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

Γεωπονικό Πανεπιστήμιο Αθηνών

Εργαστήριο Ζωοτεχνίας

MIS 380231

Δράση 3η : Ποιότητα σφαγίου και κρέατος ορνίθων

Παραδοτέα: D3_PUBL_3

Impact of the dietary supplementation with flavonoids on the metabolic fingerprint of chicken plasma. An NMR-Based Metabolomic Study.

Υποβλήθηκε για παρουσίαση στο 9th Aegean Analytical Chemistry Days (AACD2014) που διοργανώθηκε από 29 Σεπτεμβρίου έως 3 Οκτωβρίου 2014 στη Χίο

ABSTRACT

Impact of the dietary supplementation with flavonoids on the metabolic fingerprint of chicken plasma. An NMR-Based Metabolomic Study.

Charalambos Fotakis [A], Panagiotis Zoumpoulakis [A,*], Ch. Baskakis [A], Michael Goliomytis [B], Panagiotis E. Simitzis [B], Stelios G. Deligeorgis [B]

[A] Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation
[B]Department of Animal Breeding and Husbandry, Faculty of Animal Science and Aquaculture, Agricultural University of Athens
*E-mail: pzoump@eie.gr

The increased awareness of consumers towards a diet rich in natural, safe and health-promoting ingredients has led to the search of alternative sources which may be used in the food and feed industry because of their valuable nutritional properties. Flavonoids have intense antioxidant and anti-inflammatory properties and could be used as dietary supplements in order to derive chicken meat products of improved quality.

An experiment was therefore conducted to examine the effects of supplementing feed with different levels of hesperidin or naringin, flavonoids that are abundant and inexpensive by-products of citrus cultivation, on the the metabolic fingerprint of chicken plasma. Sixty, eleven day old, broilers were randomly assigned into 6 treatment groups of ten chickens each. One of the groups served as control (C) and was given a commercial basal diet, without flavonoid supplementation, whereas the other five groups were given the same diet further supplemented with hesperidin at low (750mg/kg of feed) (H1) or high (1500mg/kg) (H2) concentration or naringin at low (750mg/kg) (N1) or high (1500mg/kg) (N2) concentration or α-tocopheryl acetate (200mg/kg) (E). Plasma samples were collected 4 and 8h after the beginning of flavonoids dietary supplementation and at the end of the experiment (42 days).

NMR based metabolomics using CPMG pulse sequence was implemented [1] in order to elicit information from the plasma. The spectral data was subjected to multivariate data analysis using SIMCA 13 software. In this context, supervised analysis (OPLS-DA) traced variations in the metabolic pattern according to the sustenance consumption which was attributed to specific metabolites with the application of the S-line plot. Particularly, control samples displayed higher concentration in unsaturated lipids, di-saccharides and threonine when compared to the VE, while the latter were characterized by the presence of HDL, dimethylamine and carnitine. Hesperidin (both E1 and E2) samples in contrast to control ones were characterised by increased levels of di-saccharide, threonine, creatinine, carnitine, dimethylamine and glutamine/glutamate. Narginin (both N1 and N2) samples also revealed the previous pattern and at the same time appeared to have increased levels of citrate and acetate when compared to controls.

Results indicated that similar metabolic patterns were observed irrespective of the type (H or N) and level (H1 or H2, N1 or N2) of the flavonoid incorporated in broiler diet.

Moreover, quantification of selected metabolites (leucine, isoleucine, valine, alanine, citrate, dimethylglycine, glutamine, lactate, threonine, glucose, fumarate, tyrosine phenylalanine, formate) was

also attempted using Chenomx software. N and VE samples exhibited the highest homogeneity among these results.

REFERENCES:
[1] NMR metabolite profiling of Greek grape marc spirits, Food Chemistry. Charalambos Fotakis, et al. Food Chemistry 138 (2013) 1837–1846.

Acknowledgment
This work is supported from the Programme THALIS 2012-2015, SECTORAL OPERATIONAL PROGRAMME, EDUCATION AND LIFELONG LEARNING –ESPA 2007-2013

KEYWORDS: Flavonoids, NMR metabolomics, Chicken plasma, OPLS-DA, S-line plot, biomarker elucidation

Impact of the dietary supplementation with flavonoids on the metabolic fingerprint of chicken plasma. An NMR-Based Metabolomic Study

Charalambos Fotakis [A], Panagiotis Zoumpoulakis [A,*], Ch. Baskakis [A], Michael Goliomytis [B], Panagiotis E. Simitzis [B], Stelios G. Deligeorgis [B]
A: Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation
B: Department of Animal Breeding and Husbandry, Faculty of Animal Science and Aquaculture, Agricultural University of Athens
*E-mail: pzoump@eie.gr

INTRODUCTION

Flavonoids have intense antioxidant and anti-inflammatory properties and could be used as dietary supplements in order to derive chicken meat products of improved quality. This work examines the effects of supplementing feed with different levels of hesperidin or naringin, flavonoids that are abundant and inexpensive by-products of citrus cultivation, on the the metabolic fingerprint of chicken plasma. Sixty, eleven day old broilers, were randomly assigned into 6 treatment groups of ten chickens each. One of the groups served as control (C) and was given a commercial basal diet, without flavonoid supplementation, whereas the other five groups were given the same diet further supplemented with hesperidin at low (75mg/kg of feed) (H1) or high (150mg/kg) (H2) concentration or naringin at low (75mg/kg) (N1) or high (150mg/kg) (N2) concentration or α-tocopheryl acetate (200mg/kg) (E). Plasma samples were collected 4 and 8h after the beginning of flavonoids dietary supplementation and at the end of the experiment (42 days).
NMR based metabolomics using CPMG pulse sequence was implemented in order to elicit information from the plasma. The spectral data was subjected to multivariate data analysis using SIMCA 13 software. In this context, supervised analysis (OPLS-DA) traced variations in the metabolic pattern according to the sustenance consumption which was attributed to specific metabolites with the application of the S-line plot.

EXPERIMENTAL

APPARATUS: Varian 600MHz NMR spectrometer with a 5H [13C – 15N] 5mm PFG Auto ID probe

SAMPLE POOL: 60 chickens 10 control samples, 10 were fed ration enriched with Vitamin E (VE), 20 were fed with ration enriched with Naringin (N) and 20 were fed nutrient enriched with Hesperidin (H).

SAMPLE PREPARATION: 400μL chicken plasma were lyophilized (~5h) + 500μL D2O containing TSP-d4 (1.54 mM) + 100μL D2O-Buffer pH 7.2. Finally the sample was centrifuged at 18.000g at 4°C for 10 min and then was put into a 5mm NMR tube.

SAMPLE ACQUISITION: The CPMG pulse sequence suppressed protein spectral liner (lock solvent= D2O, Spectral width= 7225 Hz (-1 to 11 ppm), PW90= 8.8, SAT PWR= 2, SAT FRQ=-217 to -221Hz, SAT DLY= 2s, d2= 0.001, ln= 8 2, Number of scans= 256, Complex points= 32K). All NMR spectra were phase and baseline corrected, reduced into spectral buckets of 0.001 ppm and aligned, with the use of the MestreNova 8.1 otr.

MULTIVARIATE DATA ANALYSIS: The SIMCA-P 13.0 software was utilized to implement Principal Component Analysis (PCA) and Orthogonal partial least squares discriminant analysis (OPLS-DA). The PCA and OPLS-DA models were extracted at a confidence level of 95%. The quality of these models is described by R2 and Q2 values. The regression has been validated by using misclassification tables and cross validation analysis of variance (CV-ANOVA), with a P-value < 0.05.

METABOLITE IDENTIFICATION

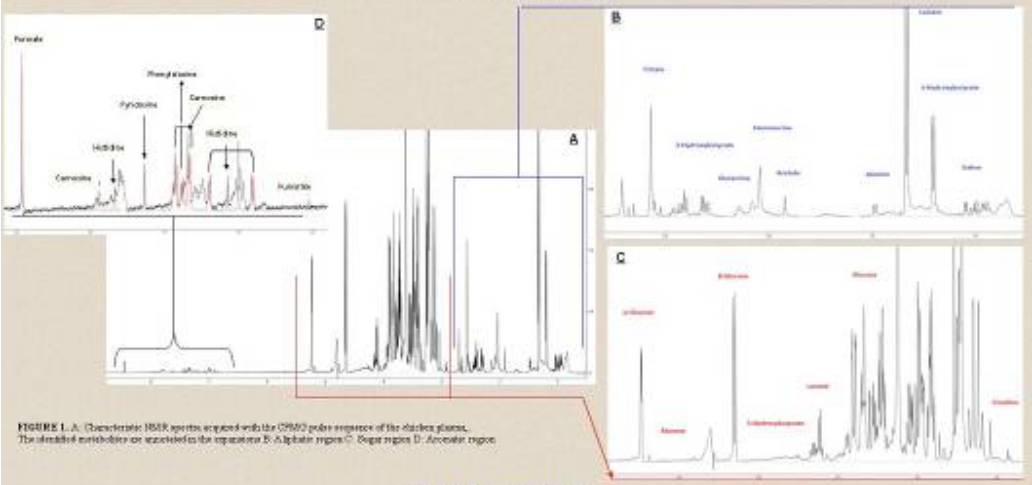

FIGURE 1. A: Characteristic NMR spectra acquired with the CPMG pulse sequence of the chicken plasma. The identified metabolites are assembled in the regions B: Aliphatic region C: Sugar region D: Aromatic region.

MARKER ELUCIDATION

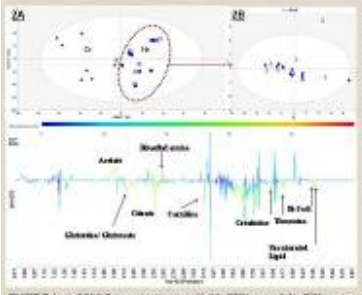

FIGURE 2. A: OPLS-DA model (A= 1+1, R=23, R²(cum)= 0.71, R²Y(cum)= 0.74, Q²(cum)= 0.42) discriminating the Control (Co) from the samples fed Naringin (Ni). B: PCA-class model of the Ni samples depicting the homogeneity among three samples. C: S-line plot highlighting the most important metabolites for the differentiation.

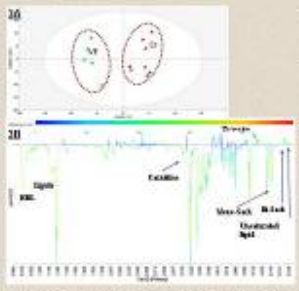

FIGURE 3. A: OPLS-DA model (A= 1+1, R=13, R²(cum)= 0.53, R²Y(cum)= 0.81, Q²(cum)= 0.68) discriminating the Control (Co) from the samples fed Vitamin E (VE). B: S-line plot highlighting the most important metabolites for the differentiation.

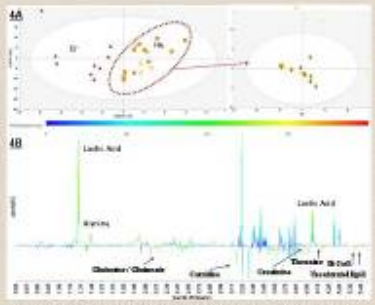

FIGURE 4. A: OPLS-DA model (A= 1+1, R=25, R²(cum)= 0.89, R²(cum)= 0.78, Q²(cum)= 0.43) discriminating the Control (Co) from the samples fed Hesperidin (Hi). B: PCA-class model of the Hi samples depicting the homogeneity among these samples. C: S-line plot highlighting the most important metabolites for the differentiation.

DISCUSSION

Results indicated that similar metabolic patterns were observed irrespective of the type (H or N) and level (H1 or H2, N1 or N2) of the flavonoid incorporated in broiler diet. Moreover, quantification of selected metabolites including aminoacids and low MW organic acids was also attempted using Chenomx software. N and VE samples exhibited the highest homogeneity among these results.
In particular, the metabolic trends of each group as framed by pairwise comparisons of OPLS-DA models probed to:
Control vs Vitamin E: Co samples displayed higher concentration in unsaturated lipids, α-saccharides and threonine, while VE samples were characterized by the presence of HDL, lipids, glucose and carnitine.
Control vs Hesperidin (Hi1 & Hi2): Co samples were characterized by increased levels of α-saccharide, threonine, creatinine, carnitine, unsaturated lipid and glutamine/glutamate, while the Hi samples exhibited higher concentrations of lactic acid and alanine.
Control vs Naringin (Ni1 & Ni2): Co samples also revealed the previous pattern and at the same time appeared to have increased levels of citrate while dimethyl amine and acetate characterized the Ni samples.

Acknowledgment
This work is supported from the Programme THALIS 2012-2015, SECTORAL OPERATIONAL PROGRAMME, EDUCATION AND LIFELONG LEARNING –ESPA 2007-2013

Η Επιτροπή Πιστοποίησης Παραδοτέων

Π. Σιμιτζής Λέκτορας	Μ. Χαρισμιάδου Λέκτορας	Π. Ζουμπουλάκης Ερευνητής