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Αξιοποίηση Φυσικών Αντιοξειδωτικών στην Εκτροφή των Αγροτικών Ζώων για Παραγωγή Προϊόντων Ποιότητας

Γεωπονικό Πανεπιστήμιο Αθηνών

Εργαστήριο Ζωοτεχνίας

MIS 380231

Δράση 7η : Επίδραση των φλαβονοειδών στην έκφραση γονιδίων σχετιζόμενων με την αντιοξειδωτική δράση

Παραδοτέα: D7_PUBL_2

Effect of dietary supplementation of broiler chickens with the natural antioxidants hesperidin and naringin on the expression of lipogenesis related genes and fatty acid profile

Υποβλήθηκε για παρουσίαση (poster) στο International Symposium on Animal Functional Genomics, 2015 Conference, Piacenza, Italy, που διοργανώθηκε στη Piacenza, Italy την 27-29 Ιουλίου 2015.

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TRANSCRIPTOMICS - Abstract N° 64

Effect of dietary supplementation of broiler chickens with the natural antioxidants hesperidin and naringin on the expression of lipogenesis related genes and fatty acid profile

Ariadne L. Hager-Theodorides, Nina A. Dragona, Katerina Moschou, Christina Kappou, Ilias Arkoumanis, Michael Goliomytis, Maria Charismiadou, Panagiotis Simitzis, Theofilos Massouras and Stelios Deligeorgis

Hesperidin and naringin, flavonoids abundant in citrus fruits, exhibit health-promoting properties notably antioxidant and modulation of lipid metabolism. Increased antioxidant capacity and favorable fatty acid profile are desirable properties for broiler meat. In chickens hesperidin lowered plasma and egg yolk cholesterol and improved broiler meat antioxidant capacity. Here the effects of broiler diet supplementation with hesperidin and naringin on the expression of the lipogenesis related genes *adiponectin, ppar-γ* and *fatty acid synthase* (*fasn)* and fatty acid profile were assessed. 240 ROSS308 broilers were divided into treatment groups that received different diets supplemented with 0.75 or 1.5g hesperidin, 0.75 or 1.5g naringin, 0.2g vitE per kg feed or unsupplemented/control diet from the 11th to 42nd day of age. Abdominal adipose tissue, liver, breast and thigh muscle samples were obtained from 10 animals per treatment and used for gene expression and/or fatty acid composition analysis. Hesperidin and naringin did not affect the expression of the genes studied in adipose tissue and hesperidin did not affect gene expression in the liver ($P>0.05$). On the contrary, naringin significantly lowered the level of *fasn* ($P<0.05$). Breast muscle fatty acid profile analysis showed a tendency for lower palmitic acid content in naringin fed compared to control chickens and increased oleic acid content in hesperidin fed animals and increased total PUFA in both hesperidin and naringin fed animals ($P<0.10$). In the abdominal fat, a tendency for lower fat percentage, increased conjugated linoleic acids (CLA) and increased PUFA was observed in naringin fed animals compared to control and a tendency for reduced oleic acid, increased CLA ($P<0.10$) and significantly reduced MUFA in hesperidin fed animals ($P<0.05$).
Increased *fasn* expression is associated with increased fat deposition therefore the observed reduction of *fasn* expression in the liver in naringin fed chickens is a putative molecular mechanism of naringin affecting lipid metabolism and fat deposition in broiler meat. In addition palmitic acid is the product of *fasn* activity therefore the observed reduced levels of *fasn* expression are consistent with the lower palmitic acid levels in breast muscle of naringin fed chickens.

Project implemented within the framework of "Thalis–The effects of antioxidant's dietary supplementation on animal product quality", MIS380231, Funding Body: Hellenic State, European Union.

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Effect of dietary supplementation of broiler chickens with the natural antioxidants hesperidin and naringin on the expression of lipogenesis related genes and fatty acid profile

Ariadne L. Hager-Theodorides[1]*, Nina A. Dragona[1], Katerina Moschou[2], Christina Kappou[1], Ilias Arkoumanis[1], Michael Goliomytis[1], Maria Charismiadou[1], Panagiotis Simitzis[1], Theofilos Massouras[2] and Stelios Deligeorgis[1]
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Introduction

Hesperidin and naringin, flavonoids abundant in citrus fruits, exhibit health-promoting properties such as antioxidant and modulation of lipid metabolism. Increased antioxidant capacity, reduced fat content and favorable fatty acid composition, i.e. reduced MUFA-increased PUFA, are desirable properties for broiler meat. Previous studies showed that in chickens hesperidin lowered plasma and egg yolk cholesterol levels and improved broiler meat antioxidant capacity.

Objectives

This study investigated the effects of broiler diet supplementation with hesperidin and naringin on lipid metabolism on the molecular level by studying the expression of the lipogenesis related genes *Adiponectin (AdipoQ)*, *PPAR-γ* and *fatty acid synthase* (*FASN*) and on the biochemical level by analysis of fatty acid composition of broiler meat.

Methods

Two hundred and forty 1d-old broiler chickens, Ross 308, as hatched, were randomly assigned into 6 dietary groups as shown in Table 1. Samples were taken at 42 d of age.

Table 1. Supplementation levels of antioxidants fed to broiler chickens from 11 d to 42 d of age.

Antioxidant	Dietary group, g/kg feed					
	C	N1	N2	E1	E2	VE
Naringin	-	0.75	1.5	-	-	-
Hesperidin	-	-	-	0.75	1.5	-
Vitamin E	-	-	-	-	-	0.2

→ **Relative gene mRNA expression** of *adiponectin (AdipoQ), adiponectin receptor (AdipoR)-2, peroxisome proliferator-activated receptor γ (PPAR-γ)* and *fatty acid synthase (FASN)* in liver and adipose tissue samples (n=4) was determined by quantitative (q)RT-PCR with SYBR green on ABI7500.
→ **% fat content** of abdominal adipose tissue, breast (*pectoralis major*) and thigh (*biceps femoris*) muscle was determined based on total lipid extraction following Folch's protocol (n=10).
→ **Fatty acid profiles** were determined by gas chromatography of methyl esterified fatty acids (FAMEs) using Shimadzu GC-17A (n=10).

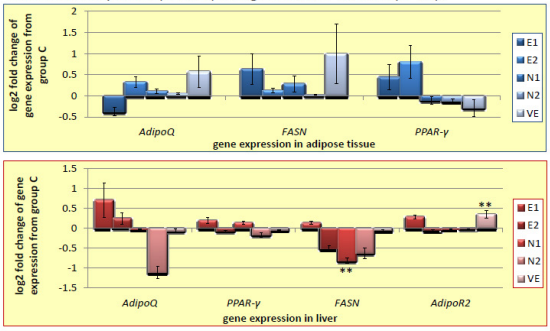

Figure 1. Fold change in mRNA expression relative to the control diet group (C) of lipogenesis related genes in adipose tissue (top) and liver (bottom).
Gene expression was normalized by *β-actin expression*. **Statistically significant differences with C, *P*<0.05, calculated using pair wise fixed reallocation randomisation test (REST-MCS© v2).

Results and discussion

- Naringin supplementation reduced *FASN* mRNA expression in the liver (Fig. 1).
- Vit E increased expression of *AdipoR2* in the liver (Fig. 1).
- Hesperidin significantly linearly decreased total MUFA content in abdominal fat (Table 1).
- Hesperidin and Naringin tended to linearly increase PUFA in both breast and abdominal fat (Table 1).
- Naringin tended to linearly decrease the palmitic acid (C16:0) content of breast muscle (Table 1).
- Hesperidin tended to linearly increase the oleic acid (C18:2n-6) content of breast muscle (Table 1).

-Observed reduction of *FASN* expression is consistent with reduced C16:0 in breast muscle of Naringin fed chickens.

Table 1 Fatty acid content of adipose tissue and breast muscle[a].

Adipose tissue	Dietary Group								
Fatty acids (FA), g/100g	C	E1	E2	N1	N2	VE	S.E.M.	P-linear C-H	P-linear C-N
C16:0	24.16	23.92	24.43	24.00	23.75	23.21	0.44	0.703	0.539
C18:1n-9	38.78	37.76	37.01	37.42	37.31	38.32	0.61	0.059	0.104
C18:2n-6	23.73	25.11	25.81	25.37	25.96	25.98	0.78	0.109	0.084
CLA	0.08	0.10	0.10	0.09	0.10	0.10	0.01	0.072	0.087
Monounsaturated fatty acids (MUFA)	43.74	42.52	41.31	42.02	42.06	42.61	0.80	0.04	0.163
Polyunsaturated fatty acids (PUFA)	26.55	28.06	28.62	28.21	28.77	28.84	0.80	0.123	0.096

Breast (*pectoralis major*)	Dietary Group								
Fatty acids, g/100g	C	E1	E2	N1	N2	VE	S.E.M.	P-linear C-H	P-linear C-N
C16:0	25.03	24.33	24.74	24.74	24.22	24.16	0.32	0.573	0.072
C18:1n-9	34.20	33.54	33.12	32.74	33.37	34.05	0.59	0.224	0.255
C18:2n-6	23.15	24.85	24.64	24.65	24.29	23.57	0.51	0.052	0.131
CLA	0.34	0.35	0.42	0.40	0.41	0.40	0.04	0.201	0.193
Monounsaturated fatty acids (MUFA)	37.56	36.97	36.26	35.78	36.66	37.05	0.72	0.227	0.332
Polyunsaturated fatty acids (PUFA)	26.17	27.82	27.67	27.87	27.49	26.63	0.55	0.073	0.095

[a]Only fatty acids that show a tendency to be linearly affected by the antioxidant supplementation are shown. Cells in red shades denote a tendency for linear FA content reduction with hesperitin or naringin supplementation, whereas cells in green shades colouring a tendency for linear FA content increase with supplementation.

Conclusions

Hesperidin and Naringin have been shown to increase broiler meat antioxidant capacity. Here we show that they can also affect lipogenesis and we identify the reduction of *FASN* expression as a putative underlying molecular mechanism.
Future plans: to assess expression of additional genes related to lipogenesis and antioxidant defense pathways using the PCR-array technology.

Acknowledgements

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.

European Union
European Social Fund
OPERATIONAL PROGRAMME EDUCATION AND LIFELONG LEARNING
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MANAGING AUTHORITY
NSRF 2007-2013
EUROPEAN SOCIAL FUND
Co- financed by Greece and the European Union

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Η Επιτροπή Πιστοποίησης Παραδοτέων

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