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# NMR-based metabolomic comparison of chicken meat from different breeds with multivariable analyses (#86)

Hyun Cheol Kim, Hyun Jung Lee, Ji Won Kim, Cheorun Jo

Seoul National University, Department of Agricultural Biotechnology, Seoul, South Korea

## Introduction

Metabolomic approaches have been widely used to elucidate different biological changes in meat and meat products using chemometric analyses. Among them, nuclear magnetic resonance (NMR)-based spectroscopy is one of the leading methods as it is rapid, time-saving, and quantifying metabolites simultaneously without chemical characteristics of target metabolites [1]. In addition, multivariable analysis (MA) using NMR could reflect overall metabolic information, elucidating metabolic changes or differences of the subject [2]. As an example, principal component analysis (PCA) and partial least squares (PLS) are the most popular methods in MA to differentiate between classes in highly complex datasets [3]. PCA is usually used to check the trend in the data, while orthogonal PLS-discriminant analysis (OPLS-DA) was used for classification of the data [4].

Korean native chicken (KNC) is an indigenous breed in Korea which has unique flavor and chewy texture [5]. It generally shows slow growth, therefore, has been developed to increase its productivity, which may possibly affect characteristic meat quality traits of their own [5]. However, their differences were not well-investigated to elucidate meat quality characteristics because of limited information of metabolites [6]. Therefore, in this study, the experiment was performed to see differences in meat between commercial KNC (CKNC), three different newly-developed crossbred KNCs, and commercial broiler using NMR-based metabolomic approach with MA models.

## Methods

*Sample preparation*

CKNC and three different newly-crossbred KNC A, B, and C (KNC-A, -B, and -C, respectively) were slaughtered at 12 weeks old (Harim Co., Iksan, Korea) and commercial broiler (CB; Cobb 500ff), which have similar carcass weight (approximately 1.3 kg) and slaughtered at the same day, was obtained from the same processing plant.

*Sample extraction*

Prior to the extraction, the samples from breast and thigh of each breed were ground and pooled, respectively. Then, 5 g sample was extracted with 0.6 M perchloric acid and lyophilized according to Kim et al. [5]. The extracts was reconstituted by $D_2O$ [20 mM phosphate buffer solution including 1 mM 3-(trimethylsilyl)propionic-2,2,3,3-$d_4$ acid (TSP)] for NMR analysis

*NMR spectrum acquisition*

One dimensional $^1H$ NMR spectra were acquired based on the method from Kim et al. [5]. Each spectrum was recorded on an AVNACE III HD 850 MHz Cryo-NMR spectrometer (Bruker Biospin GmbH, Rheinstetten, Baden-Württemberg, Germany). Spectra were obtained using zg30 pulse sequence with the lock on the $D_2O$ resonance and 128 scans were recorded. Spectra were processed using Topspin 3.5p7 (Bruker Biospin GmbH). The chemical shifts (δ) were referenced to the TSP.

*Multivariable analysis*

Processed spectra were used on an AMIX (Analysis of MIXtures software v.3.0, Bruker Biospin GmbH) for MA. The spectra were bucketed by 0.02 ppm for data clustering from 0 to 9.5 ppm and normalized ($H_2O$ peak was excluded). The multivariable analyses (PCA and OPLS-DA) were processed by Simca 15.0 (Umetrics, Umeå, Sweden). Pathway analysis was performed using MetaboAnalyst (http://metaboanalyst.ca).

## Results

*Multivariable analysis of chicken meat extracts*

Bucketed data from each chicken breeds was acquired and processed into MA (Fig. 1). PCA cannot separated well within the breeds from KNCs. The most noticeable differences was observed between KNCs and CB (Fig. 1a and 1b). Based on the result of PCA, we applied OPLS-DA to distinguish metabolic differences (Fig. 1c and 1d), KNCs were clustered and separated from CB. Especially in the thigh meat, CB, CKNC, KNC A and other breeds (KNC B and C) were well clustered. In general, KNCs and CB had different metabolic characteristics in both breast and thigh.

*Pathway analysis and major metabolites*

To identify the exact metabolic differences, pathway analysis was performed and major metabolism which shows high impact is shown in Fig. 2. β-Alanine metabolism, arginine and proline metabolism, and alanine, aspartate and glutamate metabolism were nominated in common. Additionally, glycine, serine, and threonine metabolism and D-glutamine and D-glutamate metabolism are discriminated between breast meat of KNCs and CB and phenylalanine metabolism for thigh meat. The metabolites related with these major pathways are quantified and listed in Table 1. KNCs shows higher anserine/carnosine content in breast meat and carnosine content in thigh meat. However, other free amino acids of KNCs showed significantly lower than CB.

## Conclusion

NMR-based metabolomic approaches discriminated the chicken meats from KNCs as well as broiler. OPLS-DA analysis separated KNCs and broilers well in both breast and thigh, and following pathway analysis and investigation of relative metabolites could provide clues for differences in biochem

Notes

ical characteristics between breeds. Therefore, this study is meaningful to see the possibility of NMR-based metabolomic approaches in meat science catching the subtle differences using multivariable analyses.

## References


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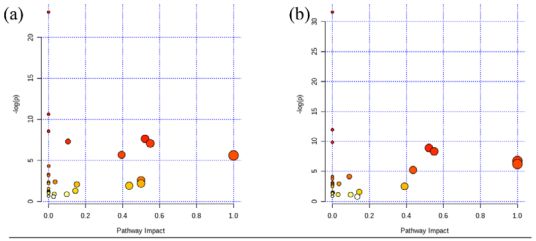
Notes

| Meat Portion | Bucket[a] | Major metabolites | VIP Score[b] | Quantitative intensities | | *P* value |
|---|---|---|---|---|---|---|
| | | | | KNCs | Broiler | |
| Breast Meat | 3.23 | Anserine/ Carnosine | 1.092 | 23.654 | 16.912 | <0.0001 |
| | 2.79 | Aspartate | 1.545 | 0.154 | 0.230 | 0.0243 |
| | 3.29 | Betaine | 1.650 | 3.372 | 7.825 | <0.0001 |
| | 3.05 | Creatine/ Phosphocreatine | 1.352 | 50.387 | 52.390 | 0.1536 |
| | 2.37 | Glutamate | 1.081 | 0.745 | 1.510 | <0.0001 |
| | 2.49 | Glutamine | 1.697 | 0.377 | 0.794 | <0.0001 |
| | 3.59 | Glycine | 1.346 | 1.830 | 2.965 | <0.0001 |
| | 4.29 | Threonine | 1.699 | 1.434 | 1.884 | <0.0001 |
| Thigh Meat | 1.51 | Alanine | 1.506 | 2.198 | 5.004 | <0.0001 |
| | 3.81 | Anserine | 1.507 | 1.829 | 4.067 | <0.0001 |
| | 2.81 | Aspartate | 1.481 | 0.304 | 0.617 | 0.0042 |
| | 4.49 | Carnosine | 1.051 | 5.608 | 4.316 | <0.0001 |
| | 2.37 | Glutamate | 1.578 | 1.263 | 3.055 | <0.0001 |
| | 2.09 | Glutamine | 1.567 | 0.988 | 2.377 | <0.0001 |
| | 7.33 | Phenylalanine | 1.590 | 0.199 | 0.602 | <0.0001 |
| | 7.15 | Tyrosine | 1.396 | 0.076 | 0.179 | 0.0107 |
| | 2.57 | β-Alanine | 1.560 | 0.670 | 1.171 | <0.0001 |

[a]Bucket indicates the integral of NMR spectrum every 0.02 ppm
[b]VIP Score indicates variable importance in projection (VIP) score from OPLS-DA

**Table 1.**
Metabolites related with major pathways discovered by OPLS-DA in breast and thigh meat from Korean native chickens and commercial broiler.




| | Hits[a] | *P* value | Home *P*[b] | Pathway Impact[c] |
|---|---|---|---|---|
| Breast meat | | | | |
| β-Alanine metabolism | 4 | <0.001 | 0.037 | 0.52 |
| Arginine and proline metabolism | 5 | <0.001 | 0.050 | 0.11 |
| Alanine, aspartate and glutamate metabolism | 4 | <0.001 | 0.062 | 0.55 |
| Glycine, serine and threonine metabolism | 4 | 0.003 | 0.248 | 0.40 |
| D-Glutamine and D-glutamate metabolism | 2 | 0.004 | 0.262 | 1.00 |
| Thigh meat | | | | |
| β-Alanine metabolism | 4 | <0.001 | 0.010 | 0.52 |
| Alanine, aspartate and glutamate metabolism | 4 | <0.001 | 0.018 | 0.55 |
| Phenylalanine metabolism | 2 | 0.005 | 0.380 | 0.44 |
| Arginine and proline metabolism | 3 | 0.016 | 1.000 | 0.09 |

[a]Hits represents the number of metabolites in one pathway
[b]Home *P* indicates the statistical *P* values that were further adjusted using the Holm-Bonferroni method for multiple tests
[c]Pathway impact is calculated as the sum of the importance measures of matched metabolites normalized by the sum of the importance measures of all metabolites in each pathway

**Fig. 2.**
Pathway analysis from OPLS-DA using breast and thigh meat of Korean native chickens and commercial broiler.

Notes

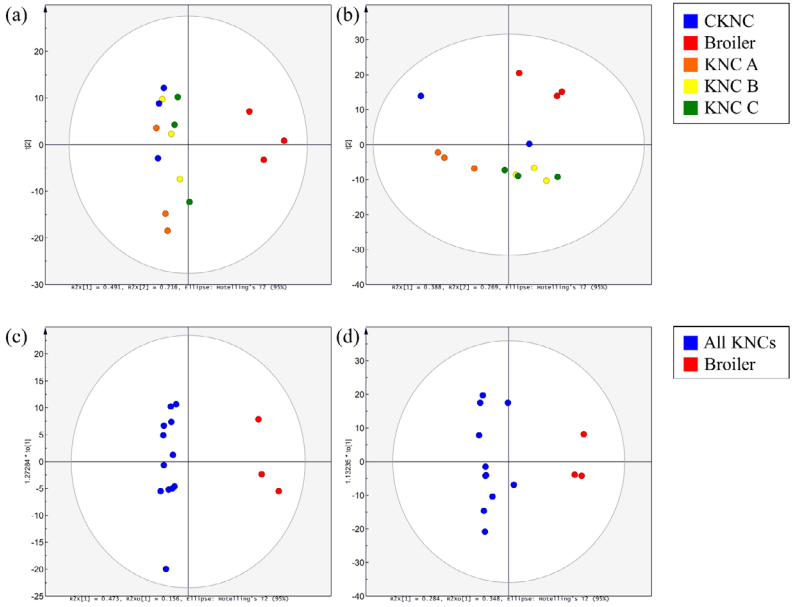



**Fig. 1.**
PCA score plot of (a) breast and (b) thigh meat and OPLS-DA score scatter plot of (c) breast and (d) thigh meat from Korean native chickens.

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