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Original Article

The Dietary Inclusion of Chickpea Seeds (Cicer Arietinum L.) Influences the Thermal Properties of Muscle Proteins, But Not the Texture of Drumstick Muscle in Broiler Chickens

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ABSTRACT

In this study, the effect of replacement of soybean meal with raw chickpea seeds as a primary protein source in the diet of broiler chicks on physical properties of drumstick muscle (m. gastrocnemius) was examined. One-day-old Ross 308 broilers (n=160) were fed soybean meal (n=80) or raw chickpea seeds (n=80) as a primary protein source for 42 days. Drumstick muscles, after dissection and storage for 24 h at 5°C, were subjected to texture profile analysis to determine changes in the structural and material integrity, and the thermal stability of muscle proteins were assessed on the basis of differential scanning calorimetry measurements. Light meromyosin, heavy meromyosin and sarcoplasmic proteins in the meat samples of broilers fed raw chickpea seeds were characterized by higher thermal stability as the higher denaturation temperatures were observed. In addition, the calorimetric enthalpy of denaturation of light meromyosin was significantly higher in the muscles of broiler fed raw chickpea seeds, while higher enthalpy of denaturation of actin was determined in those fed soybean meal. Muscle weight, hardness, and the other evaluated textural traits, did not differ between treatments. In summary, dietary protein source affected only thermal stability of muscle proteins; however, the physiological consequences of these alterations are yet to be determined. This study also showed that thermal analysis can be a useful tool for analyzing the effect of nutrition on the development and structural changes in the muscle tissues of poultry.

INTRODUCTION

Chickpea seeds (CPS) is one of the most important grain legumes. CPS have been proposed as alternative protein source in livestock feeding (Bampidis & Christodoulou, 2011). However, information on the effects of CPS-based diets on animal health and growth performance are limited. The dietary inclusion of CPS influenced the mechanical properties of tendons and thermal stability of tendon collagen proteins in broiler chickens (Muszyński *et al.*, 2018a). On the other hand, there was no effect of CPS inclusion on thermal properties of collagen structures of the articular cartilage of the tibia (Muszyński *et al.*, 2018b).

Leg muscles, bones and tendons, as main elements of the musculoskeletal system, provide body support and stability, and give the possibility of movement. The mechanical efficiency of the skeletal muscles depends on the number, diameter, orientation, packing, and type of muscle fibers, which are determined mainly by the lifestyle. The physiological ability of the muscle to produce force depends both on muscle volume, determined by its morphological composition, and influenced by nutritional factors (Rennie *et al.*,

2004), as well as on the biochemical properties of specific muscle fibers (metabolic fatigue and muscle protein breakdown).

Structural properties of raw muscle tissue can be assessed by texture profile analysis (TPA) (Ramírez *et al.*, 2004; Wattanachant *et al.*, 2004). Similarly, differential scanning calorimetry (DSC) has been shown to be an efficient technique to analyze the thermal properties of muscle proteins (Kijowski & Mast, 1988; Wattanachant *et al.*, 2005; Voutila *et al.*, 2009). However, to the best of our knowledge, there are no published studies that applied TPA or DSC techniques to assess the dietary effects on the physical properties of the muscle tissue.

Drumstick muscles are continually involved in maintaining postural stability and movement. In this study, the relationship between thermal properties of proteins forming the muscle tissue, indicating protein stability, and structural properties of the drumstick muscles of broilers fed raw chickpea seeds as a primary source of dietary protein was assessed.

MATERIALS AND METHODS

Birds and management

The experimental protocol was approved by the 2nd Local Ethics Committee at the University of Life Sciences in Lublin, Poland.

Upon arrival, 160 one-day-old male broilers were randomly divided in two treatments, with four replicate pens with 20 birds each. The treatments consisted of diets containing either soybean meal (SBM group, n =80) or raw chickpea seeds (CPS group, n=80) as a primary protein source.

The birds were housed in wire-floored cages, in a force-ventilated, light-controlled poultry house at the Experimental Station of the University of Life Sciences in Lublin, Poland. The animals were kept under standard rearing conditions and air temperature set at the optimal level depending on age according to the Council Directive 2007/43/EC (European Council, 2007). During the first week, the chickens were kept at 33°C, which was reduced by 2°C weekly, until the final temperature of 24°C. Relative humidity cycled from 50 to 60% and light was provided for 23 h per 24 h day/night cycle. From 1 to 42 d of age, all the chickens were provided with fresh water and feed ad libitum, in accordance with the stage of the rearing. The starter (d1-21), grower (d 22-35), and finisher (d 36-42) diets were formulated to meet or exceed the nutritional requirements of broiler chickens (Smulikowska & Rutkowski, 2005), and to be isonitrogenous and isoenergetic (Table 1) (Muszyński *et al.*, 2018b).

On d 42, eight birds per treatment (two birds per replicate) were fasted for 18h, stunned by mechanical methods and then decapitated. The drumstick muscles (m. gastrocnemius) from the both legs were dissected, weighed, and stored at 5°C for 24 h.

Muscle protein thermal stability measurements

Muscle samples were minced with a razor blade. A sample weighing 15-20 mg was placed in 40-μL aluminum DSC pans and sealed. Thermal analysis was performed using a DSC-1 calorimeter (Mettler-Toledo GmbH, Switzerland) at a heating rate of 10°C/min from 20°C to 90°C. An empty pan was used as reference. For each transition peak, onset temperature (Ton), temperature at maximum heat absorption (Tmax), and net enthalpy (ΔH) of denaturation were determined using a software integrated with the calorimeter. Finally, the punctured pans were dried for 24 h at 105°C to determine sample moisture content (Blicharski *et al.*, 2017). One analysis for each muscle sample was performed. Proteins were identified referring to data presented in other studies (Kijowski & Mast, 1988; Wattanachant *et al.*, 2005; Voutila *et al.*, 2009).

Muscle texture profile analysis

A 16mm-thick slab were cut transversely to the fibers from the middle part of muscle. Subsequently, 16-mm diameter cylinders were cut out with a plug cutter. Measurements were performed using a TA-XT2i Texture Analyser (Stable Micro Systems, UK). Seven texture traits were calculated, describing both compression textural parameters (hardness, adhesiveness, springiness and cohesiveness) and tensile properties (fracturability, gumminess and resilience) (Bourne, 2002).

Statistical analysis

The normality of data distribution was tested using the Shapiro–Wilk test. Data with normal distribution were compared by Student's t-test. When variables were not normally distributed, the Mann-Whitney U test was applied. The data were analyzed using Statistica 12 software (TIBCO Software Inc., USA) with $p<0.05$ considered statistically significant.

Table 1 – Composition and nutritive value of the experimental diets.

Ingredient (%)	Starter (days 1-21)		Grower (days 22-35)		Finisher (days 36-42)	
	SBM	CPS	SBM	CPS	SBM	CPS
Corn	10.00	10.00	10.00	10.00	15.00	10.00
Wheat	53.75	21.40	44.91	19.41	35.25	19.95
Soybean meal 46% CP	28.65		21.50	-	19.40	-
Chickpea seeds 21% CP	-	45.00	-	45.00		45.00
Triticale	-	10.00	10.00	10.00	15.00	10.00
Rapeseed meal	-	2.00	4.00	-	5.00	
Soybean oil	2.40	2.40	4.40	4.40	5.20	5.20
Monocalcium phosphate	0.88	0.88	0.83	0.83	0.80	0.80
Limestone	1.35	1.35	1.31	1.31	1.30	1.30
Sodium bicarbonate	0.08	0.08	0.08	0.08	0.08	0.08
Sodium chloride	0.30	0.30	0.27	0.27	0.27	0.27
Fat-protein concentrate	1.00	1.00	1.00	1.00	1.00	1.00
Premix vita-min	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	-	4.00	-	6.00	-	4.70
DL-methionine 99%	0.09	0.09	0.10	0.10	0.10	0.10
L-lysine HCl 78%	0.30	0.30	0.30	0.30	0.30	0.30
L-threonine 99%	0.50	0.50	0.50	0.50	0.50	0.50
Carbovet (90% airy charcoal)	0.20	0.20	0.30	0.30	0.30	0.30
Nutritional value per kg of feed:						
a Metabolizable energy, MJ/kg	12.4	12.5	12.9	13.0	13.1	13.1
b Crude protein, %	21.1	21.2	19.0	19.1	18.0	18.1
b Crude fat, %	4.28	5.21	6.23	8.23	7.09	9.0
b Crude fiber, %	3.12	1.32	3.34	1.23	3.37	1.24
b Total Ca, %	0.93	0.83	0.91	0.82	0.82	0.81
b Total P, %	0.69	0.51	0.69	0.45	0.68	0.44
a Bioavailable P, %	0.44	0.35	0.42	0.34	0.41	0.33
a Total Ca / bioavailable P	2.12	2.32	2.14	2.40	2.17	2.41

[a]calculated values

[b] analyzed values

RESULTS

There was no effect of the diets on drumstick muscle weight (p=0.134) or muscle moisture content (74.11±1.43% and 72.81±1.74% for the SBM and the CPS groups, respectively; p=0.124).

The thermograms obtained with both treatments show three endothermic peaks (Figure 1), which are characteristic of the leg skeletal muscles of poultry (Kijowski & Mast, 1988; Wołoszyn, 2002; Voutila *et al.*, 2009). In the first peak (denaturation of light meromyosin), Tmax and ΔH were lower in the SBM group than in the CPS group (p<0.001, p<0.05, respectively, Table 2). The second peak showed that Tmax of heavy meromyosin/sarcoplasmic proteins was also lower in the SBM group compared with the CPS group (p<0.05). Finally, for the third peak, which corresponds to the thermal denaturation of actin, and possibly of titin, as shown for protein isolated from porcine and bovine muscles (Pośpiech *et al.*, 2002), the enthalpy of actin denaturation was lower in the CPS group than in SBM group (p<0.001).

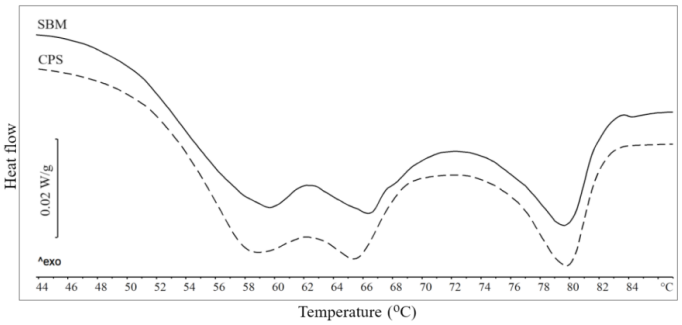

Figure 1 – Example of DSC thermograms (exothermally upwards) of drumstick muscle samples of broilers fed SBM (solid) or CPS (dotted) as primary protein source. Three peaks, corresponding to light meromyosin, heavy meromyosin/sarcoplasmic proteins and actin(from left to right) denaturation can be observed.

No significant muscle textural differences between treatments were detected (p>0.05, Figure 2).

DISCUSSION

It has been shown that the thermal properties of chicken muscles are influenced by genetics, sex, age or different experimental protocols (Wattanachant *et al.*, 2005). In the present experiment, broiler derived from

Table 2 – Quantitative analysis of drumstick muscle thermal data. The values of denaturation onset temperature (T_{on}; °C), maximum temperature of denaturation (T_{max};°C) and enthalpy of denaturation(ΔH; J/g) of drumstick proteins of broilers fed SBM or CPS as primary protein source.

		Group		
		SBM	CPS	*p* value
Peak 1 (light meromyosin)	Ton	52.89±0.25	52.08±0.34	0.076
	T_{max}	57.81±0.13	59.05±0.14	<0.001
	ΔH	0.927±0.11	1.305±0.126	0.014
Peak 2 (heavy meromyosin and sarcoplasmic proteins)	Ton	63.63±0.35	64.13±0.53	0.443
	T_{max}	65.47±0.03	65.87±0.14	0.016
	ΔH	0.845±0.037	0.894±0.040	0.097
Peak 3 (actin)	Ton	76.19±0.13	76.18±0.18	0.973
	T_{max}	79.60±0.05	79.75±0.06	0.095
	ΔH	1.307±0.036	0.914±0.072	<0.001

Values are presented as means (n=8) ± standard error;

Denaturation enthalpy (ΔH) values are normalized for sample dry weight;

SBM: broilers fed soybean meal as a primary protein source; CPS: – broilers fed raw chickpea seeds as a primary protein source.

a same breeder flock, housed in the same conditions, and slaughtered at the same age. The muscle samples were stored in the same conditions, and evaluated using the same experimental protocol. Therefore, the differences in the denaturation patterns of muscle proteins may have resulted from differences in the internal structure of the muscle tissue. The elevated Tmax values of heavy meromyosin/sarcoplasmic proteins in the drumstick of broilers fed CPS suggests additional protection of heavy meromyosin by some heat shock proteins (Ueda *et al.*, 2015; Rios-Mera *et al.*, 2017).

In post-mortem studies, it is not possible to determine directly the mechanical properties of muscles. However, hardness, one of the texture profile traits, is correlated with the mean fiber cross-sectional area and the thickness of peri-and endomysium, parameters with are strictly related to muscle mechanical performance (Lachowicz *et al.*, 2003; Żochowska-Kujawska *et al.*, 2007). In the present experiment, as no differences in hardness and other texture traits were observed, the mean cross-sectional area of muscle fibers and the mechanical strength of muscles were probably the same. However, TPA analysis results should not be considered fully reliable as indications of differences in muscle composition or structure between groups. The texture properties of raw muscles depend not only on the protein fraction (including structure, composition

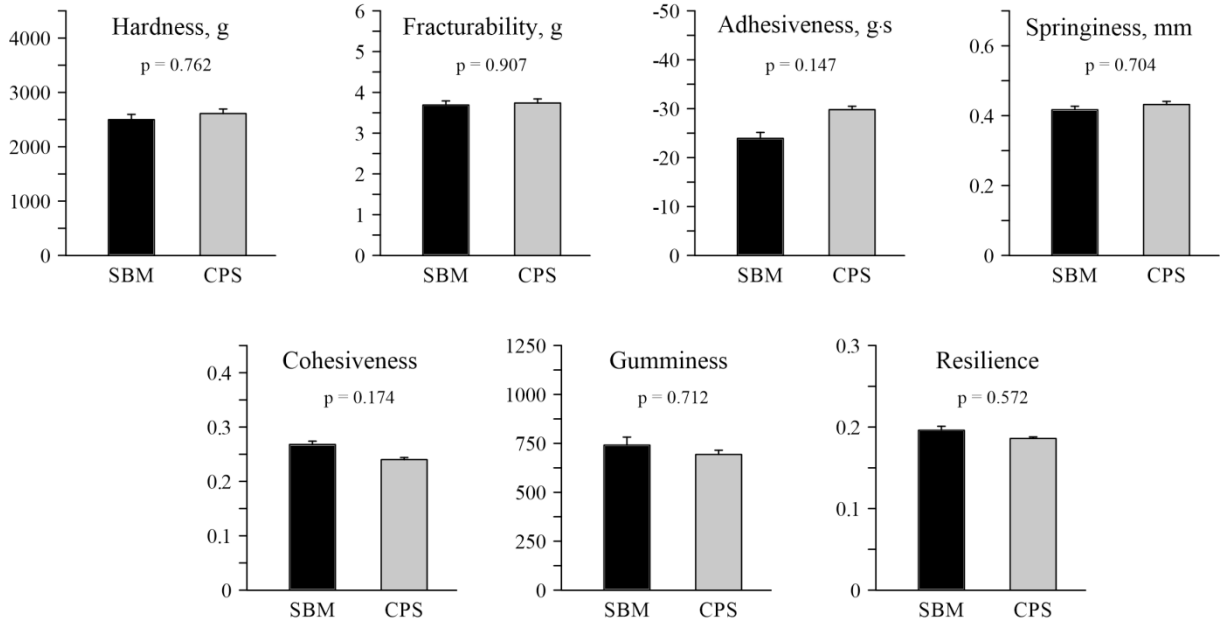

Figure 2 – Results of TPA analysis of drumstick muscle samples of broilers fed SBM or CPS as primary protein source.Values are presented as means ± standard error.

and amount of intramuscular connective tissue or structural properties of intramuscular connective tissue and soluble collagen content), but also on fat and water content, water holding and binding capacity, and enzyme activity (Wattanachant *et al.*, 2004; Bhat *et al.*, 2018). While muscle water content was the same in both groups, water holding capacity, binding capacity, or fat content were analyzed in the present study. Moreover, different seeds used as protein sources may affect lipid fatty acid composition and lipid stability (Cherian *et al.*, 2002). In addition, the disintegration of intramuscular connective tissue influences tenderization of raw meat (Nishimura, 2010). Nevertheless, despite the detected differences in muscle protein composition, these did not affect texture traits in the TPA analyses in the present study.

To the best of our knowledge, this is the first study about the influence of the diet on thermal characteristic of muscle proteins. However, the mechanism by which protein source affected thermal stability of muscle proteins and physiological consequences of such changes remains to be determined. It is unclear whether it could alter muscle contraction and force generation capacities, which directly depend on actin and myosin. It may be a consequence of the differences in the profile of proteinogenic amino acids present in SBM and CPS. Therefore, the main limitation of our study was that the amino acids of the muscle tissues were not analyzed. Moreover, we suggest that in future studies, muscle texture analysis should be complemented with other methods, such as histology, tensile tests or spectroscopic techniques. Nevertheless, we believe that those limitations do not invalidate the originality of our study.

In conclusion, we showed that the thermal properties of chicken drumstick muscle proteins were affected by diet type, although protein source did not influence the textural integrity of the muscle. Furthermore, we demonstrated that DSC can be a useful tool for studying the structural changes in the muscle tissues in poultry.

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