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Short communication

High resolution magic angle spinning NMR spectroscopy reveals that pectoralis muscle dystrophy in chicken is associated with reduced muscle content of anserine and carnosine

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ABSTRACT

Increased incidences of pectoralis muscle dystrophy are observed in commercial chicken products, but the muscle physiological causes for the condition remain to be identified. In the present study a high-resolution magic angle spinning (HR-MAS) proton (^{1}H) NMR spectroscopic examination of intact pectoralis muscle samples (n = 77) were conducted to explore metabolite perturbations associated with the muscle dystrophy condition for the very first time. Both in chicken with an age of 21 and 31 days, respectively, pectoralis muscle dystrophy was associated with a significantly lower content of anserine ($p = 0.034$), carnosine ($p = 0.019$) and creatine ($p = 0.049$). These findings must be considered intriguing as they corroborate that characteristic muscle di-peptides composed of β-alanine and histidine derivatives such as anserine are extremely important in homeostasis of contractile muscles as a results of their role as buffering, anti-oxidative, and anti-glycation capacities.

1. Introduction

Globally there is an increasing demand for poultry meat, which can be ascribed to its attractive nutritional profile. Concomitantly, commercial chicken production is facing increasing challenges with high incidences of abnormalities observed in chicken breast muscles. The abnormalities are characterized by pale and bulging areas of substantial hardness and are referred to as wooden breast. The high incidences of wooden breast has been ascribed to efficient breeding work, which has led to progressive improvements to produce fast-growing broilers with a high proportion of breast meat (Petracci, Mudalal, Soglia, & Cavani, 2015). However, the exact underlying biochemical mechanisms involved in the induction and progression of the muscle abnormalities are far from cracked. Nuclear magnetic resonance (NMR) is an extremely useful technique in food science due to its versatility. High resolution magic angle spinning (HR-MAS) NMR spectroscopy is an appealing analytical technique for semi-solid foods and tissues as it enable measurement on intact samples. HR-MAS NMR studies of a variety of foods and by-products have shown how the technique can provide important information about intrinsic metabolites (Mucci, Parenti, Righi, & Schenetti, 2013; Pereira et al., 2014; Vermathen, Marzorati, Baumgartner, Good, & Vermathen, 2011) and thereby pave the way for new food and nutraceutical products development. In the study of muscle-based tissues, HR-MAS techniques have also shown great promise, in particular in the biomedical field (Diserens et al., 2015; Muench et al., 2015), but also in relation to meat quality (Bertram, Hu, Rommereim, Wind, & Andersen, 2004). On the basis of this, we aimed to investigate the potential of HR-MAS ^{1}H NMR spectroscopy to shed light on the metabolic perturbations associated with muscle dystrophy in chicken pectoralis. Pectoralis muscle dystrophy currently presents a challenge that heavily affects poultry industry as the condition is accompanied by meat quality-deteriorating effects resulting in high incidences of wooden breast (Mudalal, Lorenzi, Soglia, Cavani, & Petracci, 2015). To the best of our knowledge, this study is the first to examine and report the metabolic perturbations associated with muscle dystrophy in chicken pectoralis.

2. Materials and methods

2.1. Chemicals

Deuterium oxide (D_2O) (99.9 D%) containing 3-(trimethylsilyl) propionic-2,2,3,3-d4 acid (TSP) were purchased from Sigma Aldrich (St. Louis, MO). All chemicals used were of analytical grade, and double-deionized water was used throughout.

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2.2. Sampling

At day 21 and 31 post hatching, 200 randomly selected chickens were collected at a commercial chicken farm in Denmark, killed by cervical dislocation, and both pectoralis major removed. Immediately after, the muscles were scored for muscle dystrophy (wooden breast) by palpation of the tissue, regarding hard and compliance muscles as muscle dystrophy/wooden breast as opposed to soft and elastance tissue were regarded normal breasts. Tissue samples were snap frozen in liquid nitrogen and stored at −80 °C until further analysis. At day 21 post hatching, a total of 15 muscles, originating from different birds, were categorized as muscle dystrophy/wooden breast. A total of 23 of the remaining muscles were randomly selected to represent control muscles. At day 31 post hatching the corresponding number were 19 muscles categorized as muscle dystrophy/wooden breast and 20 muscles randomly selected as control.

2.3. HR MAS ^{1}H NMR spectroscopic analyses

Frozen tissue samples (n = 77) of chicken pectoralis major were cut on a dry-ice bench to fit into 30 μL HR-MAS rotor inserts. The inserts were subsequently added D_2O containing 3-(trimethylsilyl) propionic acid-d4 (TSP) and weighed prior to NMR spectroscopy. The inserts were placed in 4 mm zirconium rotors and HR-MAS ^{1}H NMR spectroscopy was carried out at 281 K using a 600 MHz spectrometer (Bruker Avance III, Fällanden, Switzerland) equipped with a ^{1}H/^{13}C/^{31}P MAS probe. All experiments were acquired with a spinning speed of 5000 Hz. One-dimensional (1D) NOESY experiments with presaturation were performed using a recycling delay of 3 s and an acquisition time of 2.25 s. A spectral width of 7288 Hz was employed. 1D CPMG experiments were performed using two different echo times, 50 ms and 400 ms, and a recycling delay of 3 s with an acquisition time of 1.57 s. A spectral width of 10,417 Hz was employed. For all experiments a total of 128 scans were collected into 32 K data points. The spectra were processed with zero-filling prior to Fourier transformation. All spectra were referenced to the internal alanine methyl doublet at 1.47 ppm.

2.4. Data analysis

The NMR spectra were subdivided into 0.01 ppm bins, reducing each spectrum into 950 separate variables in the regions 10.00–5.00 and 4.75–0.5 ppm. Principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were performed in order to identify differences in the metabolite profiles of dystrophic and normal muscle tissue. The OPLS-DA models were cross-validated using segmentation with seven splits. Covariances were investigated by analysis of OPLS-DA regression coefficients back-transformed to original data and color-coded by the loading weights. The multivariate data analysis was performed using SIMCA-P + 14 (Umetrics AB, Umeå, Sweden). Binning and analysis of OPLS-DA plots were performed in MATLAB 2014a (MathWorks Inc., Natick, MA, USA) using in-house developed scripts. Selected metabolites were quantified using Chenomx NMR Suite 8.1.2 (Chenomx Inc, Edmonton, AB, Canada). Statistical significance was evaluated by Student's *t*-test using the Statistics Toolbox in MATLAB 2014a (MathWorks Inc.).

3. Results and discussion

A representative HR MAS ^{1}H NMR spectrum obtained on chicken pectoralis muscle is shown in Fig. 1. A total of 20 metabolites were detected and assigned. Most of these metabolites have also been detected on beef meat (Castejon, Garcia-Segura, Escudero, Herrera, & Cambero, 2015). Multivariate data analysis including PCA and O-PLS-DA revealed that intensities of lipid signals (0.9 and 1.3 ppm) were increased in dystrophic muscles (Fig. 2). Analysis of NOESY spectra (data not shown) showed a similar increase in lipid signals in dystrophic muscles, which suggests that it is the total amount of intracellular lipid that is increased in dystrophic. Multivariate data analysis also showed that variables originating from anserine (2.71 ppm, 3.21 ppm, 4.50 ppm, 7.25 ppm and 8.59 ppm), carnosine (2.69 ppm, 3.21 ppm, 4.49 ppm, 7.25 ppm and 8.55 ppm) and creatine (3.03 ppm and 3.93 ppm) were lowered in dystrophic muscles (Fig. 2). Consequently, muscle content of anserine, carnosine and creatine were quantitated from the HR MAS ^{1}H NMR spectra, and statistical analysis revealed that pectoralis muscle dystrophy was associated with a significantly lower content of anserine ($p = 0.034$), carnosine ($p = 0.019$) and creatine ($p = 0.049$) (Fig. 3). Carnosine and anserine are representing characteristic muscle dipeptides composed of β-alanine and histidine derivatives. As a result of the presence of an imidazole ring in histidine, the dipeptides act as buffers, and because the pK values of anserine and carnosine are close to 7, their proton sequestering capacity is high (Sale, Saunders, & Harris, 2010). The dipeptides also possess

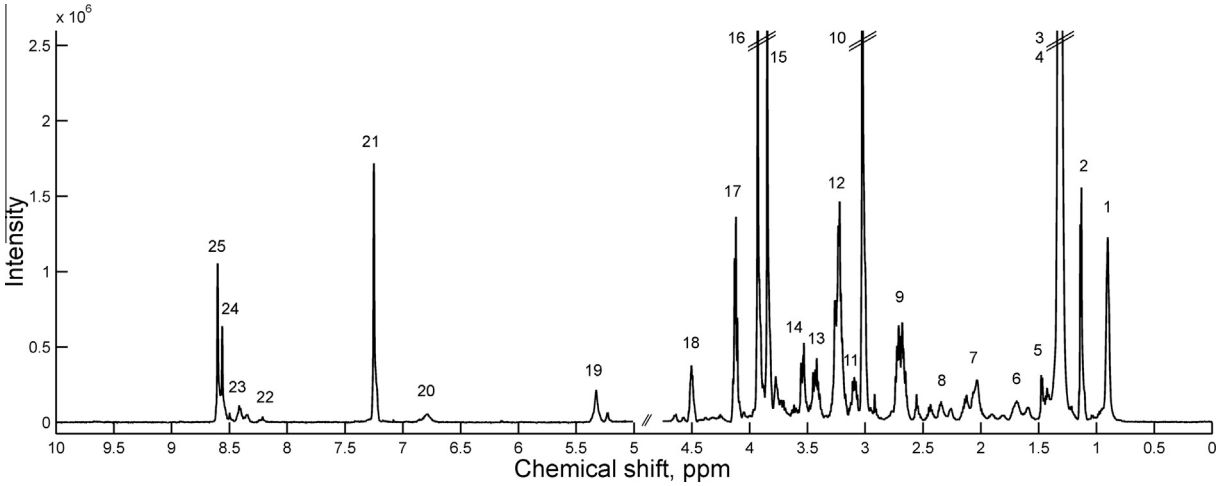

Fig. 1. Representative high-resolution magic angle spinning ^{1}H NMR spectrum of chicken muscle tissue. The spectrum were acquired using a CPMG pulse sequence with a spin echo time of 50 ms. Assignments: 1: CH_3 of fatty acids, 2: 2,3-butanediol, 3: CH_2 of fatty acids, 4: lactate, 5: Alanine, 6: leucine/isoleucine, 7: CH_2 adjacent to CH=CH in fatty acids, 8: carnitine, 9: anserine/carnosine, 10: creatine, 11: choline/phosphocholine, 12: taurine, 13: glycerol, 14: anserine, 15: creatine, 16: lactate, 17: carnosine/anserine, 18: CH=CH in fatty acids, 19: tyrosine, 20: anserine/carnosine, 21: inosine, 22: nucleotides, 23: carnosine, 24: anserine.