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**THE EFFECTS OF DIETARY HESPERIDIN AND NARINGIN SUPPLEMENTATION ON LAMB PERFORMANCE AND MEAT CHARACTERISTICS**

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## The effects of dietary hesperidin and naringin supplementation on lamb performance and meat characteristics

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### ABSTRACT

An experiment was conducted to examine the effects of supplementing feed with hesperidin or naringin, bioflavonoids that are abundant and inexpensive by-products of citrus cultivation, on lambs' growth performance, carcass and meat characteristics. Forty-four male Chios lambs were randomly assigned to 4 groups. One of the groups served as control (C) and was given a basal diet, whereas the other 3 groups were given the same diet further supplemented with hesperidin at 2500 mg (H), or naringin at 2500 mg (N), or  $\alpha$ -tocopheryl acetate at 200 mg (E) per kg feed. At the end of the experiment (35th day), lambs were fasted, weighed and slaughtered. After overnight chilling, samples of longissimus thoracis muscle were taken and were used for meat quality evaluation. No significant differences were observed in final body weight, body weight gain and edible organ weights among the four experimental groups. pH, colour parameters (L, a\*, b\*), water holding capacity and shear force value of longissimus thoracis muscle were also not significantly influenced by the dietary treatments. Measurement of lipid oxidation values showed that hesperidin or naringin supplementation positively influenced meat antioxidant properties during the refrigerated storage at 4°C for up to 8 days, however to a lesser extent compared to  $\alpha$ -tocopheryl acetate.

Nowadays, there is a strong interest in isolating antioxidants from natural sources and using them in animal nutrition with the intention to minimize lipid oxidation in meat products. According to the findings of the present study, flavonoids appeared as a great alternative, since they resulted in an improvement of meat antioxidant capacity leading to a prolongation of its shelf-life and an increase of its acceptability in the market.

*Keywords: hesperidin; naringin; lambs; meat quality; lipid oxidation*

### INTRODUCTION

Oxidation by free radicals is one of the primary mechanisms of quality deterioration in foods and especially in meat products. It is initiated in the highly-unsaturated fatty acid fraction of membrane phospholipids, leading to the production of hydroperoxides, which are susceptible to further oxidation or decomposition to secondary reaction products such as short-chain aldehydes, ketones and other oxygenated compounds that may adversely affect lipids, pigments, proteins, carbohydrates, vitamins and the overall quality of products by causing loss of flavour, colour and nutritive value and limiting shelf-life (Kanner, 1994). In the past, synthetic antioxidants were used with the intention to prevent lipid oxidation by scavenging chain-carrying peroxy radicals or diminishing the formation of lipid radicals. The last decade, considerable interest has arisen in the use of natural antioxidants that would serve as alternatives to synthetic supplements on purpose to improve meat quality, without leaving residues in the product or the environment (Wenk, 2003). The use of natural antioxidants can prolong the shelf life and increase the acceptability of meat and its economic value in the marketplace. Moreover, nutritional approaches are often more effective than direct addition of the additive to the muscle food since the compound is preferably deposited where it is most needed (Govaris et al., 2004). Antioxidant effects of  $\alpha$ -tocopheryl acetate supplementation on ruminant muscles are well established. Dietary supplementation of the lipid soluble antioxidant vitamin E increases the endogenous content of tissues and allows uniform incorporation of  $\alpha$ -tocopheryl into the subcellular



membranes where it can effectively inhibit the oxidative reactions at their localized sites and improve meat quality due to delayed lipid oxidation (Gobert et al., 2010).

Dried citrus pulp is the main by-product from the citrus-processing industry and produced after extraction of the juice from citrus fruits and drying of the residues. Citrus pulp is a mixture of peel, inside portions and culled fruits of the citrus family and is rich source of energy, fiber and calcium. Fibers from citrus fruits have an additional advantage over dietary fibers from other sources due to the presence of associated bioactive compounds (i.e. flavonoids). These compounds usually contain one or more aromatic hydroxyl groups, which actively scavenge free radicals and are responsible for the antioxidant activity. Flavonoids and especially their subgroup flavanones, which contain hesperidin and naringin, are health-promoting molecules with multifunctional biological activities; they have been shown to attenuate inflammation, to quench active oxygen species and their intake appears to be inversely related to risk of cardiovascular disease and several form of cancer (Erlund, 2004). At the same time, citrus pulp constitutes a cheap feed particularly during the dry summer when grass land is very limited in countries around the Mediterranean and diminishes dependence of livestock on grains that can be consumed by humans (Bampidis & Robinson, 2006; Volanis et al., 2004).

The combination of increased disposal costs in many parts of the world with the antioxidant properties of citrus by-products have increased interest in their utilization as alternative feeds for ruminants. The objective of the present study was the evaluation and comparison of the effects of hesperidin or naringin dietary supplementation on lamb growth performance, carcass and meat characteristics.

## MATERIALS & METHODS

Forty-four male weaned 3 months-old lambs of the Chios breed were weighed and randomly assigned into 4 equal groups. One of the groups served as a control (C) and was given a commercial basal diet, whereas the other three groups were given the same diet further supplemented with hesperidin at 2500 mg/kg (H), or naringin at 2500 mg/kg (N), or  $\alpha$ -tocopheryl acetate at 200 mg/kg (E). We have used these levels of supplementation with the intention to reach the maximum levels of flavonoids ingestion by the lamb when it consumes a diet supplemented with citrus pulp. Methods used in the present experiment were approved by the bioethical committee of the Agricultural University of Athens under the guidelines of "Council Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes".

Fresh feed was prepared daily in order to minimize the losses of hesperidin or naringin properties as a result of its exposure to air and light. Feed intake and weight of lambs were weekly recorded. Water was available ad libitum and diet, composed of the concentrated feed and alfalfa hay, was provided twice daily at 08.00 and 15.00 hours. Comparison of growth performance parameters was based on feed intake and liveweight recorded during the experiment. At day 35 of the experiment period, lambs were fasted for 18 h (water was allowed), weighed and slaughtered. Weights of edible parts and carcass were measured. After refrigerated storage for 24 h at 4°C, carcasses were again weighed and the longissimus dorsi muscle thoracic region (6th–13th rib) was then excised and used for the analyses. pH (HI 99163 Meat Temperature Meter, Hanna instruments, Romania), colour parameters (Miniscan XE, HunterLab, Reston, VA), water holding capacity (Sierra, 1973) and tenderness (shear force values) (Zwick Model Z2.5/TN1S, Germany) were directly measured. Measurement of lipid oxidation (Botsoglou et al., 1994) was implemented on days 1, 3, 6 and 9 and 3, 6 and 9 months after storage at 4°C and -20°C, respectively.

Growth performance, carcass parameters and meat quality characteristics, such as pH<sub>24</sub>, colour parameters (L\*, a\*, b\*), cooking loss (%), water holding capacity (%), shear force value (N) and malondialdehyde (MDA) concentration for the longissimus thoracis muscle were analyzed using a Mixed Model procedure which contained the fixed effect of nutritional treatment. All model analyses were performed by SAS/STAT (2005).

## RESULTS & DISCUSSION

Feed intake was influenced nor by flavonoids, neither by the  $\alpha$ -tocopheryl acetate dietary supplementation (Table 1). Similarly, lambs consuming a diet supplemented with hesperidin at the levels of 1.5 or 3.0 mg/kg (Simitzis et al.,



2013) or solar-dried citrus pulp at a concentration of 30-45% (Caparra et al., 2007) or citrus pulp silage at a level of 30% of the total diet (Scerra et al., 2001) did not eat less feed compared to controls. No significant differences were also observed in final body, carcass and edible parts weights (Table 1). Previous studies similarly demonstrated no effect of hesperidin (Simitzis et al., 2013), dietary citrus pulp (Caparra et al., 2007; Scerra et al., 2001) and orange pulp (10%) (Lanza et al., 2001) supplementation on growing performance and carcass characteristics in lambs.

No significant differences in pH<sub>24</sub> of lamb meat were illustrated among the dietary treatment groups (Table 1). Solar dried citrus pulp dietary supplementation (30-45%) (Caparra et al., 2007) or incorporation of citrus pulp silage in lamb diet (30%) (Scerra et al., 2001) also appear not to influence lamb muscle pH. In addition, incorporation of hesperidin in lambs' diet at a level of 1.5 or 3.0 g/kg does not seem to influence pH value (Simitzis et al., 2013). At the same time, longissimus thoracis muscle colour parameters were not significantly different among the treatment groups (Table 1). In a previous experiment implemented in lambs, meat from lambs fed with a hesperidin supplemented diet (1.5 or 3.0 g/kg) had similar colour values with the control group (Simitzis et al., 2013). Moreover, incorporation of orange pulp (10%) in lamb diets increased lightness (L\*) meat colour value (Lanza et al. 2001). On the other hand, addition of citrus pulp silage in lamb diets (Scerra et al., 2001) did not have any significant effect on L\*, a\* and b\* values.

Hesperidin or naringin or α-tocopheryl acetate supplementation did not influence the meat shear force value (Table 1). There is no evidence of naturally occurring additives dietary supplementation. Moreover, hesperidin (Simitzis et al., 2013) and citrus pulp (Caparra et al., 2007; Scerra et al., 2001) dietary supplementation does not appear to influence lamb shear force values. Cooking loss and water holding capacity were also not significantly affected by the dietary treatment (Table 1). No differences in meat water holding capacity were also found, when hesperidin (1.5 or 3.0 g/kg) (Simitzis et al., 2103) or solar-dried citrus pulp (30-45%) (Caparra et al., 2007) was incorporated in lambs' diet.

**Table 1.** Effect of hesperidin or naringin dietary supplementation on growth performance parameters and meat quality characteristics in lambs

Parameters		Treatment				S.E.M.
		C	H	N	E	
Mean daily feed intake (0-35d) (g)		710	714	714	712	14
Final Body Weight (kg)		30.2	30.8	30.9	29.9	0.67
Cold Carcass Weight (kg)		14.1	14.4	14.6	14.3	0.37
Edible Parts (kg)		1.83	1.81	1.86	1.83	0.05
pH <sub>24</sub>		5.59	5.57	5.57	5.61	0.02
Colour	L	41.5	40.7	40.9	40.4	0.75
	a*	12.6	12.9	13.2	13.1	0.32
	b*	13.5	13.0	11.8	11.6	0.35
Water Holding Capacity (%)		11.0	12.7	11.4	11.4	0.74
Cooking loss (%)		20.3	18.8	19.8	20.4	0.90
Shear force (N)		59.1	61.5	65.4	58.6	3.77

The extent of lipid oxidation in raw longissimus thoracis muscle stored at 4°C for up to 9 days or at -20°C for up to 9 months varied with the storage time and the dietary treatment (Figure 1). Refrigerated and long-term frozen storage increased the levels of malondialdehyde (MDA), the compound used as an index of lipid oxidation, the increase being higher in the control group. Results indicated that incorporation of hesperidin and naringin into the lambs diets at the level of 2.5 g/kg delayed (P<0.05) lipid oxidation in the refrigerated longissimus thoracis muscle samples; the delay was, however, lower than that occurred when hesperidin and naringin were substituted by α-



tocopheryl acetate at the level of 0.2 g/kg. The inhibition of lipid oxidation in lamb meat after dietary supplementation with hesperidin or naringin was probably the result of flavonoids' properties. Hesperidin and naringin and their metabolites entered the circulatory system, distributed and possibly retained in lamb tissues. However, according to the present results, there was not a significant effect of dietary hesperidin or naringin supplementation on the MDA values of long-term frozen longissimus thoracis muscle (Figure 1). It seems that flavonoids and their metabolites did not remain functional in lamb tissues during long-term frozen storage. According to previous studies, flavonoids can take over the role of  $\alpha$ -tocopheryl acetate as a chain-breaking antioxidant in liver microsomal membranes (van Acker et al., 2000). Dietary supplementation has been proved to be a simple and convenient strategy to uniformly introduce a natural antioxidant into phospholipid membranes where it may effectively inhibit the oxidative reactions at their localized sites (Lauridsen et al., 1997). The bioavailability of flavonoids could not be directly demonstrated, because adequate analytical methodology for their identification and quantification is not yet available for animal tissues.

Previous experiments demonstrated that serum superoxide dismutase level was increased by the flavonoids hesperidin and naringin dietary supplementation. Additionally, total antioxidant activity and scavenging superoxide ability were enhanced, and serum TBARS level was decreased by flavonoids supplementation (Lien et al., 2008). These results are in accordance with the results of Jeon et al. (2001), Seo et al. (2003) and Kim et al. (2004), who found an antioxidant activity of flavonoid additives in rabbits, rats and mice, respectively. Hesperidin dietary incorporation (1.5-3.0 mg/kg) reduced MDA values in lamb meat (Simitzis et al., 2013). At the same time, the dietary administration of different levels of hesperidin exerts significant effect on broiler breast meat antioxidative capacity, probably indicating that hesperidin is introduced into the cell phospholipids membranes and protect them from oxidation in broiler muscles (Simitzis et al., 2011).



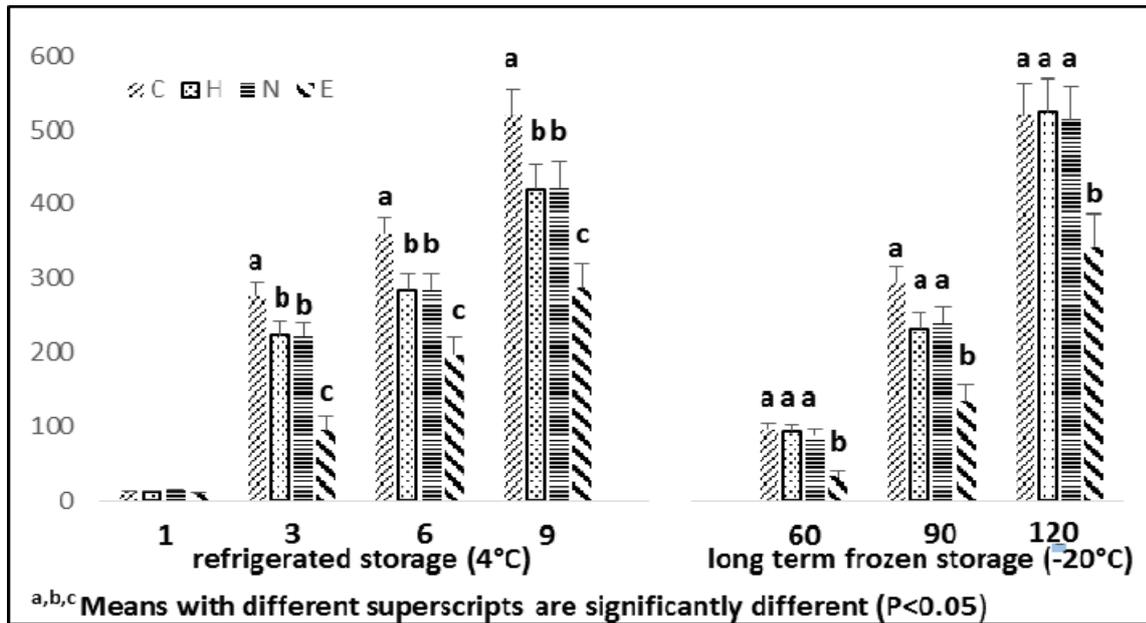


Figure 1. Effect of refrigerated (4°C) and long-term frozen (-20°C) storage on lipid oxidation of raw lamb longissimus thoracis muscle as a function of hesperidin, naringin and vitamin E dietary supplementation

## CONCLUSION

Nowadays, there is a strong interest in isolating antioxidants from natural sources and using them in animal nutrition with the intention to minimize lipid oxidation in products. In this experiment, the dietary administration of hesperidin or naringin exerts significant effect on lamb meat antioxidative capacity during refrigerated storage. Further experimentation is, among others, needed to elucidate their exact action into the cell phospholipids membranes in lamb muscles and establish their regular use.

## ACKNOWLEDGEMENTS

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## The Effects of Dietary Hesperidin and Naringin Supplementation on Lamb Performance and Meat Characteristics

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### Introduction

Hesperidin and naringin are natural occurring flavonoids, well known for their antioxidant and anti-inflammatory properties. They are contained in citrus pulp that represents a cheap, but rich source of energy, fiber and calcium for sheep diets around the Mediterranean

### Objective

The aim of the present study was to investigate the effects of dietary supplementation with hesperidin or naringin on growth performance parameters, quality characteristics (pH, colour, water holding capacity, cooking loss, tenderness) and oxidative stability (MDA assay) of meat in lambs

### Materials & Methods

**44** 3-months old male Chios lambs were assigned into 4 experimental groups:

- 1.(C), without dietary supplementation
- 2.(H), supplemented with hesperidin at 2.5 g/kg
- 3.(N), supplemented with naringin at 2.5 g/kg
- 4.(E), supplemented with α-tocopheryl acetate at 0.2 g/kg

Comparison of growth performance parameters was based on **feed intake** and **live weight** weekly recorded. At day 35 of the experiment, lambs were slaughtered and weights of **carcass** and **edible parts** were measured. After refrigerated storage for 24 h at 4°C, **quality characteristics** were determined in *longissimus thoracis* muscle. **MDA concentration** was also measured 1, 3, 6 and 8 days and 2, 3 and 4 months after storage at 4°C and -20°C, respectively

### Conclusions

Hesperidin or naringin dietary supplementation do not seem to negatively influence growth performance parameters and meat quality characteristics in lambs



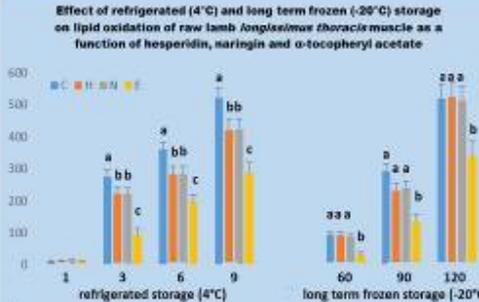
On the other hand, antioxidant properties of lamb meat were improved after the dietary supplementation with hesperidin or naringin (storage at 4°C)

### Results

Effect of hesperidin or naringin dietary supplementation on growth performance parameters and meat quality characteristics in lambs

Parameters	Treatment				S.E.M.	
	C	H	N	E		
Mean daily feed intake (0-35d) (g)	710	714	714	712	14	
Final Body Weight (kg)	30.2	30.8	30.9	29.9	0.87	
Cold Carcass Weight (kg)	14.1	14.4	14.6	14.3	0.37	
Edible Parts (kg)	1.83	1.81	1.86	1.83	0.06	
pH <sub>24</sub>	5.59	5.57	5.57	5.61	0.02	
	41.8	48.7	46.9	40.4	0.75	
Colour	a*	12.6	12.9	12.2	13.1	0.32
	b*	13.6	13.6	11.8	11.8	0.38
Water Holding Capacity (%)	11.0	12.7	11.4	11.4	0.74	
Cooking loss (%)	20.3	18.8	19.8	20.4	0.90	
Shear force (N)	59.1	61.5	65.4	58.6	3.77	

Effect of refrigerated (4°C) and long term frozen (-20°C) storage on lipid oxidation of raw lamb *Longissimus thoracis* muscle as a function of hesperidin, naringin and α-tocopheryl acetate



\*a,b,c Means with different superscripts are significantly different (P<0.05)







This research project was implemented within the framework of the Project "Thalis - The effects of antioxidant's dietary supplementation on animal product quality", MIS 380231, Funding Body: Hellenic State and European Union



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ΥΠΟΥΡΓΕΙΟ ΠΑΙΔΕΙΑΣ & ΘΡΗΣΚΕΥΜΑΤΩΝ, ΠΟΛΙΤΙΣΜΟΥ & ΑΘΛΗΤΙΣΜΟΥ  
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