

Πρόγραμμα Θαλής-«Αξιοποίηση Φυσικών Αντιοξειδωτικών στην Εκτροφή των Αγροτικών Ζώων για Παραγωγή Προϊόντων Ποιότητας»

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

Γεωπονικό Πανεπιστήμιο Αθηνών

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

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ABSTRACT

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

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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.



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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

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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 on various flavonoids that are abundant and inexpensive by-products of citrus cultivation, on 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 conventional basal diet, without flavonoid supplementation, whereas the other five groups were given the same diet further supplemented with hesperidin at low (75mg/kg) (H1) or high (150mg/kg) (H2) concentrations or naringin at low (75mg/kg) (N1) or high (150mg/kg) (N2) concentrations or a biochaninA extract (200mg/kg) (E). Plasma samples were collected 4 and 8h after the beginning of flavonoid dietary supplementation and at the end of the experiment (82 days). NMR based metabolomics using CPMG pulse sequence was implemented in order to obtain information from the plasma. The spectral data was subjected to multivariate data analysis using SIMCA 15 software. In this context, supervised analysis (OPLS-DA) traced variations in the metabolic pattern according to the substrate consumption which was attributed to specific metabolites with the application of the S-line plot.

EXPERIMENTAL

APPARATUS: Varian-600MHz NMR spectrometer with a ^1H (13C – 15N) Inveo PFG Probe QNP
SAMPLE POOL: 60 chickens (30 control samples, 10 were fed ration enriched with Vitamin E (VE), 20 were fed with ration enriched with Naringin (N) and 20 were fed with ration enriched with Hesperidin (H)
SAMPLE PREPARATION: 480µL chicken plasma were lyophilized (-50 + 50kPa D2O containing TSP-d4 (0.54 mM) + 180µL D2O Buffer pH 7.1. Finally the sample was centrifuged in 10 000g at 4°C for 10 min and then was put into a clean NMR tube.
SAMPLE ACQUISITION: The CPMG pulse sequence suppressed protein spectral lines (back solvent= D2O, Spectral width= 7225 Hz (-1 to 11 ppm), PWSW= 1-8, SAT PWR= 2, SAT FREQ= 217 to -221Hz, SAT DLY= 2s, 42= 0.001, 10= 8.2, Number of scans= 256, Sample position= 32K). All NMR spectra were phase and baseline corrected, reduced into spectral buckets of 0.081 ppm and aligned with the use of the MestreNova 8.1.01r.
MULTIVARIATE DATA ANALYSIS: The SIMCA-15 software was utilized to implement Principal Component Analysis (PCA) and Orthogonal partial least square discriminant analysis (OPLS-DA). The PCA and OPLS-DA models were validated 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 classification NMR and cross validation analysis of variance (CV-ANOVA), with a F-value < 0.05.

METABOLITE IDENTIFICATION

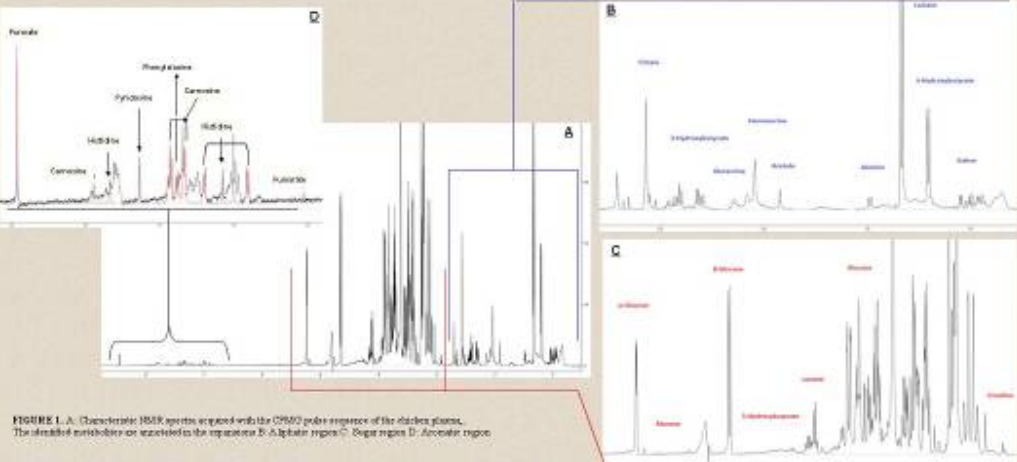
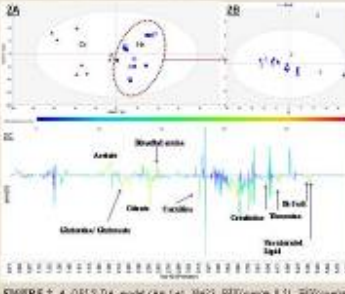
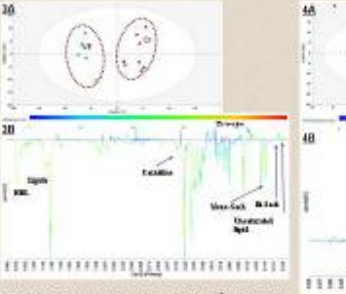


FIGURE 1. A: Overlaid NMR spectra acquired with the CPMG pulse sequence of the chicken plasma. The identified metabolites are annotated in the spectrum. B: Alpha region. C: Beta region. D: Aromatic region.

MARKER ELUCIDATION





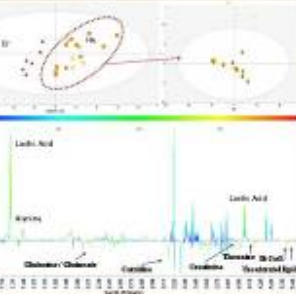


FIGURE 2. A: OPLS-DA model (X=14, Y=23, R²(cum)=0.31, R²(Ycum)=0.54, Q²(cum)=0.42) discriminating the Control (C) from the samples fed Hesperidin (H). B: PCA class model of the H samples depicting the homogeneity among these samples. C: S-line plot highlighting the most important metabolites for the differentiation.

FIGURE 3. A: OPLS-DA model (X=14, Y=13, R²(cum)=0.55, R²(Ycum)=0.81, Q²(cum)=0.48) discriminating the Control (C) from the samples fed Naringin (N). B: PCA class model of the N samples depicting the homogeneity among these samples. C: S-line plot highlighting the most important metabolites for the differentiation.


FIGURE 4. A: OPLS-DA model (X=14, Y=25, R²(cum)=0.59, R²(Ycum)=0.78, Q²(cum)=0.42) discriminating the Control (C) from the samples fed Hesperidin (H) and Naringin (N). B: PCA class model of the H 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, identification of selected metabolites including aminoacids and low MW organic acids was also attempted using Chemnitz software. H and VE samples exhibited the highest homogeneity among these results.

In particular, the metabolite trends of each group as traced by pairwise comparison of OPLS-DA models probed by:

- Control vs Hesperidin (H1 & H2):** Cx samples were characterized by increased levels of 4-sarcosine, threonine, creatinine, carnitine, unsaturated lipid and glucose/glycates, while the Hx samples exhibited higher concentration of lactic acid and alanine.
- Control vs Naringin (N1 & N2):** Cx samples also revealed the previous pattern and at the same time appeared to have increased levels of citrate while identifying oxalic acid and acetate characterized the Nx samples.




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