



## Scientific Research

### The effect of adding calcium salts of unsaturated fatty acids to the diet on the meat quality of Sangsari male lambs

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## ABSTRACT

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One of the effective strategies for improving production efficiency in fattening male animals is the use of nutritional additives, since dietary energy is known to be one of the main limiting factors in animal nutrition and plays a fundamental role in the digestion and utilization of other nutrients. This study aimed to investigate the effect of adding calcium salts of unsaturated fatty acids (omega 3, 6, and 9) on the quality characteristics of meat of male Sangsari lambs. In this study, 28 lambs were divided into four nutritional groups with different levels of fatty acids and received the corresponding diets for 75 days. Meat quality was evaluated using indices such as color ( $L^*$ ,  $a^*$ ,  $b^*$ ), lipid oxidation (MDA), hardness, cooking loss, and water-holding capacity. The results showed that the addition of omega 3 led to a decrease in transparency and an increase in the yellowness of the meat, while the hardness of the texture decreased and its tenderness improved ( $P < 0.05$ ). Although MDA concentration increased, these values remained within acceptable limits. Also, unsaturated fatty acid supplements reduced cooking loss and improved water retention capacity, indicating higher meat stability during storage. In general, adding these supplements to lamb diets can be used as an effective strategy to improve meat quality and shelf life. However, its effects depend on the type and amount of fatty acid consumed.

## 1.Introduction

Mehdishahr, also known as Sangsar, is located in the northwest of Semnan province, 20 km from Semnan city [1]. This region, which was known for its nomadic lifestyle in the past, is now largely urbanized. Animal husbandry, especially sheep milk processing, is one of the main pillars of the Sangsar nomads' lives and has a history of several thousand years. The nomads of this region produce more than 30 types of unique dairy products with high skill, such as yogurt, doogh, arshe, cheese, lor, chikoo, kashk and wareon, etc [2]. The Sangsar sheep breed is one of the indigenous and resistant breeds of Iran that is raised in the Sangsar region in a nomadic, semi-nomadic, and rural way. This breed is one of the small to medium-sized and colorful breeds that can be seen in black, white, blond, and light to dark brown colors. The Sangsari breed of sheep has a small body size, delicate limbs, short tails, and a small, arched tail, and is hornless. In addition, Sangsari sheep are known for their excellent fattening quality, with approximately 60% of their weight being meat before slaughter. Sangsari sheep meat has a delicious and delicious taste due to its balanced fat and soft texture. This meat is of high quality due to its high fattening efficiency and appropriate muscle and fat composition. In addition to its excellent taste, the meat of this breed is known as a product with desirable nutritional properties in domestic and foreign markets [3].

One of the effective solutions for improving production efficiency in fattening male animals is the use of nutritional additives, since dietary energy is known to be one of the main limiting factors in animal nutrition and plays a fundamental role in the digestion and utilization of other nutrients. Therefore, various nutritional approaches are used to increase the energy density of the diet [4]. The use of unrefined fats in livestock diets can cause problems such as the inhibition of long-chain unsaturated fatty acids on the activity of rumen microbes. Increased

unsaturation of fats, although increasing digestibility, may impair rumen fermentation and reduce dry matter intake, milk fat and fiber digestion. In addition, rumen microorganisms hydrogenate unsaturated fatty acids, but their complete saturation can impair the fermentation process [5].

According to studies conducted in this field, to reduce the negative effects of fats, preserved fats can be used. These fats include types such as formaldehyde-preserved fats, crystalline fats, amide fatty acids, hydrogenated fats and calcium salts of long-chain fatty acids CSFA. Research has shown that adding preserved fats to the diet of lactating cows increases milk production without reducing its fat content [6]. In general, fatty acid supplements not only increase dietary energy, but also act as an effective supplement in livestock and poultry nutrition by improving the absorption of fat-soluble vitamins, improving production efficiency, reducing carcass fat and increasing essential fatty acids in meat [4]. Among the protected fats, CSFA are of particular importance due to their greater stability in the rumen and preservation of nutrient digestibility [7, 8]. These salts are produced by processing oils such as soybean, fish, flax or palm, in which triacylglycerols are converted to calcium salts of fatty acids with calcium compounds. These compounds protect against rumen fermentation by increasing the melting point of unsaturated fatty acids and resistance at pH above 6.5 and reducing negative effects on microbial metabolism [5]. Calcium salts, passing through the rumen in an intact soapy form, break the calcium and fatty acid bonds in the later stages of digestion and both components are absorbed separately. These salts include saturated fatty acids such as palmitic and stearic and unsaturated fatty acids such as oleic, linoleic and linolenic. Their melting point of approximately 38°C allows for effective dissolution and absorption in digestive fluids, improving feed efficiency by

reducing energy loss through feces and cation absorption [5].

According to Alba et al. (2021), the addition of CSFA to ruminant diets can improve energy balance and production performance. This supplementation increases the proportion of unsaturated fatty acids in meat, which are considered nutritionally healthier for humans than saturated fatty acids. These changes occur due to the partial protection of unsaturated fatty acids from the biohydrogenation process in the rumen [9]. Research has also shown that CSFA can improve meat tenderness, juiciness, and color, although the exact mechanism of these effects is not yet fully understood [10]. In addition, studies have shown that the interaction of CSFA with other dietary components, such as forages, can have positive effects on nutrient digestibility and meat quality. For example, He et al. (2018) reported that the simultaneous use of CSFA and alfalfa can improve rumen fermentation and meat quality traits in beef cattle. Overall, the evidence suggests that the targeted use of CSFA in ruminant diets has the potential to improve the nutritional value and sensory quality of meat, but further research is needed to precisely identify the mechanisms and optimize the application of this approach[11].

According to a study by Daghkia et al. (2015), the use of calcium salts of omega-3 and omega-6 fatty acid sources in ewes resulted in an increased in the number of lambs born. This increase was associated with higher estrogen concentrations during estrus and pre-estrus follicular phases, which in turn stimulates the secretion of gonadotropins through positive feedback [12]. In another study, Mohtashami et al. (1400) investigated the effect of bioactive fatty acids on the growth performance of suckling Holstein calves under cold stress. The results showed that the addition of calcium salt to fish oil under these conditions can play an effective role in improving the health of the animal and ensuring optimal

growth [4]. In addition, other researchers reported a 2-10% increase in milk production in cows fed with fat supplements compared to cows fed diets without fat supplements [13]. Also, Ganjkanloo et al. (2005) investigated the effect of adding different types of fat sources such as cottonseed, animal tallow, calcium salts of long-chain fatty acids, waste oils from restaurants and preserved fats in tablet form during the lactation period. In most cases, these sources had a positive effect on increasing milk production [14].

Given that previous studies have mainly evaluated the effect of fatty acid supplements on weight parameters and carcass yield, and the quality of the extracted meat has been less studied, and also that no research has been conducted in this field on Sangsari breed lambs so far, this study evaluates the effect of adding calcium salts of unsaturated fatty acids to the diet on the meat quality of Sangsari male lambs.

## 2. Materials and Methods

### 2.1. Materials

High-purity calcium salts of fatty acids imported under the brand LADOR were obtained from Aala Rogen Sepahan Company and the chemical compounds used were from Merck, Germany.

### 2.2. Method

In this study, 32 male Sangsari lambs, aged four to five months and with an average weight of 16.5 kg, were used at the livestock breeding station of the Faculty of Veterinary Medicine, Semnan University. The lambs were randomly placed in individual pens and had free access to water and feed. After completing the 14-day habituation period, the lambs were randomly fed one of the experimental diets for 84 days according to Table 1 and observed the principle of animal welfare with the ethics code number 15652496. The feed was completely mixed and provided to the lambs at the level of appetite two times, at 7:00 and

17:00. The amount of metabolizable energy and crude protein in the diets was the same. At the end of the fattening period, the animals were slaughtered and the carcasses were dissected. In

addition to measuring the cross-sectional area of the loin and the thickness of the backfat, the weights of the various carcass parts and viscera were also recorded [15].

**Table 1.** Feed ingredients and chemical composition of experimental diets (percentage of dry matter).

Ingredients (by dry matter)	Rations			
	Control	Omega 3 (g)	Omega 6 (g)	Omega 9 (g)
Alfalfa	14.60	16.20	16.20	16.20
Corn silage	15.40	18.00	18.00	18.00
Barley	41.26	35.00	35.00	35.00
Corn	11.04	9.00	9.00	9.00
Rapeseed meal	3.06	3.80	3.80	3.80
Soybean meal	3.72	3.95	3.95	3.95
Wheat bran	7.64	7.50	7.50	7.50
Fatty acid calcium salts	0	2	2	2
Calcium carbonate	1.10	1.05	1.05	1.05
Vitamin-mineral supplement*	0.84	1.20	1.20	1.20
Sodium bicarbonate	0.84	0.90	0.90	0.90
Salt	0.51	0.71	0.71	0.71
Dicalcium phosphate	0	0.7	0.7	0.7
Metabolizable energy (Mcal/kg)	2.68	2.68	2.68	2.68
Dry matter (percent)	69.92	69.92	69.92	69.92
Crude protein	14.00	14.00	14.00	14.00
Cell wall (percent dry matter)	28.90	31.70	31.70	31.70
Neutral detergent fiber(NDF) (percent dry matter)	41.30	42.33	42.33	42.33
Calcium (percent dry matter)	0.75	0.75	0.75	0.75
Phosphorus (percent dry matter)	0.42	0.42	0.42	0.42

\* A kilogram of vitamin supplement contains 600,000 international units of vitamin A, 200,000 international units of vitamin D, 200 mg of vitamin E, 2,500 mg of antioxidants, 195 g of calcium, 80 g of phosphorus, 21,000 mg of magnesium, 2,200 mg of manganese, 3,000 mg of iron, 300 mg of copper, 300 mg of zinc, 100 mg of cobalt, 120 mg of iodine, and 1.1 mg of selenium.

## 2.2. Cooking Loss (CL)

to measure cooking loss, the coded samples were weighed and then placed on a greased rack in an oven at 163°C. A digital probe thermometer was used to record the core temperature of the samples during cooking; the probe was placed in the center of the sample and the cooking process continued until the core temperature reached 77°C. After removing the samples from the oven, they were exposed to cold air until they reached ambient temperature and then reweighed. The percentage of loss on cooking for each sample was calculated using formula (1) [15].

$$Cl = \frac{w1 - w2}{w1}$$

(CL: Loss due to cooking, W<sub>1</sub>: Sample weight before cooking, W<sub>2</sub>: Sample weight after cooking)

## 2.3. Colorimetry

After slaughtering animals, to measure meat color indices based on L\* (lightness), a\* (redness) and b\* (yellowness), the rectus muscle (located between ribs 6 and 10) is selected due to the accumulation of fatty acids and the desirable quality of muscle fibers, which makes it a suitable option for colorimetry was selected and described. The lightness index L\* varies from

black (0) to white (100), the redness index  $a^*$  from green (negative values) to red (positive values), and the yellowness index  $b^*$  from blue (negative values) to yellow (positive values). The color quality assessment was performed using a Hunterlab device and in triplicate for each sample, 24 hours after slaughter. Before colorimetry, the device was calibrated using a standard white plate, which was adjusted according to the guidelines of the International Group of Indicators for  $L^*$ ,  $a^*$ , and  $b^*$  indices [15].

#### 2.4. Determination of lipid oxidation rate (TBARS)

At the specified times, the fat of the meat samples was extracted and the resistance of the meat to oxidation was calculated by measuring the TBARS number according to the method of Nam and Ahan (2003). To determine thiobarbituric acid, 5 g of the sample was homogenized with 31 ml of an extraction solution containing 4% perchloric acid and 1 ml of butylated hydroxyanisole (1 g/l concentration) stored at 4°C for 1 min at 13,500 rpm. The resulting mixture was passed through Whatman filter paper and the volume of the filtered solution was adjusted to 50 ml by adding 4% perchloric acid. Then, 5 ml of this solution was mixed with 5 ml of 0.02 mol/l TBA solution, vortexed and placed in a water bath at 100°C for 60 min to form a malonaldehyde complex. The optical absorbance ( $A_s$ ) of the solution was read by a spectrophotometer at a wavelength of 530 nm against a distilled water control ( $A_b$ ) and was calculated based on the following equation. The TBARS number was reported as mg malonaldehyde per kg of a sample [16].

$$TBA = \frac{A_s - A_b \times 50}{500}$$

#### 2.5. Texture Profile Analysis (TPA)

In order to examine the texture profile of meat samples, first, straight muscle meat samples were cut into dimensions (1.9 × 1.8 × 0.8 cm) and

placed in the center of the TPA plate for a two-cycle compression test. The texture profile analysis test of the meat sample was performed using a texture analyzer with a 500 N load cell at ambient temperature, under two compression conditions with a pre-test and post-test speed of 5 mm/s and an exit speed of 1 mm/s, a percentage change of 50%, and an initial loading force of 10 g. In this test, the sample was subjected to 50% axial tension to prevent the meat samples from being damaged during the test [16].

#### 2.6. Water Holding Capacity Measurement (WHC)

The water-holding capacity of the samples was evaluated according to the method of Heimonides et al. (1999). For this purpose, first, 5 g of the sample was carefully weighed. Then, the sample was wrapped in two filter papers and placed in a falcon. Falcon was centrifuged in a refrigerated centrifuge at 3600 g and 8°C for 30 minutes. After centrifugation, the sample was removed from the filter paper and the weight of the paper and sample was measured separately. The water holding capacity of the sample was also calculated using the following formula [17].

$$WHC \text{ g/kg} = [(1 - M_w / M_s) 1000]$$

$M_s$  = Initial weight of sample in grams

$M_w$  = Weight of water removed from sample in grams after centrifugation

#### 2.7. Statistical analysis

All experiments were performed in at least three replicates using a completely randomized factorial design. Data were analyzed using one-way analysis of variance (ANOVA) and Tukey's mean comparison test with a 95% confidence level for significant variables. All data were analyzed with SPSS version 29 (SPSS Inc., Chicago, IL, USA) and the results were presented as mean ± standard deviation. Graphs were drawn with Excel 2016.

### 3. Results and discussion

#### 3.1. Color evaluation

Color characteristics play an important role in determining the overall appearance of foods and affecting consumer acceptance. According to Table 2, it was observed that the highest and lowest transparency levels were related to the control and omega-3 groups, respectively, and this difference between these two groups was significant ( $P<0.05$ ). It should also be noted that the transparency level in the omega-6 and omega-9 groups did not differ significantly from the control group, but these two groups had significantly higher transparency than the omega-3 group ( $P<0.05$ ). Also, no significant difference was observed in the transparency level during the storage days 0, 3, and 7 ( $P<0.05$ ). As shown in Table 2, the highest and lowest  $a^*$  index corresponded to the seventh day of the control group and the seventh day of the omega-3 group, respectively, and this difference between these two groups was significant ( $P<0.05$ ). It should also be noted that the  $a^*$  index level in the omega-6 and omega-3 groups.

Omega 9 did not differ significantly from the control group on different days, but these two groups had significantly higher redness than the omega-3 group ( $P<0.05$ ).

According to Table 2, it was observed that the highest and lowest  $b^*$  index was related to the seventh day of omega 3 and the day of the start of the experiment in the control group, respectively, and this difference between these two groups was significant ( $P<0.05$ ). It should also be noted that the level of  $b^*$  index in the omega 6 and omega 9 groups did not differ significantly from the control group on different days, but these two groups had significantly higher yellowness than

the omega 3 group ( $P<0.05$ ). On the day of the start of the experiment, the yellowness of the meat in the omega 3 group was significantly higher than the control group and the two omega 6 and omega 9 groups ( $P<0.05$ ). It should be noted that with the increase in the amount of unsaturated fatty acids, the yellowness of the meat decreased.

In the present study, it was found that the control group had the highest level of transparency and red color on all days, while the omega-3 group consistently showed the lowest transparency and the highest yellowness. Also, increasing the number of fatty acids affected the transparency and color of meat. Color is a visual characteristic of meat and carcasses that is known as the first influential criterion in the selection and evaluation of meat and carcasses [18]. In general, there are three main sources of color differences in meat, which are the morphology of muscle myoglobin, the ratio of color pigments in the muscle, and the metabolism of glucose in the muscle after death. It seems that these factors did not affect the tendency to redness and yellowness of meat. Brightness is an index that determines the reflection of light and can also be affected by the composition of fatty acids in the carcass [19]. Differences in fatty acid composition can affect color because unsaturated fatty acids reflect less light than saturated fatty acids. On the other hand, the oxidation of fatty acids can also cause color changes in addition to reducing meat stability and reducing meat color quality by reducing its redness. The meat brightness ( $L^*$ ) seems to be affected by higher fat deposition in muscle tissue [20]. The increased yellowness could be a result of higher yellow pigment content, which was confirmed by higher fat content [21].

**Table 2.** Results of evaluation of optical parameters of meat during the study.

Day	Indicators	Sample			
		Control	Omega 3	Omega 6	Omega 9

0	b*	13.96 ± 0.61 <sup>d</sup>	15.26 ± 0.57 <sup>bcd</sup>	14.26 ± 0.40 <sup>d</sup>	14.20 ± 0.40 <sup>d</sup>
	a*	13.43 ± 0.45 <sup>ab</sup>	13.73 ± 0.42 <sup>bcd</sup>	14.46 ± 0.47 <sup>bc</sup>	14.62 ± 0.25 <sup>ab</sup>
	L*	32.40 ± 0.47 <sup>a</sup>	30.26 ± 0.45 <sup>bc</sup>	31.61 ± 0.51 <sup>ab</sup>	31.64 ± 0.70 <sup>ab</sup>
3	b*	14.73 ± 0.40 <sup>bcd</sup>	16.03 ± 0.21 <sup>ab</sup>	15.20 ± 0.46 <sup>cd</sup>	14.40 ± 0.44 <sup>bcd</sup>
	a*	15.20 ± 0.36 <sup>a</sup>	12.56 ± 0.50 <sup>cde</sup>	14.23 ± 0.45 <sup>ab</sup>	14.33 ± 0.45 <sup>ab</sup>
	L*	31.63 ± 0.50 <sup>ab</sup>	29.70 ± 0.56 <sup>cd</sup>	30.83 ± 0.50 <sup>bc</sup>	31.53 ± 0.31 <sup>ab</sup>
7	b*	15.66 ± 0.55 <sup>abc</sup>	16.83 ± 0.40 <sup>a</sup>	15.73 ± 0.57 <sup>abc</sup>	15.26 ± 0.50 <sup>bcd</sup>
	a*	15.43 ± 0.57 <sup>a</sup>	11.37 ± 0.45 <sup>e</sup>	12.43 ± 0.26 <sup>de</sup>	15.43 ± 0.32 <sup>e</sup>
	L*	31.40 ± 0.36 <sup>ab</sup>	28.66 ± 0.78 <sup>d</sup>	30.46 ± 0.60 <sup>bc</sup>	30.90 ± 0.26 <sup>abc</sup>

a-e: Different letters in each column and row indicate statistical differences between doses on different days ( $p < 0.05$ ).

### 3.2. Oxidative resistance

The results of the study showed that on the day of the start of the experiment, no significant difference was observed between the treated and control groups. However, with the increase in storage time from the day of the start of the experiment to three days, the concentration of malondialdehyde in the treated groups increased significantly compared to the control group. Among them, only the group treated with omega-3 showed a statistically significant increase compared to the control group, while the changes observed in the groups treated with omega-6 and omega-9 were not statistically significant compared to the control group. According to Ozpinar et al. 2003, an increase in the concentration of malondialdehyde indicates a decrease in the oxidative stability of meat [22].

According to the data presented in Table 3, on the seventh day of storage, the lowest and highest concentrations of malondialdehyde were observed in the control group and the group treated with omega-3, respectively. Furthermore, on the same day, the omega-3 and omega-6 groups showed a significant increase in

malondialdehyde concentration compared to the control group, while the omega-9 group did not show a statistically significant change compared to the control group. The significant changes in malondialdehyde concentration between the treated groups can be attributed to the difference in the content of unsaturated fatty acids, since polyunsaturated fatty acids (PUFA) are more susceptible to oxidation than saturated fatty acids [23]. In general, malondialdehyde concentration increased significantly in all groups over time, which can be attributed to the increase in lipid oxidation. Among all samples, the highest malondialdehyde concentration on day 7 was in the omega-3-treated sample. However, this value was still below the established standard limit [24]. Similarly, Ozpinar et al. (2003) reported an increase in malondialdehyde concentration after fish oil supplementation in broiler diets [22]. In contrast, Bialek et al. (2021) reported that omega-3 fatty acid supplementation in lamb diets decreased malondialdehyde concentration, which they attributed to improved oxidative stress markers [25].

**Table 3.** Results of assessment of malondialdehyde concentration during the study.

	Day 0	Day 3	Day 7
Control	0.403 ± 0.040 <sup>f</sup>	0.700 ± 0.015 <sup>e</sup>	0.890 ± 0.020 <sup>c</sup>
Omega 3	0.403 ± 0.015 <sup>f</sup>	0.810 ± 0.026 <sup>cd</sup>	1.210 ± 0.030 <sup>a</sup>
Omega 6	0.406 ± 0.020 <sup>f</sup>	0.780 ± 0.035 <sup>de</sup>	1.040 ± 0.031 <sup>b</sup>
Omega 9	0.380 ± 0.040 <sup>f</sup>	0.740 ± 0.040 <sup>de</sup>	0.890 ± 0.046 <sup>c</sup>

a-e: Different letters in each column and row indicate statistical differences between doses on different days ( $p < 0.05$ ).



### 3.3. Meat texture evaluation

Among the textural parameters, hardness is used to describe the physical characteristics of meat. According to Figure 1, the highest hardness of meat was observed in the control group on the day of the experiment, while the lowest hardness was observed in the omega-3 group on the seventh day. This pattern remained constant on all measurement days (0, 3, and 7 days), which indicates the stability of the effect of supplements in reducing meat hardness. The results showed that all treatment groups showed a significant decrease in meat hardness compared to the control sample. This decrease can be attributed to the reaction of compounds resulting from lipid oxidation with proteins and changes in cross-links, which subsequently reduces the performance of proteins due to denaturation. Several studies have also reported a positive relationship between hardness and textural sensory properties of meat and meat products [26-28].

On the other hand, in all groups, a significant decrease in meat hardness was observed with increasing storage time. This decrease is likely related to the increase in oxidation during storage, which can affect the protein structure and textural properties of meat [20].

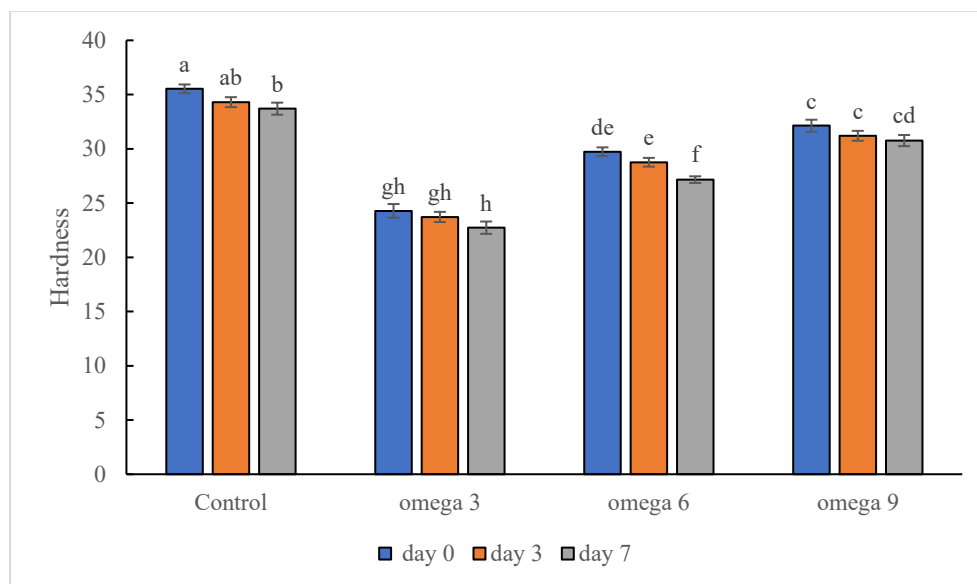
On the other hand, among the treatment groups, the lowest hardness was found in the omega-3 group, followed by the omega-6 and omega-9 groups, respectively. The decrease in hardness in the omega-3 group can be attributed to the increase in fat fluidity. According to Enser and Wood (2017), the addition of omega-3 fatty acids, especially ALA, EPA and DHA, leads to an increase in the content of polyunsaturated fatty

acids in meat, which can lead to a decrease in meat hardness by increasing fat fluidity. On the other hand, the addition of excessive omega-3 fatty acids leads to fat oxidation, which negatively affects flavor and color and can counteract the benefits of improving tenderness [20].

The increased stiffness of the omega-6 treatment groups can be attributed to the presence and consumption of high levels of omega-6 in the diet of animals without sufficient omega-6. According to Alagawani 2021, a balanced intake of omega-6 alongside omega-3 is very important [29]. Similarly, Herdman et al. 2010 reported that omega-6 fatty acids, although essential, can lead to increased stiffness if consumed in excess, as they may promote the deposition of saturated fat in the muscle [30]. On the other hand, the omega-9 fatty acid group had higher stiffness than the omega-6 and omega-3 groups. This difference can be attributed to the structural characteristics of omega-3 and omega-6 fatty acids, which are unsaturated and contain multiple double bonds [31]. This is because the presence of these double bonds creates complexity in the molecular structure of these acids, resulting in more fluid fats that can help increase the sensitivity of muscle tissue. In contrast, the double bond in omega-9 creates a more stable structure that helps make tissues stronger [32].

Studies have shown that the intake of omega-3, omega-6, and omega-9 fatty acids in the diet has a significant impact on the toughness and overall quality of meat. The balance between these fatty acids affects not only the nutritional profile of meat but also its physical properties, including tenderness and firmness [30, 33].





**Fig 1.** Results of meat hardness assessment during the study.

### 3.4. Water holding capacity

On the day of the experiment, the highest WHC was observed in the omega-6 group, while the lowest was in the control group. However, these differences were not statistically significant (Table 4). On the third and seventh days of storage, the highest and lowest WHC were observed in the omega-3 and omega-9 groups, respectively. Among all samples, the omega-3 group showed the highest WHC on the seventh day. The increase in WHC in the treatment groups may be related to the moderate oxidation of myosin, since the oxidation process causes polymerization, which can lead to an improvement in WHC [34]. The mechanism of water-holding capacity is mainly dependent on the function of myofibrillar proteins and structures associated with binding and trapping water, such as myofibrils. Studies have shown

that factors such as pH, ionic strength, and oxidation have a direct impact on the ability of myofibrillar proteins and muscle cells to retain and trap water [35]. The results also showed that WHC increased with increasing storage time in all groups. The increase in WHC can be attributed to increased pH, protein degradation, and intramolecular rearrangement [36]. Similarly, Chang et al. (2015) reported that meat samples stored for 0 and 14 days had higher WHC than samples stored for 7 days. These researchers stated that with the increase in pH during the maturation process, protein degradation and changes in electrical charges cause intramolecular rearrangement, resulting in an increasing in WHC. These results emphasize that biochemical changes and intracellular or intercellular lipids that are more unsaturated increase the storage capacity during storage, playing a key role in maintaining meat quality [37, 38].

**Table 4.** Results of water holding capacity assessment during the study.

	Day 0	Day 3	Day 7
Control	0.133± 0.015 <sup>f</sup>	0.180± 0.020 <sup>bcd<sup>ef</sup></sup>	0.197± 0.021 <sup>bcd</sup>
Omega 3	0.143± 0.0150 <sup>ef</sup>	0.210± 0.020 <sup>bc</sup>	0.273± 0.021 <sup>a</sup>
Omega 6	0.157± 0.012 <sup>def</sup>	0.197± 0.021 <sup>bcd</sup>	0.230± 0.020 <sup>ab</sup>

Omega 9	0.137± 0.012 <sup>f</sup>	0.170± 0.010 <sup>cdef</sup>	0.193± 0.015 <sup>bcd</sup>
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a-e: Different letters in each column and row indicate statistical differences between doses on different days ( $P < 0.05$ ).

### 3.5. Cooking loss

As shown in Table 5, on the day of the experiment, the highest and lowest loss due to cooking meat was related to the control and omega-6 groups, respectively. None of the treatment groups had a significant difference compared to each other and the control group ( $P > 0.05$ ). The decrease in the loss due to cooking the omega-6 sample on the day of the experiment can be attributed to the presence of omega-6 fatty acids, which can prevent the loss due to cooking to some extent. On the third day, the highest and lowest loss due to cooking was related to the control and omega-3 groups, respectively ( $P < 0.05$ ); it should also be noted that the two omega-3 and omega-6 groups did not have a significant difference in loss due to cooking compared to each other. on the seventh day, the highest and lowest loss due to cooking was related to the control and omega-3 groups, respectively, which had a significant difference ( $P < 0.05$ ).

The results showed that all treatment groups had a significant decrease compared to the control group in cooking loss ( $P < 0.05$ ); also, in different types of fatty acid treatments, an increase in cooking loss was created and all treatment groups had a significant difference compared to each other ( $P < 0.05$ ).

In the control group, no significant difference was observed over time on days 0, 3, and 7 ( $P > 0.05$ ). In the omega-3 group, a significant decrease in cooking loss of meat was created over time, in such a way that the highest cooking loss was for day 0 and the lowest was for day 7 ( $P < 0.05$ ). In

this group, a decreasing trend was also observed with increasing time, in such a way that cooking loss was significantly different from each other on all days ( $P < 0.05$ ). In the omega-6 group, a significant decrease in cooking loss of meat was created over time, in such a way that the highest cooking loss was for day 0 and the lowest was for day 7 ( $P < 0.05$ ). However, in this group, only days 3 and 7 were significantly different from day 0, but they did not show significant differences from each other ( $P > 0.05$ ). The reduction in cooking loss in samples treated with omega-3 fatty acids during the third and seventh days is related to the production of leachate and changes in myofibril volume. In the cooking process, the melting of fats can play a key role in maintaining or reducing moisture, depending on their chemical structure. Omega-3 fatty acids, due to their lower melting point than saturated fats, remain liquid at cooking temperatures. Also, the polyunsaturated and long-chain nature of these acids (PUFA) along with multiple double bonds, create greater fluidity and, by integrating into the cell membrane, form a protective barrier around muscle fibers that prevents cooking loss [20].

In contrast, the difference in cooking loss between treatment groups can be attributed to the role of omega-9 fatty acids, such as oleic acid. These monounsaturated fatty acids, by creating a firmer fat tissue within the muscle, lead to a decrease in the ability to retain moisture during the cooking process. The findings of this study indicate that the presence of omega-3 fatty acids, due to their physical and chemical properties, has a positive effect on reducing cooking loss and maintaining meat quality [39].

**Table 5.** Results of the evaluation of cooking loss during the study period.

	Day 0	Day 3	Day 7
Control	30.86± 0.68 <sup>a</sup>	30.86± 0.55 <sup>a</sup>	30.86± 0.30 <sup>a</sup>

Omega 3	30.07± 0.75 <sup>ab</sup>	25.67± 0.40 <sup>c</sup>	20.83± 0.25 <sup>d</sup>
Omega 6	29.80± 0.81 <sup>ab</sup>	26.23± 0.51 <sup>c</sup>	25.00± 0.62 <sup>c</sup>
Omega 9	30.67± 0.77 <sup>ab</sup>	28.73± 0.40 <sup>b</sup>	28.73± 0.55 <sup>b</sup>

a-e: Different letters in each column and row indicate statistical differences between doses on different days ( $p < 0.05$ ).

## 4. Conclusion

The results of this study indicate that the addition of calcium salts and unsaturated fatty acids to the diet of male Sangsari lambs can lead to improved meat quality. These effects include improved meat color indices, and reduced hardness and shear strength of meat, all of which indicate higher meat quality. Also, the increase in malondialdehyde concentration in the omega-3 group requires attention, as it may indicate oxidative processes that can affect the final meat quality. Overall, the findings of this study confirm the importance of using these supplements to improve meat quality and can be a guide for improving nutritional systems in the livestock industry.

## 5-References

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## اثر افزودن نمک‌های کلسیمی اسیدهای چرب غیراشباع در جیره بر کیفیت گوشت بره‌های نر سنگسری

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## چکیده

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## کلمات کلیدی:

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یکی از راهکارهای مؤثر برای بهبود راندمان تولید در دام‌های نر پرواری، استفاده از مواد افزودنی تغذیه‌ای است چراکه انرژی جیره غذایی به‌عنوان یکی از عوامل اصلی محدودکننده در تغذیه دام‌ها شناخته شده و نقشی اساسی در هضم و بهره‌وری سایر مواد مغذی ایفا می‌کند. این مطالعه با هدف بررسی تأثیر افزودن نمک‌های کلسیمی اسیدهای چرب غیر اشباع (امگا ۳، ۶ و ۹) بر ویژگی‌های کیفی گوشت بره‌های نر سنگسری انجام شد. در این پژوهش، ۲۸ رأس بره به چهار گروه تغذیه‌ای با سطوح مختلف اسیدهای چرب تقسیم شدند و به مدت ۷۵ روز جیره‌های مربوطه دریافت کردند. کیفیت گوشت با استفاده از شاخص‌هایی نظیر رنگ ( $L^*$ ,  $a^*$ ,  $b^*$ ), اکسیداسیون لیپیدها (MDA)، سختی، افت پخت، و ظرفیت نگهداری آب ارزیابی شد. نتایج نشان داد افزودن امگا ۳ منجر به کاهش شفافیت و افزایش زردی گوشت شد، در حالی که سختی بافت کاهش و نرمی آن بهبود یافت ( $P < 0.05$ ). هرچند غلظت MDA افزایش یافت، این مقادیر همچنان در محدوده قابل قبول باقی ماند. همچنین، مکمل‌های اسید چرب غیر اشباع افت پخت را کاهش و ظرفیت نگهداری آب را بهبود بخشیدند که نشان‌دهنده پایداری بالاتر گوشت در طول نگهداری است. به طور کلی، افزودن این مکمل‌ها به جیره غذایی بره‌ها می‌تواند به عنوان راهکاری مؤثر برای بهبود کیفیت و ماندگاری گوشت مورد استفاده قرار گیرد. با این حال، اثرات آن وابسته به نوع و مقدار اسید چرب مصرفی است.