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Fiber Growth Dynamics in Biceps Brachii Muscle of Developing Chick in Relation to Somatic Growth Rate

Mayalata Dimpal and Rahul Kundu*

UGC-CAS Department of Biosciences, Saurashtra University, Rajkot-360005, Gujarat, India

ABSTRACT

Present communication reports the fibre orientation in relation to functional activity and the fiber growth dynamics in relation to the somatic growth rate of the *Biceps brachii* muscle of developing male white Leghorn chick. Muscle samples were obtained from the chicks of 7 to 56 days age groups at 7 days of interval. Fresh frozen sections were stained for fiber identification and their morphometrical characteristics. The study tested three hypotheses. Results revealed presence of all three basic fiber types i.e. red, pink and white. Results also showed that the growth of all three fiber types occurred by hypertrophy exclusively. True hyperplasia did not evident as it was possibly seized in the late embryonic stage. The growth dynamics of this muscle in this flightless bird is typically by hypertrophy only. Fiber growth by hyperplasia was not at all evident in any age group. Splitting of large fibers into smaller fibers was evident in some cases of pink and white fibers only. Observed results showed that all three basic fiber types grew by hypertrophy almost exclusively irrespective of their location and functional activities. Muscle fiber growth in this muscle mass was definitely in relation to the somatic growth rate of the chick.

Keywords: Muscle fibers, Growth dynamics; Chick, Biceps brachii, Hypertrophy, Hyperplasia

**** Corresponding author***

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INTRODUCTION

Skeletal muscle consists of red pink and white fiber types in almost all vertebrates [33]. These muscle types are identified on the basis of histochemical staining with myosin ATPase [1, 28]. Their colour is indicative of the level of circulation each muscle types receive [25, 26, 23]. In fish musculature different fiber types are not intermingled but are separated into different muscle masses [40]. Most of the muscles in mammalian and avian species contain a mixture of different types of skeletal muscle fibers [34]. Muscle fibers are adapted morphologically and biochemically to support a specific functional requirement. These fiber types undergo transformation from one fiber type to another according to the functional demand [7] and become different muscles which adapt to specific contractile and metabolic activities required for performance. Fiber growth by hypertrophy is positively correlated to age of the animal [7]. Muscle inactivity is followed by an increase in white fibers [38] and results in muscle atrophy. Many species of birds show annual cycles of atrophy and reconstitution of muscles [5]. In such cases, the pectoralis muscles are catabolized to produce energy and undergo atrophy as a consequence of inactivity.

Muscle is one of highly specialized and organized post mitotic tissue. Previous studies on fishes have shown that muscles grow either by recruitment of fibers or increase in diameter [40]. Muscle growth in fishes begins early in their development and continues their life span [37]. The maximum diameter of fibers is genetically fixed after which the fibers start splitting into several small fibers [18]. In higher vertebrates including Aves where the post-embryonic muscle growth is entirely by hypertrophy the source of additional nuclei are normally derived from myosatellite cells [17]. Skeletal muscle growth in post-hatch birds is determined by hypertrophy and accumulation of nuclei. In avian species post-hatch muscle growth is achieved by an increase in fiber size (hypertrophy), which is associated with an increase in the number of nuclei per fiber [34]. Traditionally described as a two-headed muscle; *Biceps brachii* is the long fusiform muscle of the upper arm on the anterior surface of the humerus, arising in two heads from the scapula. It flexes the arm and the forearm and supinates the forelimb. Also called biceps, biceps flexor cubiti. The aims of the present study were to identify the different muscle fiber types based on their histochemical and histological nature, distribution pattern in Biceps brachii muscle in relation to their functional activities and to study the muscle fiber growth in relation to the somatic development. The results obtained could be useful in animal agricultural science to improve the meat quality as well as quantity by knowing the strategies of growth in skeletal muscles of agricultural animals.

MATERIALS AND METHODS

Male chick (White leghorn strain, “Broiler”), *Gallus gallus* was selected as experimental animal model. *Biceps brachii* muscle with eight age groups (7 days to 56 days at the interval of 7 days) was selected for the study. A total of 32 animals were used for the study. They were obtained from a poultry farm situated in the Rajkot city and maintained in the departmental animal house facilities in iron cage (36"×24"×24") in highly hygienic condition. Growing animals were fed with a poultry starter mash (ingredients-cereal, soybean meal, wheat, grain, corn,

pulses) manufactured by Hindustan lever Ltd., and tap water was always made available *ad libitum*. All experiments were conducted according to ethical norms provided by CPCSEA India *(CPCSEA/CH/RF/ACK-2003).* Required muscles were sampled and mounted on pre-chilled tissue holder and frozen in cryostat at –18˚C. T.S. of around 10-15µm were cut on a Cryostat Microtome and histochemical staining of SDH was done. Muscle fibers were identified by their physiological and histochemical properties according to the method of Lojda [27]. Sections observed under Carl Zeiss Axioscope – II microscope at desired magnification and desired areas of the muscle section were photographed digitally [24]. Since large pink fibers and larger white fibers are not always circular in shape, diameter of each pink and white fibers was measured at least thrice from three different angles and the mean value is taken as standard diameter of that fiber. At least 100 fibers of each fiber type in each myotomal region were measured for their diameter from each size class. All morphometrical measurements were done using the Carl-Zeiss Image Analysis Software and Carl Zeiss Axioscope – II microscope. Collected data were subjected to different statistical analysis like Regression analysis and Correlation Coefficient analysis [35].

RESULTS

Fiber identification

Stained sections when observed under light microscope, the fibers showed variations in color, shape, size, distribution and orientation. The recognition and identification of these fibers was dependent on staining for the oxidative enzyme SDH. Different fibers showed different intensity of color but the three general divisions can be made; red, pink and white. The fibers were increasing in diameter during the growth periods. Red fibers appeared to be more round and smaller than pink and white fibers. Nile blue sulfate staining showed coloration for various kinds of lipids. Phospholipids were stained blue whereas, neutral lipids stained as red droplets. As it appears, the fibre, which are smaller in size and almost round shaped were stained heavily for lipid. The larger fibre were lightly stained. Neutral lipids in the form of red droplets were found only in the interstitial spaces. Glycogen was stained brilliant red and nuclei stained blue. Lipids are present in red fibers while glycogens were found in white fibers.

Fibre Distribution and Orientation

Table: 1 Distribution of fiber types in Biceps brachii muscle of chick

Sr.no	Muscle Name	Fiber types (% frequency)		
		Red	Pink	White
1	Biceps brachii	15.78 ± 2.88	45.77 ± 5.44	38.42 ± 5.40

The result obtained in terms of fibre distribution is presented in (Table 1). From the table it appears that the selected muscle showed higher proportion of pink fibers and white fibers instead if red fibers. Chickens have proportionally more type II (pink) "fast-contracting"

fibers than others. On the other hand, the muscle fibers were found to be oriented in different manner. As a general observation the red fibers were found to be more concentrated in the deeper regions near the bone, the white fibers were found abundantly at the periphery. Pink fibers were found to be scattered randomly within the muscle mass. Results of the histochemical experiment on this muscle, which is located in the forelimb, showed presence of less amount of red fiber (15.78%). Pink (45.77%) and white (38.42%) fibers were the main component of the muscle mass. Both pink and white fibers were large and somewhat rounded in shape. The Biceps muscle is one of the principle muscles of the forelimb in vertebrates. In case of flightless birds, the wings are rarely used. Biceps muscle is mostly composed of white fibers, which is characteristic of sudden and fast movement for short period. Chick, the experimental animal does not exhibit flight therefore the orientation of the muscle fiber in this muscle is corresponding with the locomotors activity of this muscle.

Fibre Growth

The present study on growth dynamics of muscle fibers types i.e. red, pink and white in developing chick indicated distinct variations in different muscle fibers of selected muscle. The diameter of red fibers ranged from 11.80 µm to 31.02 µm in the lowest age class and from 35.13µm to maximum of 106.88 µm in the highest age class in the developing chick. The mean diameter was 21.83 ± 3.70 µm in the lowest age class which increased to 74.91 ± 13.65 µm in the highest age class. The results clearly show an increase in the fiber diameter from lower to higher age class as the animal grows. Hypertrophy or an increase in fiber diameter was clearly evident in the red fibers of Biceps muscle (Table – 2). Red fibers in this muscle showed high frequency values in intermediate diameter modes in all age groups studied. Lower diameter (11-30µm) modes were observed in lower age classes. The frequency of this diameter mode was declined as the shifting of modal frequency values to next higher diameter mode was evident. The maximum frequency was observed in 31-50 µm modes in all age groups. The shifting of modal frequency values of next higher diameter mode was seen in higher age classes. Fiber diameter was increased to its maximum (up to 100 µm) at the age of 35 days and later remained unchanged from 35 to 56 days. It can be concluded that red fibers showed recruitment of small new fibers up to certain age groups i.e. 21 days, after that there is decline in fiber frequency values of lower diameter mode.

There was negative correlation observed between age and fiber frequency values towards higher diameter modes. The result is well supported by regression analysis (Fig.2) with high positive correlation coefficient value (R^2=0.958). The diameter of pink fibers ranged from 14.19 µm to 31.19 µm in the lowest age class and a minimum of 52.21 µm to maximum of 106.17 µm in the highest age class in the developing chick. The mean diameter was 20.57 ± 3.33 µm in the lowest age class which increased to 80.12 ± 12.23 µm in the highest age class. The results clearly show an increase in the fiber diameter from lower to higher age class as the animal grows (Table – 2). In case of pink fibers, there was recruitment of small new fibers in high frequency was observed. Only up to 14 days small (11-20 µm) new pink fibers were seen. Fiber diameter mode of 11-20 µm was not represented in next age groups which is indicative of no recruitment of small new pink fibers. Fiber area was increased to its maximum diameter

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(≥110 µm) up to 42 days of age then it was maintained until its higher age group. Lower age groups represented higher fiber frequency in the range of 21-40 µm while higher age groups represented higher fiber frequency in the range of 51-70 µm. A gradual shifting of fiber frequency values towards higher diameter modes (hypertrophy) was evident in this muscle.

Table– 2. Mean diameter of different fiber types in ***Biceps brachii*** muscle of Chick. Values expressed are in µm. The muscle is showing gradual increase in mean fiber diameter from the subsequent age classes up to 42 days in all the three fiber types. At 49th day it was declined suddenly but again it was risen up.

Fiber Type	Age	Min Diameter	Max Diameter	Mean Diameter ± SD
Red fiber	7th Day	11.80	31.02	21.83 ± 3.70
Pink fiber		14.19	31.19	20.57 ± 3.33
White fiber		13.46	29.67	21.40 ± 3.06
Red fiber	14th Day	12.84	45.18	28.66 ± 6.60
Pink fiber		17.68	42.99	30.45 ± 4.19
White fiber		16.14	53.67	30.57 ± 5.33
Red fiber	21st Day	16.87	45.84	33.78 ± 5.75
Pink fiber		20.88	44.18	32.69 ± 4.36
White fiber		20.76	48.69	33.30 ± 4.10
Red fiber	28th Day	28.51	62.62	43.47 ± 8.23
Pink fiber		25.29	56.03	38.71 ± 5.83
White fiber		27.04	62.67	38.63 ± 5.29
Red fiber	35th Day	34.05	91.46	58.97 ± 10.25
Pink fiber		36.67	67.73	52.78 ± 6.73
White fiber		25.72	77.88	46.07 ± 9.73
Red fiber	42nd Day	39.59	94.09	67.67 ± 11.54
Pink fiber		50.35	100.96	71.92 ± 9.69
White fiber		44.16	86.35	67.77 ± 8.29
Red fiber	49th Day	35.91	81.66	64.86 ± 8.21
Pink fiber		47.70	92.70	66.95 ± 7.68
White fiber		42.30	86.20	59.68 ± 7.65
Red fiber	56th Day	35.13	106.88	74.91 ± 13.65
Pink fiber		52.21	106.17	80.10 ± 12.23
White fiber		42.16	109.54	73.04 ± 12.23

The diameter of white fibers ranged from 13.46 µm to 29.67 µm in the lowest age class and a minimum of 42.16 µm to maximum of 109.54 µm in the highest age class in the developing chick. The mean diameter was 21.40 ± 3.06 µm in the lowest age class which increased to 73.04 ± 12.23 µm in the highest age class. The results clearly show an increase in the fiber diameter from lower to higher age class as the animal grows (Table –2). Recruitment of small new fibers was observed in lower age groups (up to 14 days). Lower age groups represented higher fiber frequencies in the range of 21-30 µm and higher age groups in the range of 61-70 µm. The maximum fiber frequency was observed in 41-50 µm modes in almost

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all age groups. White fibers of biceps muscle showed the fiber growth dynamics extremely by hypertrophy (Fig.1). Shifting of modal frequency values towards higher diameter modes was very high and started early in age class. Very large fiber with diameter around 110 µm was also observed in this muscle. This trend was well supported by regression analysis (Fig.2), where appropriate regression slope was evident (R^2 = 0.9275).

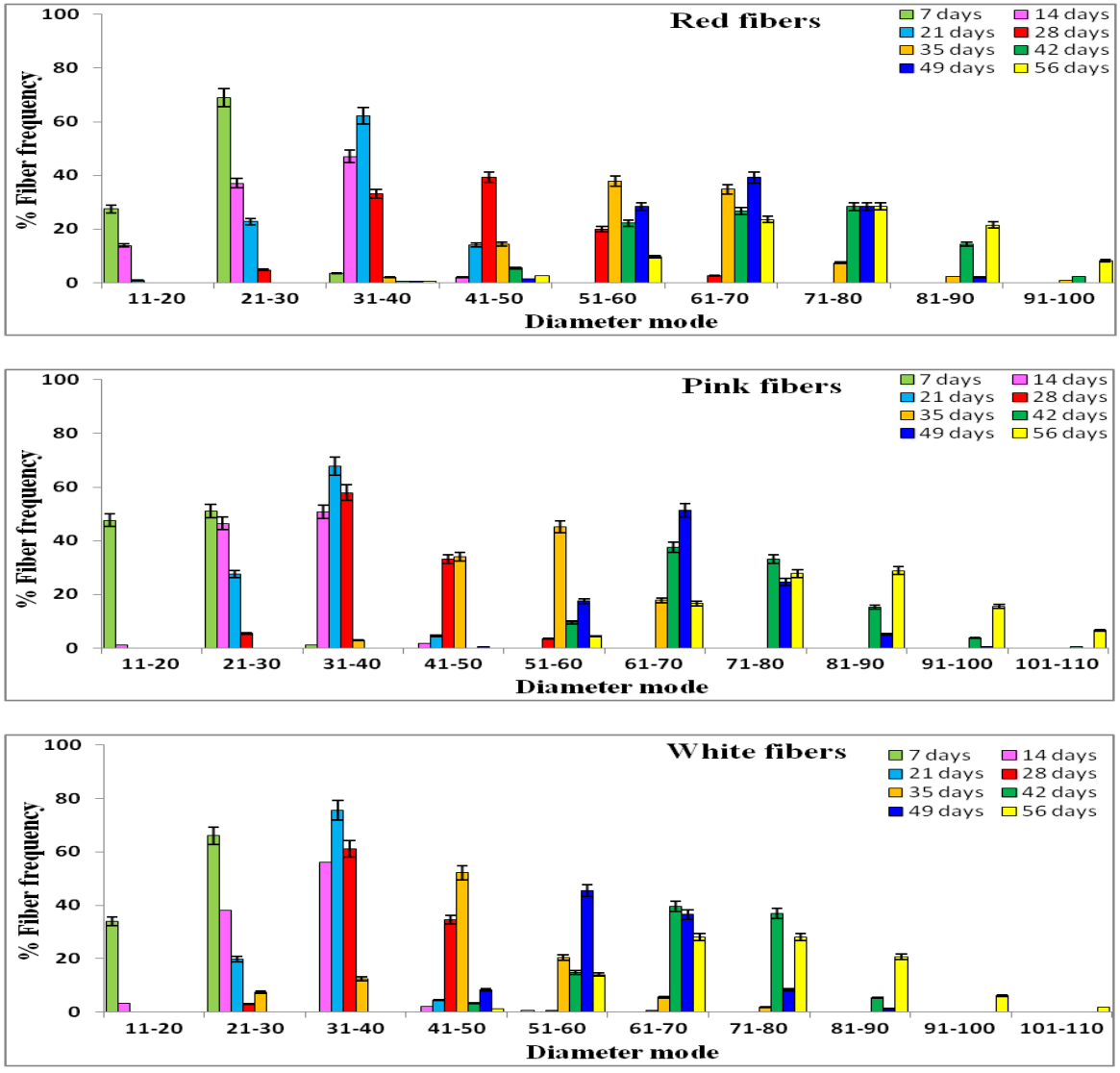

Fig.1. Graphs showing duration dependent percent fiber frequency distribution against diameter modes in red, pink and white fibers of ***Biceps brachii*** muscle of chick. Error bars represent the standard deviation of means.

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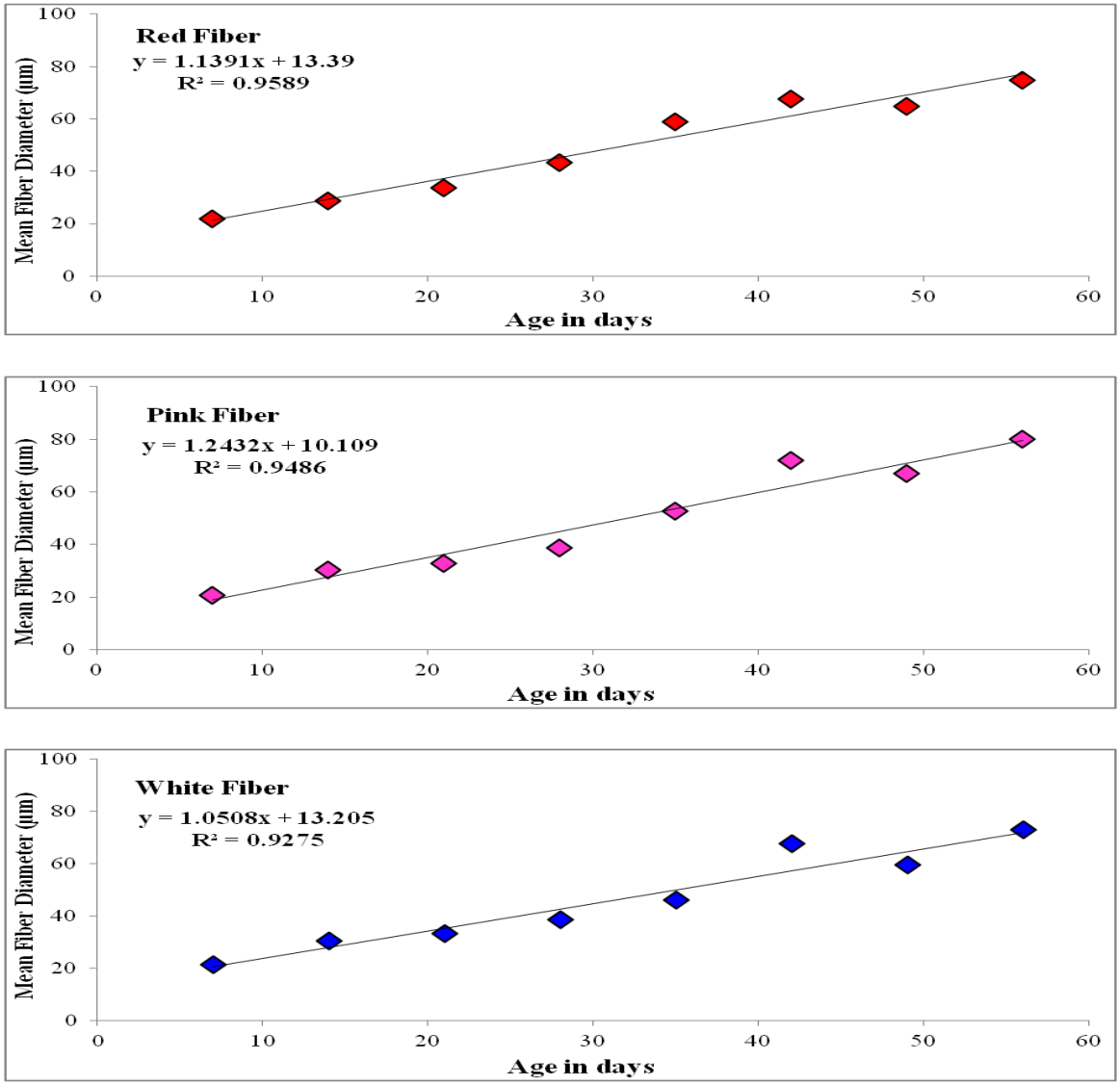

Fig. 2. Regression analysis of red, pink and white fibers of *Biceps brachii* muscle of developing chick. Regression equations and Correlation Coefficient values are given.

DISCUSSION

Fiber identification

In the light of the results obtained from the histochemical localization of lipid, glycogen, SDH and LDH in the present study, three basic fiber types of muscle were identified. The selected muscle in the chick studied, showed the basic pattern found in other species. The smaller fibers having almost round shape was Red fibers or Types I fibers. This was followed by larger Pink fibers or Intermediate fibers. However, the major bulk of most of the muscles was composed of very large irregular shaped White fibers or Type II fibers. These results are in agreement with Peter *et al*. [33], who concluded that red fibers are the smallest ones and pink fibers are intermediate in size while white fibers are the largest ones. The results of the

histochemical experiments showed very strong difference the physiological nature of the three fiber types. The high activity of the oxidative enzyme (SDH) and the low activity of the glycolytic enzyme (LDH) in red fibers indicate the metabolism of this fiber types is mainly aerobic or oxidative. This is well supported by high lipid content. Red fibers are therefore associated with aerobic metabolism using lipid, glycogen and myoglobin as fuel and perform sustained slow contraction [24]. On the other hand, very low SDH activity and a high LDH activity in white are suggestive of a mainly anaerobic metabolism through the glycolytic pathway. Thus the white fibers are associated with anaerobic metabolism using glycogen as fuel and perform burst or fast but rapidly fatiguing contraction. In red fibers oxidative enzyme activity is low and glycogen content is high [36]. In pink fibers oxidative enzyme activity is usually greater [25]. In red fibers high levels of oxidative enzymes like succinic dehydrogenase are present [36]. Similar results were obtained in fish myotomal muscles [32].

Fibre Distribution and Orientation

The obtained results revealed different orientation pattern of the fibers in the muscle mass studied. The muscle mass of the chick was found to be composed of all three basic fiber types with predominance of the pink and white fibers. This suggests an active role of this muscle in the locomotion of the animal as it is capable of all kinds of movements represented by all these fiber types. Muscle size is assumed to be proportional to the size of the whole organism. As such, growth of the muscle is used as an estimate of whole organism growth rate. Chickens and turkeys have proportionally more type II "fast-contracting" fibers. The different appearances of muscles are due to the presence and predominance of different fiber types in the muscles i.e. the red appearance of is due to the presence of more cytochrome and myoglobin [3]; while pale muscles are composed predominantly of white fibers with relatively little myoglobin. Intermediate fibers are usually intermediate in almost all characteristics as well as in color. The color of the muscle depends on the predominance of any fibers but in the fowl no muscle has been described which consists entirely of one or another. Most muscle of the fowl contains all three types of fiber [2,6,9,11,12]. Fiber type composition varies according to the type of animal and muscle and that this is related to the function performed by the muscle. Red fibers are designed for slow body movements and they are significantly present in postural muscles, the musculature of the forelimbs in larger animals appears to be more involved with maintaining a standing position than in smaller animals.

Fibre Growth

The present study on growth dynamics of muscle fibers in developing chick indicated distinct variations in all the fiber types of the muscle studied. The growth pattern was discussed according to the muscle fiber types in relation to somatic growth of the species selected for the study.

Red fibers

In early stage the growth was by hyperplasia as well as hypertrophy but at the later stages the growth was by hypertrophy and hyperplasia resulted by splitting of larger existing fibers or activation of myosatellite cells as there were few small fibers in higher age class. There was negative correlation observed between age and fiber frequency values towards higher diameter modes. The result is well supported by regression analysis (Fig.2) with high positive correlation coefficient value (R^2=0.958). In the present investigation, it is clear that the main mode of growth of red fibers is mainly by hypertrophy [40]. However, hyperplasia also plays an important role in lower age classes [19].

Pink fibers

The growth dynamics of pink fibers showed similarity with that of red fibers [20,22]. From the regression analysis it is clear that hypertrophy took the major role in the fiber growth (Fig. 2). The almost cessation of recruitment of small new fibers and almost similar frequency values in several higher diameter modes indicating that the hypertrophy of existing fibers was to be the most suitable means of the growth in the all age classes studied [15, 18]. The obtained result is well supported by the regression value (R^2 = 0.9486).

White fibers

It appears from the Fig. that the recruitment of small new white fibers was seen in lower age classes and hypertrophy was observed in almost all size classes. It is indicative of the growth principally by the method of hypertrophy [18]. White fibers showed an extended array of fiber diameter with peak diameter modes. This is suggestive of the splitting of large fibers into smaller ones [28]. This trend was well supported by regression analysis (Fig.2), where appropriate regression slope was evident (R^2=0.9275).

It is evident from the present investigation that the modes of growth of red, pink and fibers are mostly by hypertrophy only. However the frequencies of some intermediate diameter modes were high in all age classes. Moreover, the white fibers showed an extended array of fiber diameter with peak diameter modes in all three fiber types. This is suggestive of the splitting of larger fibers into smaller ones (Mascarello *et al*., 1995). The addition of persistent myoblasts or myosatellite cells [19, 14] also attributed towards the overall growth and development of muscle fibers in all the selected muscles [13, 10]. As it appear from present investigation that the growth dynamics of all three fiber types in the Biceps muscle is by hypertrophy exclusively [31]. The recruitment of small new fiber is not at all evident. The growth dynamics of this less used muscle is typical with growth by muscle fiber diameter only.

Increase in red and pink fiber area in physically active individuals has been reported in mammals [7] and birds [30, 5]. Thus, the increased hypertrophy of the fibers from 28 days of age in the selected muscle of broilers may be related to higher muscle activity during the experiment, the broilers interacted more with each other. Our results showed increase in the

diameter of the red, pink and white fibers only until 42 days of age. This absence of hypertrophy from 42 to 56 days of age for the three types of fibers indicate that the muscle fibers reached their maximum growth at 42 days but why the hypertrophic growth of the muscle fibers stopped during 42 to 49 days of age remains to be understood. Similar growth pattern was observed in Pectoralis major and Sartorius muscles of broilers [29] where growth of muscle fibers were assessed in response to enclosure sizes. There was no effect of enclosure sizes on growth of muscle fibers.

It has been observed that the diameter of the muscle fibers of broilers were increased until 42 days of age and decreased from 42 to 49 days of age which clearly demonstrates muscle atrophy at this period but there was a sudden and tremendous increase in their diameter. According to Urso *et al.* [38], lack of physical activity causes decrease in the protein accretion in the muscle extracellular matrix. Therefore, the absence of hypertrophy from 42 to 49 days of age for the three types of muscle fibers and the simultaneous reduction in the diameter of the muscle appears to indicate that the atrophy in the muscle involved reduction in extracellular connective tissue. Considering that the slaughter age for the broiler line used in this experiment is 42 days of age, the results showed that food and space competition in a congested place and may have influenced the performance if the broilers were raised for a longer period. Considering that lack of exercise might lead to muscle atrophy and vice versa [4] the results in our current experiment suggest that the atrophy seen in broilers is a result of the low physical activity that was a consequence of the small space available for locomotion.

CONCLUSIONS

The present study revealed the presence of three basic fiber types red, pink and white in the selected muscle mass. The growth of the muscle was found to be exclusively by hypertrophy only which increases the muscle mass by increase in diameter of the fiber type. As the selected animal is one of the major agricultural animal the data can be used to improve the quantity as well as the meat quality of the animal.

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