



## Onion (*Allium cepa L.*) Extract Supplementation Improved Meat Quality, Oxidative Stability and Some Hematological Parameters in Suckling Lambs

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**Key words:** Hematological parameters, meat quality, onion extract, suckling lambs

**Abstract:** The antioxidant properties of common onion (*Allium cepa L.*) have already been investigated and documented. This study was conducted to evaluate the effects of two doses of onion extract on meat oxidative stability, meat quality and some hematological parameters in suckling lambs. Eighteen suckling lambs (30±3 days old Turkey-Ghashghai) were selected, weighed and randomly allocated into 3 groups as control (regular diet+sheep milk), treatment 1 and treatment 2 (fed same as control plus 150 or 250 mg kg<sup>-1</sup> onion extract, respectively) and treated for 60 days. Blood was collected on days 30 and 60 of the experiment. The liver, spleen and thigh muscles were collected after slaughtering the animals. The effects of storage time on meat quality were determined after 3 or 6 months of storage at -20°C. The pH, moisture, Water-Holding Capacity (WHC), crude fat and Malondialdehyde (MDA) of the meat were evaluated. Administration of onion extract significantly decreased the amounts of crude fat and MDA concentration in both treatment groups (p<0.05). Lymphocyte's numbers increased significantly in the treatment 2 group compared with other groups (p<0.05). A significant increase in Lightness (L) of meat in the treatment 2 group compared to the control group was observed (p<0.05). No significant difference noticed between the groups in the remained evaluated parameters of meat, neutrophil counts, the neutrophil/lymphocyte ratio, monocyte's numbers and liver and spleen weight. Conclusion, onion extract supplementation improved meat quality, oxidative stability and lymphocyte numbers in suckling lambs.

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## INTRODUCTION

One of the main aims of animal nutrition is to produce high-quality meat by manipulating rations. Previous studies have shown that supplementing sheep rations with conventional antioxidants such as vitamin E, has a positive effect on meat quality properties<sup>[1]</sup>. There are also many reports insisting on the beneficial effects of herbal antioxidants in sheep rations<sup>[2]</sup>. Therefore, there is a growing interest to use complementary nutrient plants for their considerable role as functional and biochemical inhibitors of oxidative damage which are induced by free radicals. Many plants have good sources of phytochemicals, notably phenolic and flavonoids<sup>[3]</sup>. However, there might be wide differences in composition, concentrations and antioxidant properties in bioactive compounds of different fruits and vegetables<sup>[4]</sup>. The addition of an antioxidant to the broiler diet has been shown to improve the shelf life of poultry meat by decreasing the rate of lipid oxidation<sup>[5]</sup>. Antioxidant properties of plants are attributed to the presence of compounds such as ascorbic acid,  $\alpha$ -tocopherol,  $\beta$ -carotene, various flavonoids and other phenolic compounds<sup>[6]</sup>. Antioxidants are usually present at low concentrations and significantly delay or prevent oxidation. Antioxidants play an important role in the body defense system against Reactive Oxygen Species (ROS) or free radicals which are the harmful byproducts generated during an aerobic activity of normal cells. Lipid oxidation is a major factor influencing meat quality. Oxidation occurs ubiquitously in the muscles and is responsible for the development of off-flavor and discoloration in a variety of meats. Additionally, oxidative reactions can decrease the nutritional quality of meat; certain oxidation products can be harmful to health<sup>[7]</sup>. Lipid oxidation in meat products can be controlled by using antioxidants in the diet such as vitamin E and rosemary extract<sup>[8]</sup>. In order to postpone the development of rancidity in the meat product different synthetic antioxidants such as Propyl Gallate (PG) and Butylated Hydroxy Anisole (BHA) are commonly used<sup>[9]</sup>. However, consumers are concerned about the safety of synthetic food additives and prefer to use fresh and wholesome meat without any synthetic additive antioxidants. For instance; many phenolic compounds from the edible plant have manifested various degrees of antioxidant effects by inhibiting hydroperoxide formation<sup>[10]</sup>. Also, catechins and extracts from sesame seeds, rosemary, green tea, coffee and grapes have been shown to have strong antioxidant effects in meat<sup>[11]</sup>. Many studies have been conducted to examine the use of herbs to improve the immune response. Liliaceous family plants such as onion (*Allium cepa* L.) and garlic (*Allium sativum*) are the sulfur compounds that have immunomodulatory properties<sup>[12]</sup>. It

was reported that allicin (a main bioactive ingredient of the onion) has various beneficial activities such as immune-stimulatory, antibacterial, anti-parasitic and anti-tumor activity<sup>[13]</sup>.

Onion antiquity has been widely used as a foodstuff and has acquired a reputation in the folklore of many cultures as a therapeutic agent. The consumption of onion has been increased due to its flavor and health benefits. These beneficial properties seem to be strongly related to the high content of sulfur compounds and flavonoids and their activity as antioxidants, anti-carcinogens, their effects on lipid metabolism, the cardiovascular system and antibiotics effects<sup>[14,15]</sup>, additionally, the highest level of quercetin (flavonol compound) among the fruits and vegetables was reported to be in onion<sup>[16]</sup>.

Therefore, the objective of this study was to investigate the effect of different levels of onion extract supplementation in the ration of suckling lambs on the oxidative stability of meat, some hematological parameters and the Water-Holding Capacity (WHC) which is the ability of meat to hold all or part of its water and is one of the most important traits of meat quality.

## MATERIALS AND METHODS

Eighteen suckling lambs (Turkey-Ghashghai 30 $\pm$ 3 days old and 10.3 $\pm$ 0.3 kg body weights) were selected and purchased from local private farms (Fars province, Iran) and after clinical examination by an expert veterinarian allocated to three treatment groups, each with 6 replicates in a completely randomized design. The trial period was 60 days. The treatments consisted of a control group (regular diet+sheep milk) and two treatment groups fed with control ration + 150 mg kg<sup>-1</sup> (treatment 1) or 250 mg kg<sup>-1</sup> onion extract (treatment 2). All investigations were conducted in accordance with the "Guiding Principles for the Care and Use of Research Animals" approved by Shiraz University.

The regular ration was adjusted according to NRC requirements<sup>[17]</sup> by the UFFDA software (Table 1). In order to adapt lambs, the regular ration was provided to the lambs for a week prior to the starting of the experiment. The animals reared during May and June in ambient temperature (25-32°C) with natural light and had free access to tap water. The animals were placed in individual pens and fed twice a day (morning and afternoon) without limitation in feed and water. The amount of onion extract for each lamb was daily prepared and administered through oral gavage.

On day 30 and 60 of the experiment, blood samples were collected from the jugular vein in EDTA containing tubes and were used for evaluation of the hematological parameters. At the end of the experimental period, all the lambs were weighed individually before slaughtering. The

Table 1: Components and nutrient content of the regular ration used for suckling lambs

Ration components	Values
Corn grain (%)	27.00
Barley grain (%)	40.00
Wheat bran (%)	16.40
Soybean Meal (%)	15.00
Mineral supplement-Vitamin (%)	0.40
Shellfish powder (%)	0.50
Di-calcium phosphate (%)	0.30
Salt (%)	0.30
Nutrient Content	
Metabolism energy (mega calories/kg)	2.95
Crude protein (%)	17.11
Calcium (%)	0.58
Phosphorus (%)	0.34

lams were slaughtered consciously without anesthesia (halal method), liver and spleen were immediately removed and weighed. The data were expressed as relative of carcass weight. Thigh muscles were immediately removed, placed inside the zipper plastic bags and kept at  $-20^{\circ}\text{C}$  for 3 and 6 months. The pH, moisture, Water Holding Capacity (WHC), crude fat and Malondialdehyde (MDA) of meats were evaluated after the mentioned time of storage. The color of fresh thigh muscles including Lvalue that is the lightness component, redness and yellowness were measured using a Hunter Lab system with a colorimeter (Konicaminolta, CR-400, Japan).

The pH of meat samples was measured in triplicate using a digital pH meter (CG824, SCHOTT, Germany). Approximately 10 g of the thigh muscle was cut into small pieces and 90 mL of distilled water was added. Homogenized using a homogenizer (Yello line, DI18B, Germany) and the pH was recorded. The moisture and crude fat contents were determined according to the AOAC methods<sup>[18]</sup>.

The Water-Holding Capacity (WHC) measured in triplicate using the Laakkonen method with slight modifications. Approximately 10 g of ground meat samples were cooked at  $70^{\circ}\text{C}$  for 30 min in a water bath. Then, it was cooled down in the room temperature for 30 min and centrifuged at  $1,000\times g$  for 10 min. WHC was measured by calculating the percentage of the total moisture and water loss.

Malondialdehyde (MDA) is considered as the main marker of lipid peroxidation. Thiobarbituric Acid (TBA) is reacted with MDA which is resulting in a color compound. TBA was evaluated according to the methods suggested by Imik *et al.*<sup>[18]</sup>.

**Statistical analysis:** All data were statistically analyzed using analysis of variance and significant differences of obtained means were determined using Duncan's multiple ranges, Post-hoc tests at the level of  $p < 0.05$ .

## RESULTS

Table 2 shows the effects of feeding different levels of the onion extract on the meat quality in lamb. Moisture WHC, pH and drip loss contents did not show significant differences among the samples ( $p > 0.05$ ).

Dietary supplementation with  $250 \text{ mg kg}^{-1}$  onion extract (treatment 2) resulted in significantly lower crude fat content in the muscles compared with the muscles from lambs fed with regular diets ( $p < 0.05$ ). There were no significant differences between treatment 1 and the control group for crude fat.

Muscles of treatment 2 group had a lower MDA value compared with the other two groups ( $p < 0.05$ ). There were no significant differences between treatment 1 and the control group for the MDA level. The relative weight of the liver and spleen were not affected by the supplementation of onion extract (Table 3).

The results of instrumental color measurements on the lamb meat are presented in Table 2. The results showed a significant increase in Lightness (L) of meat in treatment 2 group compared to the control group ( $p < 0.05$ ). There was no significant difference between redness (a) and yellowness (b) of meat samples.

Feeding of the lambs with onion did not affect lymphoid organs weights ( $p > 0.05$ ). Treatment 2 significantly had higher lymphocyte than other groups ( $p < 0.05$ ). Although, the administration of  $250 \text{ mg kg}^{-1}$  onion extract (treatment 2) decreased neutrophil/lymphocyte ratio, it was not statistically significant in comparison with other groups (Table 3).

## DISCUSSION

Our results showed that additions of onion extract to the ration will not alter WHC, drip loss and moisture in the meat. Similar results were already reported following the supplying of broiler diet with garlic powder<sup>[19]</sup> or  $\alpha$ -tocopherol<sup>[20]</sup>. The pH of meat also did not change by feeding the lamb with onion extract supplement. Pre-slaughter stress of animal and post-slaughter management of meat are known to be of important factors affecting the pH of meat. The pre-slaughter glycogen reservation might be reflected in the pH of meat<sup>[21]</sup>. The addition of onion and garlic to ground beef also did not affect the pH<sup>[22]</sup>.

The use of onion extract significantly decreased the amounts of crude fat in lamb's meat. Due to the effects of onion in reducing lipogenesis and increasing fat catabolism, energy requirements for lambs are provided by burning more fat which could be the reason for the reduction in meat fat<sup>[23]</sup>. Decreased of crude fat content in thigh muscles of broiler chickens was reported following garlic supplementation<sup>[19]</sup>. Metwally reported that crude fat content decreased significantly with the same sources of *allium sativum* in fish<sup>[24]</sup>. It has been reported that some medicinal plants decreased the crude fat content in broiler

Table 2: The effect of dietary onion extract supplementation on the quality of thigh muscles meat in suckling lambs (n = 6 each group)

Variables	Control	Treatment 1	Treatment 2	SEM	p-values
<b>On slaughter day</b>					
Drip loss (%)	2.08	1.93	2.21	0.34	0.8
pH	6.28	6.3	6.23	0.09	0.8
<b>Meat color score</b>					
L (lightness)	26.83 <sup>b</sup>	27.58 <sup>ab</sup>	29.25 <sup>a</sup>	0.6	0.05
a (redness)	8.56	8.63	8.72	0.35	0.9
b (yellowness)	2.7	3.05	3.17	0.3	0.6
<b>After 3 months of freezing</b>					
Crude fat (%)	12.5 <sup>a</sup>	12.1 <sup>a</sup>	9.75 <sup>b</sup>	0.58	0.03
Moisture (%)	76.15	76.73	77.39	0.65	0.42
WHC* (%)	60.67	60.73	62.01	0.55	0.2
pH	5.31	5.34	5.35	0.28	0.8
MDA(μg/g)	1.44 <sup>a</sup>	1.43 <sup>a</sup>	1.19 <sup>b</sup>	0.09	0.01
<b>After 6 months of freezing</b>					
Moisture (%)	72.5	73.57	74.53	0.58	0.13
WHC (%)	50.77	51.47	52.07	0.82	0.5
pH	5.73	5.74	5.72	0.05	0.9
MDA (μg g <sup>-1</sup> )	5.2 <sup>a</sup>	5.13 <sup>a</sup>	4.58 <sup>b</sup>	0.09	0.001

Means with different superscript small letters (a, b) in a row are significantly different (p< 0.05). \*Water holding capacity

Table 3: The effect of dietary onion extract supplementation on the liver and spleen weights and hematological parameters of suckling lambs (n = 6 each group)

Variables	Control	Treatment 1	Treatment 2	SEM	p-values
Liver weight (%)	2.63	2.75	2.84	0.1	0.3
Spleen weight (%)	0.25	0.25	0.26	0.02	0.8
Day 30th of breeding					
Lymphocyte (%)	75.75	76.25	77.75	1.3	0.5
Neutrophils (%)	12.25	11.75	11.25	1.01	0.8
N/L	0.16	0.15	0.14	0.02	0.8
Monocyte (%)	12	12	11	0.61	0.4
Day 60th of breeding					
Lymphocytes (%)	76.75 <sup>b</sup>	77.5 <sup>b</sup>	81 <sup>a</sup>	1.06	0.04
Neutrophils (%)	11	10.5	9.7	0.75	0.3
N/L	0.14	0.13	0.12	0.02	0.3
Monocyte (%)	12.2	11.5	9.8	0.9	0.12

Means with different superscript small letters (a, b,) in a row are significantly different (p< 0.05)

chickens<sup>[25]</sup>. Feeding of lambs with onion extract significantly decreased the amounts of MDA in the meat.

A decrease in TBARS value of both non-irradiated and irradiated beef after 7 days of storage was reported following the direct addition of onion to beef patty<sup>[26]</sup>. Applying onion extracts in the turkey breast rolls significantly decreased the lipid oxidation after the refrigerated storage<sup>[27]</sup>. According to the results demonstrated by Serdaroglu and Felekoglu, the use of onion juice (1 mL 100 g) to the frozen-sardine mince (Sardinapilchardus) delayed lipid oxidation for 3 months<sup>[28]</sup>. In the study of Olusola et al TBARS decreased in the meat of broiler chickens fed with onion skin meal and extract. Onion has high quercetin content which is responsible for its antioxidant properties<sup>[29]</sup>. The decrease in the TBARS values of meat in this study is in agreement with a report by Sohaib *et al.*<sup>[30]</sup> who reported a decrease of meat lipid oxidation by dietary quercetin. This finding is also in agreement with Schwarz *et al.*<sup>[31]</sup> study who reported that aromatic and medicinal herbs were rich sources for natural radical scavenging compounds like industrially-used antioxidants which inhibit the oxidative

chain reaction by inactivating free radicals formed during the peroxidation of lipids. It has been reported that the major flavonoid of onion is quercetin which exerts the strongest antioxidant potential, especially for delaying the meat oxidation<sup>[32]</sup>.

Also, it has been confirmed that the dietary supplementation of quercetin could decrease the TBARS values of beef loin<sup>[33]</sup> and pork patties<sup>[34]</sup>. Absorbition and accumulation of dietary quercetin in the meat is followed adose-dependent manner. Besides, accumulation of the phenolic compounds in the meat act as anti-oxidation<sup>[35]</sup>. The increase in TBARS values in meat following the increase in storage time is normal<sup>[36]</sup>. Therefore, from the above findings, it could be concluded that the dietary supplementation of onion extract was effective in protecting the meat from oxidation.

Our result showed a significant increase in Lightness (L) and lymphocytes count in the treatment 2 compare to other groups (p<0.05). The degree of meat oxidation will affect the color of meat and the development of red color is associated with the generation of carboxymyoglobin and reduced myoglobin<sup>[37]</sup>. We didn't see a significant

difference between any of the measured instrumental color parameters including redness and yellowness values but meat lightness values in the treatment 2 showed a significant increase ( $p < 0.05$ ). Similar results were reported following the addition of garlic and onion to ground beef but the L value of ground beef was gradually increased<sup>[26]</sup>.

Feeding of onion extract was found not to affect the weight of lymphoid organs in this study. But there was a significant increase in the percentage of lymphocytes in treatment 2. Similar results have reported on the liver and spleen weights by other studies<sup>[29, 35]</sup>. That allium species enhancing immune response activities through the promotion of lymphocyte synthesis, cytokine release, phagocytosis and natural killer cell activity<sup>[38]</sup>. Previous studies have found that garlic and its contents could activate some immune functions such as the proliferation of lymphocytes, cytokines release, natural killer cell activity and phagocytosis<sup>[13, 39]</sup>. Furthermore, it has been proposed that allicin supplementation exerted a positive effect on improving the immunity of early age animals<sup>[39]</sup>. Onions have high levels of selenium. Therefore, part of the positive effect of onion extract in increasing the percentage of lymphocytes can be due to the presence of selenium<sup>[40]</sup>.

## CONCLUSION

The findings of this study demonstrate that lamb's meat fed with 250 mg kg<sup>-1</sup> onion extract had lower crude fat and MDA contents compared with lambs fed with a regular diet. Also, the use of onion extract led to an increase in lymphocytes and a decrease in the neutrophil/lymphocyte ratio which can be the cause of improvement of immunity in lambs.

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