PROTEIN EXPRESSION KINETICS OF CHICKEN BREAST MUSCLE

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Introduction

Chickens (Gallus gallus) selected for meat production have led to establishment of lines with increased growth rate, particularly enhanced growth rate of the pectoralis muscle. A number of disorders have been associated with accelerated growth of this muscle including ascites and pulmonary hypertension syndrome(Julian 1998). The pectoralis muscle comprises of white fast twitch fibers and thus provides a, selectively, simple tissue system to assess protein expression and dynamics. Protein accumulation reflects the balance between two opposing processes of protein synthesis and degradation (Doherty et al. 2004). Korean native chicken is a slow growing broiler, normally reaching 1.2 kg at 10 weeks. This implies that protein expression and deposition differs from a fast growing ones. Keeping this in view, this study was designed to document the expression of proteins and their pattern in pectoralis muscle of chickens at 5 and 10 weeks of age.

Materials and Methods

Five male slow growing Korean native chickens, each from 5 and 10 week group birds were randomly selected; humanely sacrificed and the left pectoralis muscles were collected and stored at -80 for use in further experiment. Protein expression was assessed in the collected pectoralis muscles as per the procedure described by Hwang et al. (2005). MS/MS spectra were generated by nano-ESI on a Q-TOF2 mass spectrometer (Micromass, Manchester, UK). All MS/MS spectra were automatically processed using Bio Analyst QS and searched against the NCBI non-redundant database (February-May 200 versions) using MASCOT 2.0. Proteins were only assigned if, for each peptide ion, ≥ 3 experimentally derived γ ions could be matched to the predicted spectra (with 0.1 Da tolerance) and over three peptides were used in the identification of a specific protein.

Results and Discussion

The primary aim of this study was to know the expression and regulation of proteins in pectoralis muscle of 5 and 10- week old chickens. For this, five 2DE gels were analyzed from each of the two groups in this study. Total 12 protein spots of interest were selected and subsequently sequenced and identified. It was found that 7 and 11 proteins were expressed in the 5 and 10-week muscles respectively. The expression pattern of different proteins in this study is presented in the table 1. Briefly, six proteins were down- regulated (two fold) in 10-week compared to that of the 5-week muscles where as 5 proteins were exclusively expressed in the 10-week muscles and desmin which was present at 5-weeks was conspicuously absent in the 10-week muscles.

The over-expression of the proteins in the 5-week muscles indicate their role during the initial muscle growth but more studies, ranging from first week to five weeks, are needed to confirm their role in growth of pectoralis muscles. Desmin, which plays a role in forming a fibrous network connecting myofibrils, was expressed only in the 5-week muscles indicating its aborted role in the later stages. The exclusive expression of 5 proteins in the 10–week muscles were mainly of myosin heavy isoforms indicating their role in the later stages of muscles growth. During early myogenesis there is increase in proteins associated with myofibrillar structure. Contrary to the findings of Doherty et al (2004) who have reported expression of myosin light chain in the early growing stages in chickens, this study revealed increased expression of myosin heavy chain protein in 5-week old chickens. This might be due to different chicken lines used in the experiments. Other than myosin forms, pyruvate kinase was found to be expressed in the 10-week muscles. Pyruvate kinase is involved in the glycogenesis pathway of muscles (Nelson and Cox, 2002) and its expression indicates increased glycogenesis in the more mature muscle (Doherty et al., 2004). Vimentin expressed in the 5-week muscles were down regulated (two fold) indicating their early role in the muscle growth.. Molero et al (2005) have reported three isoforms of vimentin and these isoforms were found to be independently regulated in their study in bovine smooth vascular muscle cells. Muscle development is also associated with a marked increase in contractile capabilities and this might be the reason for early regulation of some contractile and structural proteins (Doherty et al 2004). Protein DJ-1 was up regulated in 10-week muscles. This protein is protective against oxidative stress and its up-regulation might be due to of more metabolic activity in the 10- week muscles. Some proteins were identified in this study but no specific role could be attributed to them because of paucity of information in the available literature.

Table 1. List of spot number of 2DE SDS-PAGE, estimated molecular weight, pI and spot density for the spots having 2-fold higher or lower density from the fair comparison between postnatal stages of 5 and 10weeks of chickens

spot No.	Identified fragments	NCBI Access No.	C/Q	Estimated MW / pI	Spot density	
					5week	10week
1513	Vimentin	Gi|114326309	25/11	52,979/5.09	39641.3	18551.5
2525	Vimentin	Gi|114326309	28/13	53,396/5.13	70914.4	19290.2
4117	MGC79481 protein	Gi|52345918	6/2	13,585/6.97	22798.2	9711.1
4220	Myosin heavy chain	Gi|13432175	5/9	222,875/5.63	8772.2	24527.7
5018	DJ-1 protein	Gi|17974316	38/5	19,930/6.32	15525.4	38080.5
6102	Hypothetical protein isoform1	Gi|118093267	23/6	30298/6.00	21580.2	9139.8
2311	Desmin	Gi|2959450	5/3	53,247/5.45	20868.0	0
2009	Myosin light chain 1	Gi|127137	25/4	21,851/5.23	0	45106.8
2608	Myosin heavy chain	Gi|13432175	2/5	222,875/5.63	0	35530.9
5327	Myosin heavy chain	Gi|13432175	4/7	222,875/5.63	0	17093.2
6114	Fast myosin heavy chain HCIII	Gi|61657934	2/6	222,833/5.61	0	12627
6208	Pyruvate kinase	Gi|45382651	14/7	57,978/7.29	0	34128.5

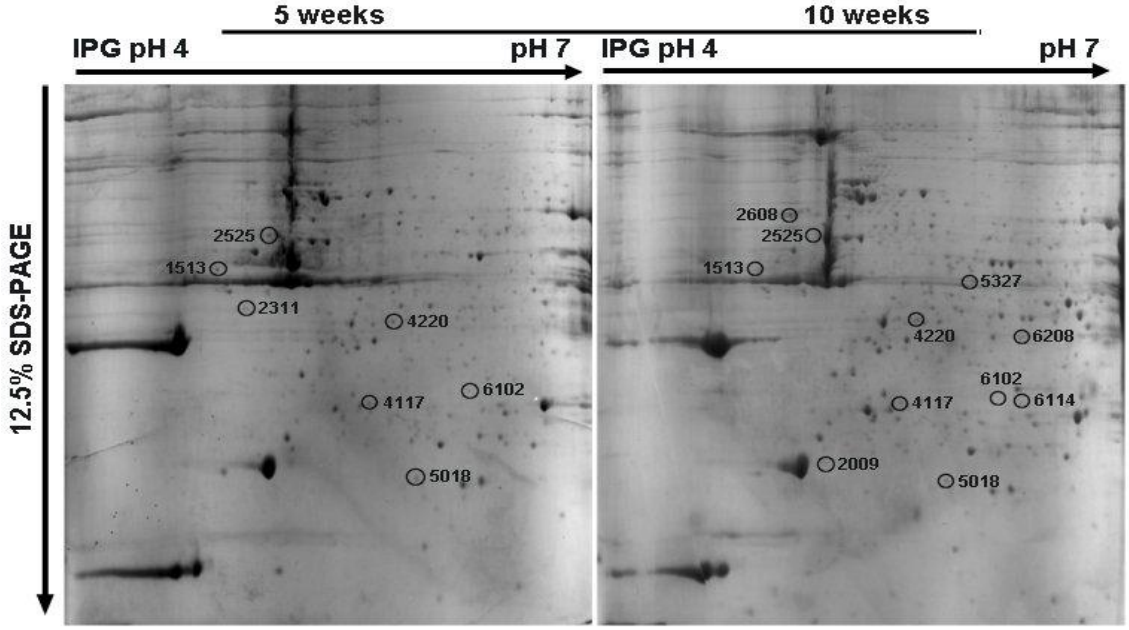

Fig. 1. Representative 2DE-gel at 5 and 10 weeks breast muscle. Proteins numbered are tabulated at Table 1.

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