Research Open

Nutrition, Dietetics & Nutraceuticals
Volume 1 Issue 1

Research Article

The effects of hesperidin or naringin dietary supplementation on yoghurt quality parameters in dairy ewes – A preliminary study

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Received: December 11, 2019; **Accepted:** December 12, 2019; **Published:** December 25, 2019

Abstract

Stakeholders that are involved in the animal production chain, such as primary producers,processors, distributors, and retailers continuously seek for alternative ways of improving health benefits and technological properties of dairy products. Hesperidin and naringin belong to flavonoids and are well-known for their multifaceted properties. The aim of this preliminary study was therefore to examine the effects of flavonoids supplementation into the diets of dairy ewes on the quality parameters and oxidative stability of yoghurt manufactured by their milk. Thirty-six Chios ewes were allocated to four groups; the control group (C) was fed concentrates without supplementation, while the other three experimental groups received the same diet further supplemented with hesperidin (6000mg/kg), naringin (6000mg/kg), or α-tocopheryl acetate (200mg/kg). As indicated, no effects on yoghurt quality parameters and oxidative stability were observed in individual samples manufactured from milk collected after 7, 21 and 28 days of flavonoids dietary supplementation. In conclusion, inclusion of flavonoids in ewes' diet does not appear to affect yoghurt quality characteristics.

Keywords: *hesperidin; naringin; yoghurt quality; oxidative stability 31*

Introduction

Dairy fermented foods, such as yoghurt, have gained a positive perception and enjoyed high popularity among the consumers due to their beneficial effects on human health [1]. Among others, consumption of these products improves immunity and results in a slight reduction in stomach pH that minimizes the risk of pathogen transit and the impacts of low gastric juice secretion [2]. At the same time, several peptides derived by proteolysis could lower blood pressure in hypertensive patients [3]. Yoghurt consists of a casein network aggregated through isoelectric precipitation by lactic acid bacteria, such as Streptococcus thermophilus and Lactobacillus delbrueckii spp. bulgaricus. Fermentation is a chemical process in which specific enzymes break down organic substances into smaller compounds resulting in more digestible, stable and flavored foods with enhanced nutritional value [4]. Enrichment of animal products with natural bioactive compounds seems to improve their quality characteristics and fortifies consumers against oxidation effects. Dietary flavonoids have received significant attention in recent years due to their antioxidant, anti-inflammatory, anti-mutagenic and anti-clotting properties that are associated with a declined risk of cardiovascular diseases and cancer development [5, 6]. In general, levels of polyphenols in yoghurt are low and its enrichment with plant-derived additives could improve its phenolic content contributing in disease prevention and correction of deficiencies with minimal side effects [1].

Several pre- and post- fermentation approaches for adding polyphenols to yoghurt have already been implemented with positive effects on the derived product. Addition of polyphenols originated from bitter orange (Citrus aurantium l.) flowers [7], berry [8], apple [9], strawberry [10], green tea [11], peppermint, dill and basil [12], pomegranate peel [13] or juice [14], or grape seed [15] significantly increased yoghurt antioxidant capacity without other significant effects on its quality. According to the literature [16], the pre-fermentation application could introduce some advantages such as the promotion of starter cultures'growth. Alternative approaches are continuously evaluated in animal production systems with the intention to improve the nutritional value and the organoleptic properties of the derived products. However, no data exist describing the effects of flavonoids inclusion into the diets of dairy ewes on the quality of the derived yoghurt. The aim of the present study was therefore to investigate the effects of hesperidin or naringin or a-tochopheryl acetate dietary supplementation on the quality characteristics (colour, pH, syneresis and texture) and oxidative stability of yoghurt manufactured by ewe milk.

Methods & Materials

Animal and diets

The experimental design is described in detail by Simitzis et al. [17]. In brief, 36 lactating Chios ewes were allocated into 4 experimental groups based on their milk yield and body weight. One of the groups served as a control (C) and was fed with a basal concentrate diet, whereas the other three groups were offered the same diet further supplemented with hesperidin (hesperidin, TSI Europe NV, Belgium) at 6000 mg/kg concentrated feed (H), or naringin (naringin hydrate 98%, Alfa Aesar GmbH & Co KG, Germany) at 6000 mg/kg concentrated feed (N), or α-tocopheryl acetate (DSM Nutritional Products Hellas, Greece) at 200 mg/kg concentrated feed (VE). Methods used in the present experiment were approved by the bioethical committee of the Agricultural University of Athens (Permit Number: 23/20032013) under the guidelines of "Council Directive 2010/63/EU on the protection of animals used for scientific purposes".

Milk samples and yoghurt preparation

Animals were milked twice a day at 6 am and 6 pm by a milking machine. Individual milk samples were collected the day before the beginning and at the 7th, 14th, 21st and 28th day of the experiment and obtained after mixing the volume of milk collected during the morning and evening milking. Individual traditional Greek yoghurt samples were separately manufactured by milk collected from each ewe during the sampling days, apart from day 14 due to technical reasons. The main stages of yoghurt production were: collection and filtration of raw ovine milk, heating to 95°C for 15 min without homogenization, transfer to closed 250 ml cups, cooling to 45 – 50°C, inoculation and mixing with 2% of a commercial thermophilic starter culture of Streptococcus thermophilus and Lactobacillus delbrueckii subsp. Bulgaricus (Chr. Hansen, Denmark), incubation at 45 °C for about 3 h and storage at 5 °C [18].

Yoghurt quality parameters

Yoghurt quality parameters were assessed after one day of refrigerated storage. Colour was measured (3 measurements per sample) using a Miniscan XE (HunterLab, Reston, USA) chromameter set on the L* (lightness), α* (redness), b* (yellowness) system (CIE 1976, Commission International de l' Eclairage). pH was determined using a pHM210 standard pHmeter (MeterLab, Radiometer, Denmark). Rheological measurements of yoghurt were implemented with a Shimadzu Testing Instrument, model AGS-500 NG (Shimadzu Corporation, Kyoto, Japan) equipped with 5 kg load cell. A plunger with a diameter of 25 mm was attached to the moving crosshead, which moved both downwards and upwards at a speed of 120 mm/min, was inserted to a depth of 20 mm below the yoghurt surface. The firmness (N) was calculated from the resulting curve and defined as the height of the peak force during the compression cycle. Syneresis of yoghurt was measured by emptying the contents of the plastic container (200 g) into a stretched cheese cloth, cutting crosswise into four pieces, draining in a funnel for 24 h at 4 °C, collecting the amount of whey drained off in a conical bottle and weighing in gram to provide an index of syneresis.

Antioxidant capacity was assessed after 10 and 20 days of refrigerated storage at 4°C on the basis of the malondialdehyde (MDA) levels formed during storage. MDA concentration was determined by using a third-order derivative spectrophotometric method [19]. In brief, 2 g of each yoghurt sample (two sub-samples per ewe) was homogenized (Edmund Buehler 7400 Tuebingen/H04, Germany) in 8 ml of aqueous trichloroacetic acid (TCA) (50 g/l) and 5 ml of butylated hydroxytoluene (BHT) in hexane (8 g/l), and the mixture was centrifuged for 3 min at 3000 × g. The top hexane layer was discarded, and a 2.5 ml of aliquot from the bottom layer was mixed with 1.5 ml of aqueous 2-thiobarbituric acid (TBA) (8 g/l) and further incubated at 70°C for 30 min. Following incubation, the mixture was cooled under tap water and submitted to third-order derivative (3D) spectrophotometry (Hitachi U3010 Spectrophotometer) in the range of 500–550 nm. The concentration of MDA (ng/ml milk) was calculated on the basis of the height of the third-order derivative peak at 521.5 nm by referring to the slope and intercept data of the computed least-squares fit of standard calibration curve prepared using 1,1,3,3-tetraethoxypropane (TEP), the MDA precursor.

Statistical analysis

The experimental unit was the animal since it was the smallest unit upon which, either the treatment was applied or the measurements were made. Data were subjected to repeated measures analysis of variance using the MIXED procedure of SAS software [20], with dietary treatment as fixed effect and the sampling day as the repeated factor. Significant differences were tested at 0.05 significance level and results are presented as least square means ± s.e.m.

Results

No significant effects of hesperidin, naringin or vitamin E dietary supplementation on yoghurt quality parameters were observed. As shown in Table 1, quality characteristics of yoghurt manufactured by ewe milk that was individually collected after 7, 21 and 28 days of flavonoids dietary inclusion were not significantly different among the experimental groups. Values for colour parameters (L, a*, b*), pH, firmness and syneresis were not influenced after 7, 21 or 28 days of flavonoids incorporation into dairy ewes' diets. At the same time, no significant effects on MDA values were found in yoghurt manufactured with milk samples collected from ewes after 7, 21 and 28 days of flavonoids dietary supplementation and stored at 4°C for 10 and 20 days (Table 2).

Discussion

There is always a challenge of improving health benefits and technological properties of dairy products. According to the literature, yoghurts inoculated with phenolic extracts display higher antioxidant capacity compared to the controls, possibly through the scavenging of free radicals [21, 22]. However, as far as the authors are aware, no data exist on the influence of antioxidants' dietary supplementation on yoghurt characteristics manufactured by ewe milk. The available literature is mainly focused on the effects of flavonoids dietary inclusion on milk characteristics of dairy cows. As indicated by the previous researchers, milk quality parameters in dairy cows

were not negatively influenced by the inclusion of propolis [23, 24] or alfalfa [25] or grape seed and grape marc meal [26] or green tea and curcuma [27] flavonoids extracts in their diets. As already pointed out, no significant effects of hesperidin and naringin dietary supplementation on the quality parameters (colour, pH, firmness and syneresis) of the derived yoghurt were observed. This finding may be partially associated with the fact that yield, composition, coagulation properties and fatty acid profile of sheep milk are not influenced by the incorporation of hesperidin or naringin in the diets of dairy ewes [17]. On the other hand, an improvement of milk oxidative stability is observed both in dairy cows [23, 24] and dairy ewes [17] after the addition of flavonoids in their diets. In contrast, no significant effects of hesperidin or naringin dietary supplementation on yoghurt oxidative stability were observed in the present study. Fermentation and post-acidification may have negatively affected the antioxidant potential of the examined flavonoids, since they are chemical processes in which enzymes break down organic substances into smaller compounds with different function and value [4]. As indicated in previous studies, the interactions between added bioactive compounds, milk proteins, polysaccharides (such as pectin) and the starter cultures might vary on a case-by-case basis [8], leading to different effects on yoghurt texture parameters [28]. At the same time, it could be suggested that dietary flavonoids supplementation did not affect bacterial growth, since no differences in yoghurt properties and especially pH values were observed.

Table 1. Effect of hesperidin and naringin on yoghurt characteristics after 0, 7, 21 and 28 days of dietary supplementation in dairy ewes

Day	Parameter	Treatment[1]				S.E.M.	*P*-value
		C	H	N	E		
0	L	95.07	94.97	95.16	94.47	0.25	0.243
	Colour[2] a*	-2.76	-2.89	-2.99	-3.00	0.07	0.084
	b*	11.46	11.33	11.84	11.58	0.35	0.790
	pH	4.24	4.23	4.25	4.49	0.14	0.542
	Firmness(N)	0.87	0.90	0.84	0.68	0.08	0.095
	Syneresis(%)	1.96	2.73	1.33	3.50	0.65	0.109
7	L	94.93	94.91	95.22	95.05	0.32	0.888
	Colour a*	-2.81	-2.73	-2.84	-2.71	0.07	0.565
	b*	10.75	10.76	10.52	10.73	0.44	0.977
	pH	4.32	4.51	4.44	4.38	0.13	0.768
	Firmness(N)	0.79	0.81	0.72	0.88	0.11	0.773
	Syneresis(%)	2.12	2.47	1.58	3.44	0.66	0.260
21	L	94.73	94.53	95.02	94.88	0.37	0.811
	Colour a*	-2.70	-2.84	-2.77	-2.66	0.07	0.282
	b*	10.32	11.00	10.84	10.80	0.57	0.854
	pH	4.30	4.19	4.18	3.94	0.23	0.720
	Firmness(N)	0.81	0.75	0.87	0.92	0.10	0.730
	Syneresis(%)	2.22	3.43	2.99	3.41	0.93	0.768
28	L	94.60	94.53	94.69	94.78	0.34	0.953
	Colour a*	-2.81	-2.67	-2.84	-2.79	0.08	0.435
	b*	10.28	11.51	11.01	11.47	0.11	0.529
	pH	4.30	4.44	4.10	4.20	0.18	0.591
	Firmness(N)	0.58	0.79	0.89	0.91	0.10	0.133
	Syneresis(%)	2.49	3.59	3.84	2.44	0.87	0.220

[1]The control group (C) was fed with a commercial basal diet, whereas the other groups consumed the same diet, with the only difference that concentrated feed was uniformly supplemented with hesperidin (H) (6000mg/kg feed) or naringin (N) (6000mg/kg feed) or vitamin E (VE) (200mg/kg feed). [2]L*; lightness, α*; redness, b*; yellowness

Table 2. MDA values (ng/g) in yoghurt manufactured from milk samples collected the day before, 7, 21 and 28 days after hesperidin and naringin dietary supplementation.

Milk Sampling (days)	Refrigerated Storage (days)	Treatment[1]				S.E.M.	*P*-value
		C	H	N	VE		
0	10	2.96	2.76	2.65	2.88	0.23	0.907
	20	3.35	3.24	3.28	3.16	0.23	
7	10	2.19	2.05	1.83	1.82	0.27	0.484
	20	3.25	2.63	2.83	3.23	0.27	
21	10	2.32	2.43	2.87	2.90	0.23	0.902
	20	2.90	2.75	3.36	3.18	0.23	
28	10	3.87	4.23	3.80	3.50	0.36	0.468
	20	4.21	4.35	4.99	4.19	0.36	

[1]The control group (C) was fed with a commercial basal diet, whereas the other groups consumed the same diet, with the only difference that concentrated feed was uniformly supplemented with hesperidin (H) (6000mg/kg feed) or naringin (N) (6000mg/kg feed) or vitamin E (VE) (200mg/kg feed).

Conclusions

As indicated by the results of the present study, dietary supplementation of dairy ewes with flavonoids at the examined levels does not improve yoghurt quality characteristics and oxidative stability.

Funding information

This research was funded by Hellenic State and European Union, within the framework of the Project "Thalis – The effects of antioxidant's dietary supplementation on animal product quality", MIS 380231.

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Citation:
Panagiotis Simitzis, Theofilos Massouras, Michael Goliomytis, Maria Charismiadou, Katerina Moschou, Christina Economou, Vagelis Papadedes, Sofia Lepesioti and Stelios Deligeorgis (2019) The effects of hesperidin or naringin dietary supplementation on yoghurt quality parameters in dairy ewes – A preliminary study. *.Nutr Diet Nutraceuticals* Volume 1(1): 1–5.