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Research Paper

Proteomic Studies of Bird Skeletal Muscle Fiber Revealed by Electrophoresis

ZOOLOGY

KEYWORDS : skeletal muscle protein, SDS-PAGE, flight muscles, G. gallus, C.livia and C.coturnix. protein bands.

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ABSTRACT

Proteins are the macromolecules of life, Their functions are the most diverse and gives a unique individuality to each organism. For the present study G. gallus C. livia and C. couturnix of Amravati region were selected and their muscle protein were analyzed by SDS-PAGE , Lamelli (1970) and total protein were estimated by folin phenol method Lowry et al (1951). The muscle proteins of three different birds species were estimated from (flight muscles - pectoralis major , pectoralis minor and leg muscles and it was found that higher concentrations of total protein were found in the three respective regions of Gallus gallus than C.livia and C.coturnix, when it compared with experimental specimens under study. G. gallus showed more protein content 62.71 (mg/g), 64.57 (mg/g) in the flight muscle as compared to C.livia free flying birds. Thus, the present study showed a significant variations in total muscle protein content from selected locomotary organs. Electrophoretic separation of muscle proteins of the selected birds showed inter specific variations. Distinct differences were observed in the intensity of the stained band. All these observations indicate that, the muscle protein electrophorograms can be used for species specificity .

Introduction :

The functions of proteins are the most diverse of any family of macromolecules. All characteristics, nature, and distinctiveness of the proteins gives a unique individuality to each organism . The genes for actin and myosin are members of gene families that encode proteins that enable movement while actin and myosin are highly conserved across all animal species, other muscle proteins show more variability.The variations in an organism's proteins are the results of random DNA mutations within the encoding genes, which have occurred over thousands to millions of years.

Thangaraj and Lipton(2004) identified the species of sea horses *Hippocampus kuda* and *H. trimaculatus* with protein markers in Indo-Pacific region. The sarcoplasmic proteins which account for 20 to 30% of the total protein in fishes have an unique property that the separation profile obtained on electrophoresis can be used for unequivocal identification of fish species with reference of authentic sample profile (Mackie , 1990).Usually fish muscle contains three main groups of proteins (Mackie,1983) which the sarcoplasmic protein component forms an ideal moiety for electrophoretic method of species identification.

To compare protein profiles between organisms, separate the mixture of protein molecules in a particular tissue (such as muscle tissue) by gel electrophoresis. This creates a unique pattern of bands for each organism, called a protein fingerprint. In this investigation use of high resolution polyacrylamide gel electrophoresis is used to know the proteins in the muscle tissues of different birds and to examine them for similarities and differences among the different species.

Materials And Methods :

For the present study, three birds species *Gallus gallus, C.livia and C.coturnix.*were procured from the local market. Birds were stunned by cerebral blow, muscles of anterior most portion of the abdominal region flight muscles and leg region were dissected out from the fresh live birds. Total protein were estimated by Folin- phenol method *Lowry et al* (1951)and analysis of muscle protein were carried out by SDS-PAGE , *Lamelli* (1970) .

Observations And Results :

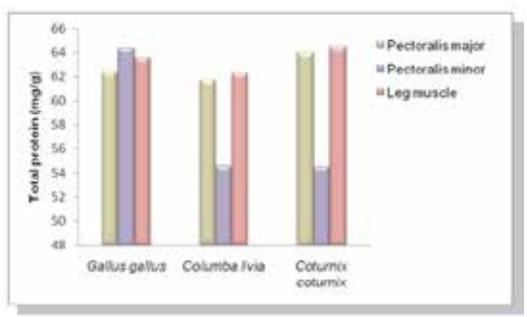

Fig.1.1.

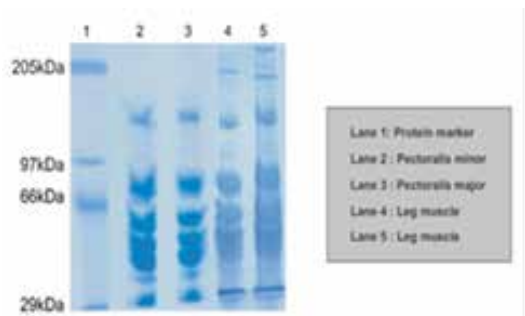

Fig. 2.1 Electrophorogram of striated muscle proteins from different regions of *Gallus gallus*

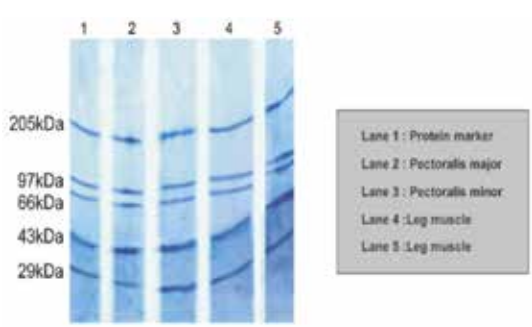

Fig. **2.2** Electrophorogram of striated muscle proteins from different regions of *Columbia livia*

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Table 1.1 showing total protein in different region of the body of experimental animals.

Experimental animal	Pectoralis major(mg/g)	Pectoralis minor (mg/g)	Leg muscle (mg/g)
Gallus gallus	62.71	64.57	63.57
Columba livia	61.71	54.57	62.28
Coturnix coturnix	63.70	54.55	63.45

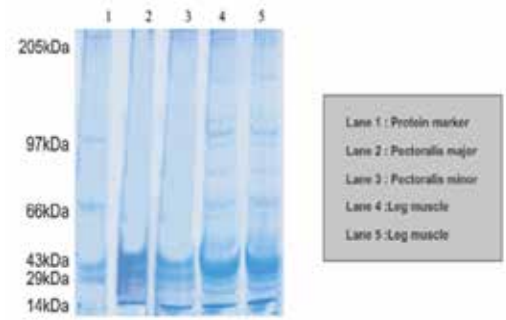

Fig 2.3 Electrophorogram of striated muscle proteins from different regions of Cotournix cotournix

Fig.1.1 shows that *Gallus gallus,* quantity of total protein extracted was 62.71, 64.57, 63.57 (mg/g) from pectoralis major, pectoralis minor and leg muscle while in *Columba livia* it was , 61.71, 54.57, 62.28 (mg/g) and in *Coturnix coturnix* it was recorded as 63.70, 54.55, 63.45 (mg/g) respectively.

In *Gallus gallus* muscle homogenates of pectoralis major resulted into seven bands exhibiting the molecular weights 140, 77, 60, 50, 45, 35, and 30 kilodaltons while in pectoralis minor muscle homogenates showed seven bands having molecular weights 145, 80, 62, 52, 45, 37, 30 kilodaltons. Leg muscle homogenates showed seven bands having 190, 140, 87, 70, 52, 32 kilodaltons molecular weights (Fig.2.1) .

In *Columba livia* pectoralis major muscle homogenates are distributed into four distinct bands having molecular weights 205, 97, 66 ,40 kilodaltons. Homogenates of pectoralis minor showed four bands having molecular weights 205 ,97 ,87 ,45 kilodaltons while leg muscle homogenates electrophoresed into four bands having 205 ,115 ,97 ,52 kilodalton molecular weights(Fig.2.2).

In *Coturnix coturnix*, muscle homogenates of three different organs, pectoralis major, pectoralis minor and leg muscles separated into protein bands which are different from one another . Pectoralis major showed four bands having molecular weights 48, 43, 29, 30 kilodaltons, pectoralis minor showed three bands exhibiting molecular weights 45, 43, 97 whereas leg muscle homogenates revealed eight distinct bands having the molecular weights 205, 175, 120 ,112, 97, 45, 35, 32 kilodaltons(Fig.2.3).

Discussion and Conclusion :

In birds , the muscle proteins were estimated from flight muscles (pectoralis major and pectoralis minor) and leg muscles of *Gallus gallus*, *Columba livia* and *Coturnix coturnix* .Higher concentrations of total protein were found in the three respective regions of *Gallus gallus* than *C.livia and C.coturnix,* when compared with each other. This may be because of high myoglobin content. Flight muscle of *Gallus gallus* and *Coturnix Coturnix* have the highest protein content than the *Columba livia* and these biochemical protein expression might be correlated with activation and relaxation kinetics. *Columba livia* (Pigeon) generally has to fly a longer distance and hence the proteins are utilized more as a source of energy resulting into less available protein of tissue when estimated.

However, *Gallus gallus* and *Coturnix coturnix* are the birds which fly only short distances and do not spend their energy in flying activity and get ready made food to consume. This might have resulted into accumulation of more proteins in their tissue *G. gallus* showed more protein content 62.71 (mg/g), 64.57 (mg/g) in the flight muscle as compared to *C.livia* free flying birds.

Thus, the present study showed a significant variations in total muscle protein content from selected locomotary organs, which proves that, the organs which are used more store proteins. In the present study muscle homogenate separations were carried out from birds. The biological specificity of protein is related to the number of amino acid residues and their sequence along with molecular weight of proteins. Using skeletal muscle protein differentiation in the present study showed that different species had different numbers of bands and it indicates inter specific variations in respect to proteins of different body parts.

Similarity and differences in the molecular weights showed a species characteristics that could be used for species identification.The species specificity of the band patterns revealed prominent variations in banding patterns would be helpful in solving the ambiguous problem related with taxonomy. Electophoresis , a technique that separates proteins on the basis of their ionic charges , permits the study of protein mixtures for taxonomic purposes (Santape,2014). In the muscle homogenates of representative animals of class aves, variations in the relative mobilities of proteins were found in pectoralis major, pectoralis minor and leg muscles. Maximum number of protein bands were estimated from the leg muscles of *C.coturnix* when compared with those of locomotary organs of *G.gallus* and *C.livia* . The proteins which got separated were also with higher molecular weights. Bulbul and Kutrup (2007) demonstrated inter specific differences in the molecular weights of the skeletal muscle myosin, actin ,troponin and tropomyosin in two amphibian species. In the present investigation also intra specific variations in molecular weights of all the striated muscle proteins are recorded , which could be the function related variations ,thus present electrophoretic technique of protein analysis is quite cheaper , time saving and more reliable.

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