decreased calpain or increased calpastatin expressions are linked to tougher meat [Ilian et al. 2001]. Therefore, muscle type, fiber type, sex, age, and feeding strategies can impact the calpain and calpastatin expression [Coria et al. 2018]. It is known that diet can impact the calpain system proteins expression, e.g., after long-term feeding restriction, the activity of calpain-1 was decreased [Therkildsen 2005]. Furthermore, differences in the composition of cattle diets affected calpastatin level in muscles [Du et al. 2004]. However, while most of the research has been conducted on pork, beef, and poultry; the effect of diet and age on expression of calpain and calpastatin genes has never been determined in rabbits. Only Wang et al. described a high rate of polymorphism at the *CAST* locus in rabbits in 2017.

Considering the above, we hypothesized that a diet containing algae or vegetable oils (sunflower or soybean) as a rich source of lipids would effectively improve the meat texture in rabbits. We also hypothesized that these supplements would change the expression of texture-associated genes, such as *CAPN1*, *CAPN3*, and *CAST2*, and these changes would be related to the rabbit's age. Thus, the study was undertaken to determine the effects of the diet with algae and vegetable oils (sunflower or soybean), and of rabbit age (12 and 18 weeks) on the meat texture, and on the expression of the *CAPN1*, *CAPN3*, and *CAST2* genes in rabbit muscle. The present study is a continuation of the previous ones that determined the effect of algae and vegetable oils (sunflower or soybean) on meat quality and reproduction of rabbits [Grzesiak et al. 2020, Maj et al. 2023].

Material and methods

General procedures

The experiment was conducted after approval by the Local Ethics Committee (No. 2/2018) respecting EU regulations (EU Directive 2010/63/EU and Council Regulation EC No. 1099/2009). A total of 160 Blanc de Termonde rabbits (80 females and 80 males) weaned on day 35 were used. One male and one female from a litter were selected for this study. The mating system was designed to avoid inbreeding. The animals were randomly sorted (40 rabbits/each group, balanced for sex) according to diet and housed in a closed building in wire cages (0.80 × 0.55 × 0.45 m) in the same environmental conditions (lighting 14L:10D, humidity 65-70%, temperature 15-20°C). The rabbits were fed isoprotein pellets, which differed in crude fat content (Farmer company, Poland). The diet was stabilized by lowering the proportion of other feed

Table 1. Dietary ingredients (%)

Components	Diets			
	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
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: C – control, A – algae, OS – sunflower oil, SO – soybean oil.

Table 2. Chemical and fatty acids composition of the diets

Item	Diets			
	C	A	OS	SO
Chemical composition (g/kg DM)				
dry matter (g/kg)	882	882	885	885
crude ash	75.22	72.71	74.71	74.71
crude protein	170	170	170	170
crude fat	33.37	42.28	49.61	49.61
crude fibre	158	158	156	156
lysine	7.01	7.42	8.85	8.85
methionine	3.45	3.42	3.38	3.38
cysteine	3.27	3.27	3.18	3.18
threonine	6.50	6.39	6.39	6.39
tryptophan	2.36	2.34	2.31	2.31
Metabolizable energy ¹ (MJ/kg DM)	9.42	9.71	9.91	9.91
Fatty acid (% of total FA)				
myristic c14:0	0.12	0.45	0.11	0.43
palmitic c16:0	4.55	12.50	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: C – control, A – algae, OS – sunflower oil, SO – soybean oil, DM – dry matter.

¹ Dietary metabolizable energy was calculated in accordance with the NRC [32].

components. Dietary metabolizable energy (ME) was calculated in accordance with the recommendations of the NRC [1977]. Feed and water were available *ad libitum* from weaning to 12 or 18 weeks of age. Throughout the experiment the rabbits in the control group (C) were fed pellets without additives. The diets in the other groups included 1% marine algae (A; DHA Gold® - *Schizochytrium* sp.), 3% sunflower oil (OS), or 3% soybean oil (SO). Tables 1 and 2 provide the diets ingredients and their chemical composition. The animals were slaughtered at 12 weeks (n = 80; 40 males and 40 females) or 18 weeks (n = 80 (40 males and 40 females) of age, at body weight around 2.5 kg and 3.4 kg, respectively.

Samples (5 g) for RNA analysis were taken from the *longissimus lumborum* muscle, frozen in liquid nitrogen, and then stored at -80°C until analyses. The carcasses were refrigerated at 4°C for 24 hours. The *longissimus lumborum* muscle was trimmed of external fat and connective tissue. Vacuum-packed samples (50 g) were delivered to the laboratory in ice boxes and frozen at -20°C (n = 20 in each group for meat texture and n = 8 in each group for gene expression).

Chemical analysis

The chemical composition of the diets and intramuscular fat (IMF) content of the *longissimus lumborum* muscle were determined following by AOAC official methods [2012]. Dry matter (method 934.01), crude protein (method 2001.11), crude fiber (method 978.10), and ash (method 967.05) were analyzed. Lipids from the diets and muscle samples were extracted with a chloroform-methanol mixture (2:1, v/v) according to Folch *et al.* [1957] as previously described by Maj *et al.* [2023].

The content of amino acids in diets was analyzed with an automatic Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc., Pleasanton, CA, USA).

Texture analysis

A Texture Profile Analysis (TPA) was conducted by a TA.XT plus texturometer (Stable Micro Systems, UK) with an adapter, which was a cylinder with a diameter of 50 mm. Cylindrical samples (10×10 mm) were cut from the *longissimus lumborum* muscle. The double compression test of samples to 70% of their height was performed. The speed of the roller moving during the test was 5 mm/s, parallel to the muscle fibers, while the interval between the presses was 5 seconds [Combes *et al.* 2003]. Using Exponent Version: 5,1,2,0, (Stable Micro Systems, UK) the following texture indicators were determined: hardness, springiness, cohesiveness, and chewiness. During serial measurements, all texture indicators were calculated automatically. Shear force was measured using the same texturometer fitted with a Warner-Bratzler shear blade with a triangular hole. The shear force of samples (10 × 10 mm) *longissimus lumborum* muscle was measured (blade speed = 2 mm/s), perpendicular to the muscle fibres.

Gene expression analysis

Frozen muscle samples were utilized for total cellular RNA isolation with TRI Reagent solution (Ambion, Austin, TX, USA) according to manufacturer's protocol. The total RNA samples concentration and purity were assessed spectrophotometrically by the NanoDrop ND2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The reverse transcription to cDNA was performed with a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) and subsequent real-time PCR was conducted as previously reported [Grzesiak *et al.* 2019] using a StepOne™ Real-Time PCR System (Applied Biosystems) and the rabbit-specific TaqMan Gene Expression Assays (Applied Biosystems) for: *CAPN1* (assay ID:Oc03396246_g1), *CAPN3* (assay ID:Oc03396993_m1), and *CAST2* (Custom PlusTaqMan RNA Assay based on rabbit *CAST2* cDNA sequence) following the manufacturer's protocol. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*, assay ID:Oc03823402_g1) and β -actin (*ACTB*; assay ID:Oc03824857_g1) were employed as endogenous controls. All qPCR reactions were carried out in duplicates. A negative control without template was conducted, and genomic DNA amplification contamination was checked by omitting reverse transcriptase during the reverse transcription reaction.

For relative mRNA quantification the real-time PCR Miner algorithm was applied [Zhao and Fernald 2005] to calculate real-time PCR expression values using reaction efficiency and the fractional cycle number at the threshold. The data obtained from the real-time PCR for *CAPN1*, *CAPN3*, and *CAST2* were normalized to those obtained for *GAPDH* and *ACTB*.

Statistical analyses

The normal distribution of the data was checked by the Kolmogorov-Smirnov test.

The data were analyzed by the least squares method and the GLM procedure (SAS 2014) according to the following linear model:

$$Y_{ijkl} = \mu + D_i + A_j + S_k + (D \times A)_{ij} + (D \times S)_{ik} + (A \times S)_{jk} + (D \times S \times A)_{ijk} + E_{ijkl},$$

where:

- Y_{ijkl} – the value of the trait;
- μ – overall mean;
- D_i – the effect of the i -th diet ($i = 1, \dots, 4$);
- A_j – the effect of the j -th age ($j = 1, 2$);
- S_k – the effect of the k -th sex ($k = 1, 2$);
- $(D \times A)_{ij}$ – the effect of the interaction between diet and age;
- $(D \times S)_{ik}$ – the effect of the interaction between diet and sex;
- $(A \times S)_{jk}$ – the effect of the interaction between age and sex;

$(D \times S \times A)_{ijk}$ – the effect of the interaction between diet, age and sex;

E_{ijkl} – a random error.

Interactions between fixed effects were removed from the model if they were not significant (diet-sex; age-sex; diet-age-sex).

The results are presented as the least squares means (LSM) and the pooled standard error of the mean (SEM) along with the significance levels of the effect. The LSMs for the studied groups were compared by the Tukey-Kramer multiple comparisons *post hoc* test. $p < 0.05$ indicates significant differences. The statistical analysis did not show significant differences between male and female rabbits; therefore, the results in Tables 3, and 4 are the averages for both sexes.

Results and discussion

Intramuscular fat (IMF) content

The algae and vegetable oils (sunflower or soybean) did not significantly influence the IMF. The IMF in rabbit meat ranged from 1.98 to 2.77 percent. The fat content decreased significantly with age and was lower in the 18-week-old rabbits than in the 12-week-old rabbits (Tab. 3).

Meat tenderness is a sensory trait related to the IMF content [Hocquette *et al.* 2010]. Noteworthy, fat content in rabbit meat is low [Daszkiewicz and Gugolek 2020] and the loin is lower in fat than other muscles. To ensure the juiciness and tenderness appealing to consumers, the optimal fat content, should be no more than 3% [Maj *et al.* 2008]. The IMF content in rabbit meat analyzed herein was low, similar in all examined groups. It decreased with age. Also, in a previous study, we found that the meat of older rabbits (21 and 31 weeks of age) contained less IMF than the meat of younger ones (12 weeks of age) - Maj *et al.* [2012].

Meat texture

The results indicated significant interaction effects between diet and age for all texture parameters. Rabbit meat hardness ranged from 8.96 to 15.35 kg (Tab. 3). The meat of the 12-week-old rabbits of the diet groups did not differ significantly in hardness, while at the 18th week, the rabbit meat from the C group was described by the highest values of this parameter and was significantly harder than the meat of rabbits from the A, OS, and SO groups. The springiness of the rabbit meat from the diet and age groups were similar, while the value of this parameter in the A group was markedly higher in older rabbits. In the case of the rabbits at the 12th week of rearing, the meat cohesiveness of the diet groups was similar, while at the 18th week, the values of this parameter were markedly lower in the SO group than in the C group. Moreover, in the SO group, meat cohesiveness decreased significantly with age. At the 12th week of rearing, the lack of differences was observed between the diet groups in the meat

Table 3. The influence of diet and age on the texture of the meat

Trait	Age-12 week			Age-18 week			Pooled SEM	p value			
	C	A	OS	SO	C	A		OS	SO	diet	age
Fat (%)	2.60	2.22	2.66	2.77	1.98	2.24	2.49	2.02	0.247	0.006	0.130
Hardness (kg)	9.65 ^{ac}	8.86 ^a	8.96 ^b	9.87 ^{ac}	15.35 ^b	10.16 ^{ac}	11.94 ^a	10.66 ^{ac}	<0.001	<0.001	<0.001
Springiness	0.50 ^{ab}	0.48 ^a	0.51 ^{ab}	0.51 ^{ab}	0.49 ^{ab}	0.53 ^b	0.51 ^{ab}	0.49 ^{ab}	0.432	0.325	0.008
Cohesiveness	0.44 ^{ab}	0.46 ^b	0.45 ^b	0.46 ^b	0.46 ^b	0.44 ^{ab}	0.44 ^{ab}	0.42 ^a	0.109	0.009	0.002
Cheewiness (kg)	2.20 ^a	2.06 ^a	2.16 ^a	2.38 ^a	3.53 ^b	2.50 ^a	2.83 ^{ab}	2.28 ^a	0.080	<0.001	0.013
Shear force (kg/cm ²)	1.65 ^a	2.68 ^b	1.97 ^a	2.41 ^b	2.68 ^b	2.52 ^b	1.98 ^a	2.03 ^a	<0.001	<0.001	<0.001

The results are presented as the least squares means (LSM) and the pooled standard error of the mean (SEM) (n = 20 per each group).

^{abc}Means bearing different superscripts in the same row are significantly different at $p < 0.05$.

C – control diet, A – diet with addition of 1% algae, OS – diet with addition of 3% sunflower oil, SO – diet with addition of 3% soybean oil, SEM – standard error of the mean.

Table 4. The influence of diet and age on expression of *CAPNI*, *CAPN3*, and *CAST2* genes in muscle of rabbits

Gene	Age-12 week			Age-18 week			Pooled SEM	p value			
	C	A	OS	SO	C	A		OS	SO	diet	age
<i>CAPNI</i>	0.0021 ^b	0.0023 ^b	0.0025 ^b	0.0011 ^c	0.0018 ^b	0.0022 ^b	0.0049 ^a	0.0019 ^b	0.0004	0.002	0.066
<i>CAPN3</i>	0.0221 ^{ab}	0.0212 ^{ab}	0.0289 ^a	0.0098 ^b	0.0224 ^{ab}	0.0185 ^{ab}	0.0211 ^{ab}	0.0302 ^a	0.0037	0.565	0.383
<i>CAST2</i>	0.00027 ^a	0.00012 ^b	0.00023 ^{ac}	0.00013 ^b	0.00019 ^{ab}	0.00022 ^a	0.00016 ^{bc}	0.00024 ^{ac}	0.00003	0.617	0.674

The results are presented as the least squares means (LSM) and the pooled standard error of the mean (SEM) (n = 8 per each group).

^{abc}Means bearing different superscripts in the same row are significantly different at $p < 0.05$.

C – control diet, A – diet with addition of 1% algae, OS – diet with addition of 3% sunflower oil, SO – diet with addition of 3% soybean oil, SEM – standard error of the mean, *CAPNI* – Calpain 1, *CAPN3* – Calpain 3, *CAST2* – Calpastatin 2.

chewiness. However, at the 18th week, the meat chewiness was significantly greater in the C group than in the A and SO groups. The chewiness of rabbit meat in the C group increased significantly with age as well. The meat shear force of 12-week-old rabbits was significantly lower in the C and OS groups compared to the A and SO groups, and in the 18-week-old rabbits, it was markedly lower in the OS and SO groups compared to the C and A groups. Moreover, meat shear force in the C group increased with age, and it decreased with age in the SO group.

There is little research on the influence of feed additives on the texture of rabbit meat or meat in general and the reports are not unanimous. Kowalska and Bielański [2009] found a better tenderness and juiciness of rabbit meat after the addition of 3% fish oil. Dal Bosco *et al.* [2004] stated that rabbits fed with pellets supplemented with linseed oil and tocopherol acetate had a better meat texture than those from the control group. On the contrary, Balaceanu-Dojana *et al.* [2014] did not observe the effect of sunflower oil enriched diet on rabbit's meat hardness. Likewise, Urrutia *et al.* [2016] did not show the effect of the flaxseed added to the feed on the hardness of lamb meat. Similarly, Rodrigues *et al.* [2022] showed that in lambs fed diets with different oil seed lipid sources, shear force of the meat was unaffected by diets. The lack of difference in the texture of cattle meat following diets ranging from high to low lipid incorporation of different fat sources was also reported [Lima *et al.* 2015]. Likewise, Costa *et al.* [2013] have found no significant changes in cattle meat texture after different inclusions of cotton seed in the diet. In our previous study, we described that the hardness, chewiness, and shear force of the rabbit meat increased with the rabbit age [Maj *et al.* 2012]. Muscle connective tissue matrix undergoes stabilization with reaching sexual maturation, thus increased collagen stability leads to increased toughness [Purslow 2018]. In the present study, the addition of algae, sunflower oil, or soybean oil to rabbit feeding resulted in reducing meat toughness in older rabbits.

The expression of *CAPN1*, *CAPN3*, and *CAST2* mRNA under different age and dietary conditions

The influence of a diet on the expression of *CAPN1*, *CAPN2*, and *CAST2* genes in the muscles of 12- and 18-week-old rabbits is shown in Table 4. At 12th week of age, the diet enriched in soybean oil led to decreased *CAPN1* mRNA expression in comparison to the C, A, and OS groups. *CAPN3* mRNA expression was lower in the SO group than in the OS group. The use of algae and soybean oil in the diet markedly diminished *CAST2* mRNA expression in comparison to the C and OS groups (Tab. 4).

In the 18-week-old animals, the sunflower oil enriched diet caused increased *CAPN1* mRNA expression when compared to the C, A, and SO groups. *CAST2* mRNA expression was lowered in the OS group in comparison to the A group. The feed with the addition of algae, sunflower or soybean oils did not change *CAPN3* mRNA expression (Tab. 4).

The source of significant variability in *CAPN1* gene expression was the diet and diet×age interaction, whereas for *CAPN3* and *CAST2* gene expression, it was only the diet×age interaction (Tab. 4).

This research shows for the first time that diet and age modulate the expression of the *CAPN1*, *CAPN3*, and *CAST2* genes in the rabbit muscle. According to our results, the influence of diet on *CAPN1*, *CAPN3*, and *CAST2* gene expression was related to rabbit age. In 12-week-old rabbits, meat shear force was greater in the diet with algae or soybean oil. In addition, the soybean oil supplement decreased *CAPN1*, *CAST2*, and *CAPN3* expression in the muscle, as the algae supplement did, too. The diet with sunflower or soybean oils in older rabbits at 18 weeks of age, led to improved meat hardness, chewiness, and shear force, and we also observed that the sunflower oil supplement increased *CAPN1* and decreased *CAST2* gene expression in the muscle.

Earlier findings on the topic referred to other species and sometimes resulted in conflicting findings, but they yielded some conclusions. Ren *et al.* [2022] showed that a high-concentrate diet increased the abundance of *CAPN1* and *CAPN2* mRNA transcripts in goat muscle, while *CAPN3* and *CAST* transcript levels were unaffected. Coria *et al.* [2020] showed that differences in meat tenderness among feeding strategies could be explained in part by alterations in the abundance of transcripts for the calpain system; a corn silage addition diminished the amount of calpains and elevated expression of calpastatin in the *longissimus dorsi* muscle of Braford steers. Giusti *et al.* [2013] indicated that the slight decrease in meat tenderness is not the result of a decreased expression of *CAPN1* and *CAPN2*, but it is likely to be associated to an elevated calpastatin expression. Furthermore, the abundance of porcine *CAST*, *CAPN1*, and *CAPN3* mRNA transcripts was analyzed in muscles characterized by diverse shear force. Greater *CAST* and *CAPN3* level were found in muscles with high shear force, confirming a direct role of calpastatin, but not calpain 3, in meat tenderization [Gandolfi *et al.* 2011]. Also, Calvo *et al.* [2014] found that meat tenderness in beef is affected by *CAST* expression, and there is a negative correlation between *CAST* expression and meat tenderness. Yang *et al.* [2012] showed a positive relation between the *CAPN3* mRNA expression in the *longissimus* muscles of pigs and tenderness. These results conflicted with those of Gandolfi *et al.* [2011], who found high *CAPN3* mRNA transcript abundance in relation to greater shear force values. Malheiros *et al.* [2018] suggested the lack of direct link between meat tenderness and *CAPN1* and *CAPN2* transcripts abundance in Nellore cattle, which could be related to the expression of *CAST2* gene. Nonetheless, the literature before our study has shown that the increasing expression and activity of calpains is valuable to the tenderness of goose, goat, and pig meat [Chang *et al.* 2020, Chaosap *et al.* 2020, Dos *et al.* 2021].

Conclusions

Our results indicate that the texture of rabbit meat depends on the age and algae, sunflower, and soybean oil supplemented diet, and on the diet-age interaction. The influence of the diet on *CAPN1*, *CAPN3*, and *CAST2* transcripts abundance depending on the rabbit age. In 12-week-old animals, meat shear force was greater following algae and soybean oil supplementation. Moreover, soybean oil decreased *CAPN1*, *CAPN3*, and *CAST2*, and algae supplement decreased *CAST2* genes expression in

rabbit muscle. In 18-week-old animals, diet supplemented with sunflower, or soybean oils led to improved meat hardness, chewiness, and shear force. Additionally, sunflower oil increased *CAPNI* and decreased *CAST2* genes expression in muscle tissue. Although the association between calpain and calpastatin mRNA expression and meat quality has been reported in other animals, in rabbits, the molecular mechanism of meat texture needs further examination.

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