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MUSCLE FIBERS CHARACTERIZATION AND GROWTH DYNAMICSIN GASTROCNEMIUS MUSCLE OF DEVELOPING WHITE LEGHORN CHICKIN RELATION TO SOMATIC GROWTH

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ABSTRACT: Present communication reports the fiber identification, fiber orientation in relation to functional activity and the fiber growth dynamics in relation to the somatic growth rate of the Gastrocnemius muscle of developing male white Leghorn chick. The study tested three hypotheses; a) muscle fibers typing on the basis of histochemical nature are similar in the muscle mass, b) distribution pattern of different muscle fibers in muscle mass are in relation to the functional activities of that particular species, c) fiber growth dynamics are related to the somatic growth rate of that species. Results of the histochemical studies revealed presence of all three basic fiber types i.e. red, pink and white which confirms the first hypothesis. Results also showed that the growth of all three fiber types occurred by hypertrophy exclusively. True hyperplasia did not evidentin any age group; it was possibly seized in the late embryonic stage. 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.
Key words: Muscle fibers, Histochemical nature, Fibre growth dynamics, Chick.

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INTRODUCTION

White Leghorn fowl is reared in the poultry firms worldwide, and known for its superior meat quality in shortest time. Muscle is one of the highly specialized and organized post mitotic tissues. Skeletal muscle is the main contributor to body mass and size in almost all vertebrates. The major components of muscles are muscle fibers which are extremely specialized cells acting as thestructural units of skeletal muscle [1]. It is well known that muscle fiber number, size, and fiber-type composition are closely related to each other [2]. These fiber types are identified on the basis of histochemical staining with SDH [3]. Their colour indicates the level of circulation each muscle type receives [4, 5,6,7]. In fish musculature fiber typesare separated into different muscle masses [8], while mammalian and avian muscles contain a mixture of different fibers types [9]. Studies show that muscle fibers are adapted morphologically and biochemically to fulfill specific functional requirement. Fiber growth by hypertrophy is positively correlated to age of the animal and further these fiber types may undergo transformations from one fiber type to another according to the functional adaptability [10]. Muscle inactivity is followed by an increase in white fibers which results in muscle atrophy [11]. Many species of birdsshow annual cycles of atrophy and reconstitution of muscles [12]. In such cases, some muscles are catabolized to produce energy and undergo atrophy as a consequence of inactivity. Previous studies on fishes have shown that muscles grow either by recruitment of new fibers or increase in diameter to existing fibers [8]. Muscle growth in fishes begins early in their development and continues throughout their life span [13].

The maximum diameter of fibers is genetically fixed after which the fibers start splitting into several small fibers [14]. 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 [15]. 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 [9].

Traditionally described as the most superficial calf muscle in the posterior part of the leg; the gastrocnemius is located with the soleus in the posterior (back) compartment of the leg. It joins the tendon of the soleus as part of the tendo-calcaneus with nerve supply from the tibial nerve, and whose action causes plantar-flexion of the foot. Some anatomists consider both to be a single muscle, the triceps surae or "calf muscle", since they share a common insertion via the Achilles tendon. The aims of the present study were to identify the different muscle fiber types based on their histochemical and histological characteristics, distribution pattern in Gastrocnemius 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 biomedical science, sports science as well as animal agricultural sciences to improve the meat quality and the quantity by knowing the growth strategies of skeletal muscles of agricultural animals.

MATERIALS AND METHODS

Male chick (White leghorn strain, “Broiler”), *Gallus gallus* was selected as experimental animal model. *Gastrocnemius* musclefromeight age groups of animals (7 days to 56 days at the interval of 7 days) was selected for the study. A total of 32 animals were used forthe study. They were obtained from a poultry farm situated in the Rajkot city and were maintained in the departmental animal house facilities in iron cage (36"×24"×24") and in highly hygienic conditions. Growing animals were fed with a poultry starter mash (ingredients-cereal, soybean meal, wheat, grain, corn, pulses) manufactured by Hindustan lever Ltd., andtap water was always made available *ad libitum*. All experiments were conducted according to ethical norms provided by CPCSEA India (757/03/a/CPCSEA). Required muscles were sampled and mounted on pre-chilled tissue holders and frozen in cryostat at –18˚C to -20^{o}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 [6]. Sections observed under microscope at desired magnification and desired areas of the muscle section were photographed digitally. 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 [16]. At least 100 fibers of each fiber type from each possible 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 [17].

RESULTS

Fiber identification: Stained sections showed variations in color, shape, size, distribution and orientationwhen observed under light microscope. 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 could be made i.e. red, pink and white. The fibers were increased in diameter during the growth periods. Red fibers appeared more round and smaller than pink and white fibers. Nile blue sulfate staining showed colorationfor various kinds of lipids. Phospholipids stained blue whereas, neutral lipids stained as red droplets and were found only in the interstitial spaces. The fibers, which are smaller in size and almost round shaped were stained heavily for lipid. The larger fibers were stainedlightly. Glycogen was stained brilliant red and nuclei stained blue. Lipids werefound to be associated more with red fibers while glycogens were found in white fibers only.

Fiber Distribution and Orientation:

Distribution: The relative proportions of red, pink and white fibers inthe selected muscle ofchick were investigated. The results obtained are presented in (Table-1). From the Table, it appears that the selected muscle showed higher proportion of pink fibers followed by white fibers and least red fibers. Chickens have proportionally more type II or pink fibers than others. The muscle fibers were found to be oriented in different manner. As a general observation, the red fibers were found to be concentrated more in the deeper regions near the bone while 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 hind limb muscleshowed the presence of less amount of red fiber (19.55%). Pink (49.19%) and white (31.19%) fibers were the major component of the muscle mass.

Both pink and white fibers were large and somewhat irregular in shape while red fibers were almost rounded in shape. The calf muscle is one of the principle muscles of the hindlimb in vertebrates; it's very much active and powerful. In case of flightless birds the animals generally use to be in standing position lot of the time and sometimes walking and running. Gastrocnemius muscle is mostly composed of white fibers in most vertebrates, 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 selected muscle corresponds with the locomotors activity of this muscle.

Fibers diameter variation and Growth:

Red fibers: The present study indicated a distinct variation in different muscle fibers of selected muscle (Fig.1). The diameter of red fibers ranged from 13.07 µm to 33.85 µm in the lowest age class and from 33.27µm to maximum of 93.75 µm in the highest age class in the developing chick. The mean diameter was 23.77 ± 4.44 µm in the lowest age class which increased to 63.87 ± 11.84 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 Gastrocnemius muscle (Table – 2). Red fibers showed successive shifting of diameter modes from lower to higher values throughout the age group. With little fluctuation fibers grew to their extreme value (up to 100 µm) at the age of 42 days. Mean fiber diameter is dependent on the age (R^2 = 0.977). After that these values were remained unchanged throughout the age group, only the intermediate fiber frequency values increased throughout the successive age groups. The frequency values in intermediate diameter modes were observed very high which declined with age. Diameter mode of 31-40 µm was represented in all age groups (Fig. 1).

Pink fibers: Almost similar trend was observed in case of pink fibers. Recruitment of small (11-30 µm) new fibers was observed in lower age classes. Maximum fiber frequency was observed in 31-50 µm modes in almost all age groups (Table – 2). There is continuous increase in mean fiber diameter with the age but fiber frequency values decrease towards higher diameter mode with the advance of age (Fig. 1). Hence, we can say that age and mean fiber diameter have strong positive correlation (R^2 = 0.991) while fiber frequency and age have negative correlation. Here the fibers grew continuously according to the age i.e. the fiber with highest diameter mode (121-130 µm) was found at the highest age (56 days) class. The frequency values in intermediate diameter modes were observed very high. Higher age classes represented almost all range of fiber diameter modes except few smaller ones (Fig.1).

White fibers: Similar trend of growth was observed in white fibers also as seen in pink fibers. Higher age classes found to possess almost all diameter modes except a few smaller ones (Table – 2). Diameter modes were increased successively throughout the age groups i.e. there was a high correlation between age and growth (R^2 = 0.980). Lowest age group represented lowest diameter modes as well as highest diameter mode was represented by highest age class. From 35 days, onward the fiber diameters were increased and maintained with the increase of intermediate diameter modes. White fibers in this giant muscle were found to contain fibers of highest diameter in the range of 131-140 µm (Table – 2). The frequency values were high in moderate diameter modes of 31-60 µm (Fig. 1).

DISCUSSION

Fiber identification:

Histochemical localization of lipid, glycogen, SDH and LDH in the present study, revealed three basic fiber types of muscle. The selected muscle in the chick studied, showed the basic pattern found in other species. The smaller fibers having almost round shape were identified as Red fibers or Types I fibers. This was followed by larger Pink fibers or Intermediate fibers. However, the major bulk of most of the muscle was composed of very large irregular shaped White fibers or Type II fibers. These results are in agreement with Peter *et al*., [18], 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 whichis well supported by high lipid content. Red fibers are therefore associated with aerobic metabolism using lipid and myoglobin as fuel and perform sustained slow contraction [16]. On the other hand, very low SDH activity and a high LDH activity in white fibers are suggestive of mainly anaerobic metabolism through the glycolytic pathway using glycogen as fuel and perform burst or fast but rapidly fatiguing contraction. In red fibers, high levels of oxidative enzymes like succinic dehydrogenase are present [19]. In pink fibers, also oxidative enzyme activity is usually greater [4]. Similar results were obtained in fish myotomal muscles also [20].

Fiber Distribution and Orientation:

Obtained results revealed different orientation pattern of the fibers in the studied muscle mass. 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 hence; growth of the muscle is used as an estimate of whole organism's growth rate. Chickens and turkeys have proportionally more type II fibers. The different appearances of muscles are due to the presence and predominance of different fiber types in the musclesi.e. the red appearance is due to more cytochrome and myoglobin [21], while pale muscles are composed predominantly of white fibers with relatively less myoglobin. Intermediate fibers are intermediate in almost allrespect. But in case of fowl no muscle has been described which consists entirely of one or another i.e. most muscle of the fowl contains all three types of fiber [22]. Fiber type composition varies according to the type of animal and to the function performed by the particular muscle. Red fibers are designed for slow and sustained body movements hence they are significantly found to be present in postural muscles while the musculature of the forelimbs in larger animals appears to be more involved with maintaining a standing position than in smaller animals.

Diameter variation and 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: Recruitment of few small new fibers (hyperplasia) was observed in early stage and thereafter increases in diameter (hypertrophy) of existing fibers throughout the age groups were observed in successive stages. Fiber frequency values declined towards higher diameter mode with increase in age which shows a negative correlation between age and fiber frequency values towards higher 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 ($R^2 = 0.977$). In 35 days, there was recruitment of few small fibers which might be due to splitting of existing larger fibers or activation of residing progenitor cells (Fig. 2). In the present investigation, it is clear that the main mode of growth of red fibers is mainly by hypertrophy. However, hyperplasia also played an important role in lower age classes. The relative importance of hypertrophy and hyperplasia to muscle growth varies markedly during ontogeny in various vertebrates to muscle growth and development declines throughout the juvenile and the adult, stages and in some species of fishes ceases at around 100% the maximum body length [8,16, 23].

Pink fibers: The growth dynamics of the pink fibers showed certain similarity with that of red fibers. A gradual shifting of fiber frequency values towards next higher diameter modes was evident in this muscle which is indicative of growth by hypertrophy (Table – 2). Hence, we can say that age and mean fiber diameter have strong positive correlation ($R^2 = 0.991$) while fiber frequency and age have negative correlation. Small new fibers observed at higher age group are may be the result of budding and splitting of larger fibers. Pink muscle appears to be intermediate between red and white muscle [23,24].

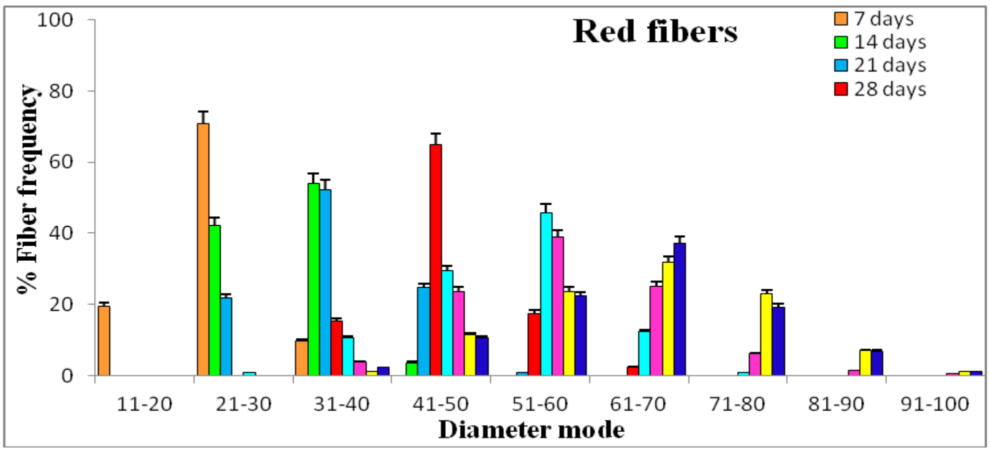
White fibers: It appears from the results (Fig. 2); recruitment of small new fibers was observed in high frequency in the lower age group and some recruitment was also observed in extremely higher age groups. Diameter modes were increased successively throughout the age groups i.e. there is perfect correlation between age and growth ($R^2 = 0.980$). The shifting of modal frequency values towards higher diameter modes was prominent in higher age groups. This is indicative of an increase in fibers having medium diameter, which is produced by splitting of existing large fibers that reached to a critical diameter [14, 25].

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

Sr.no	Muscle Name	Fiber types (% frequency)		
		Red	Pink	White
1	*Gastrocnemius*	19.55 ± 2.47	49.19 ± 2.48	31.19 ± 0.62

Table–2: Mean diameter of different fiber types in *Gastrocnemius* muscle of Chick. Values expressed are in µm.The muscle is showing gradual increase in mean fiber diameter from the subsequent age classes upto 42 days in all the three fiber types. Thereafter there is a decline upto 56 days.

Fiber Type	Age	Min Diameter	Max Diameter	Mean Diameter ± SD
Red	7th Days	13.07	33.85	23.77 ± 4.44
Pink		17.00	35.95	26.37 ± 4.36
White		13.96	35.07	23.58 ± 4.81
Red	14th Days	22.63	44.11	31.54 ± 4.03
Pink		20.09	45.49	36.78 ± 3.98
White		22.36	53.85	37.19 ± 4.86
Red	21st Days	22.30	50.07	35.83 ± 6.56
Pink		24.23	62.13	40.28 ± 5.32
White		22.04	54.37	40.11 ± 4.71
Red	28th Days	32.03	68.65	45.84 ± 5.98
Pink		33.25	59.24	46.50 ± 5.34
White		28.76	79.68	47.09 ± 7.42
Red	35th Days	28.19	71.79	50.80 ± 8.34
Pink		39.54	78.88	55.02 ± 7.23
White		39.57	75.80	54.70 ± 8.08
Red	42nd Days	35.73	90.84	56.46 ± 9.87
Pink		40.27	87.41	61.41 ± 8.92
White		36.51	89.74	62.75 ± 13.67
Red	49th Days	37.20	96.42	64.44 ± 11.75
Pink		44.07	111.67	71.80 ± 15.10
White		42.56	120.05	75.33 ± 17.96
Red	56th Days	33.27	93.75	63.87 ± 11.84
Pink		35.75	136.18	78.54 ± 19.70
White		28.00	131.28	76.21 ± 22.16



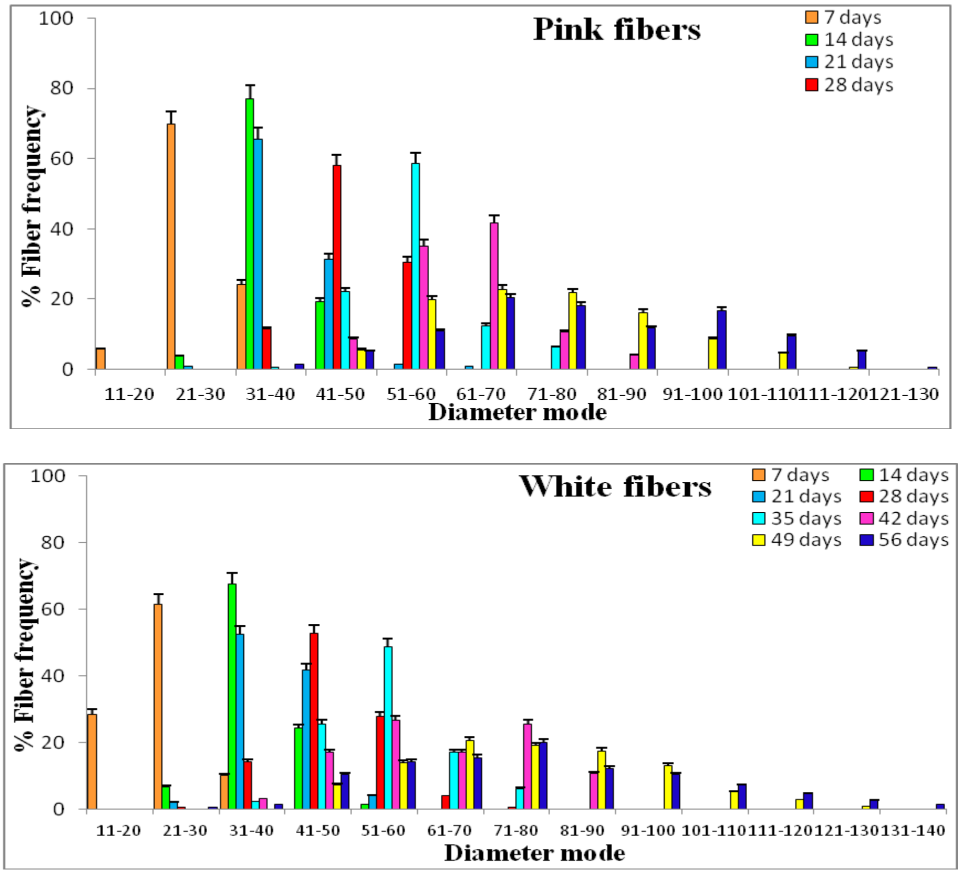
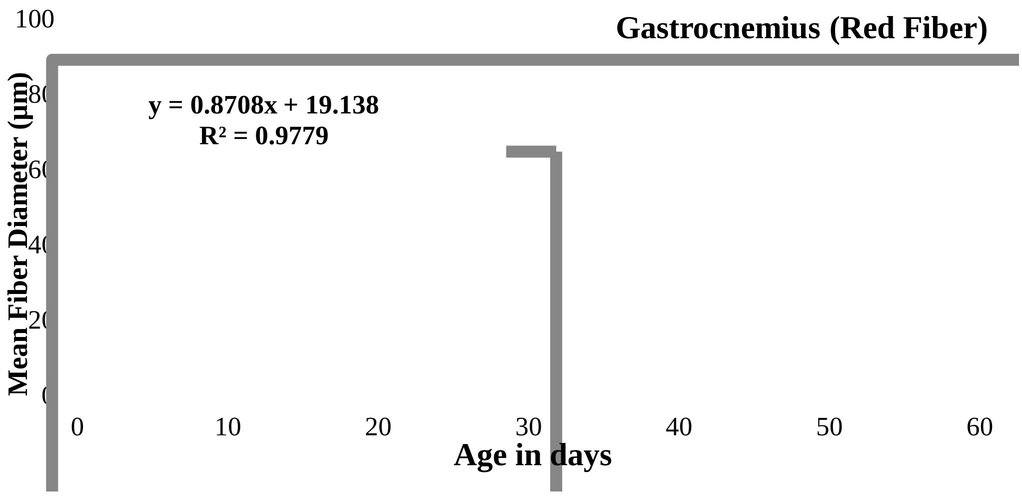
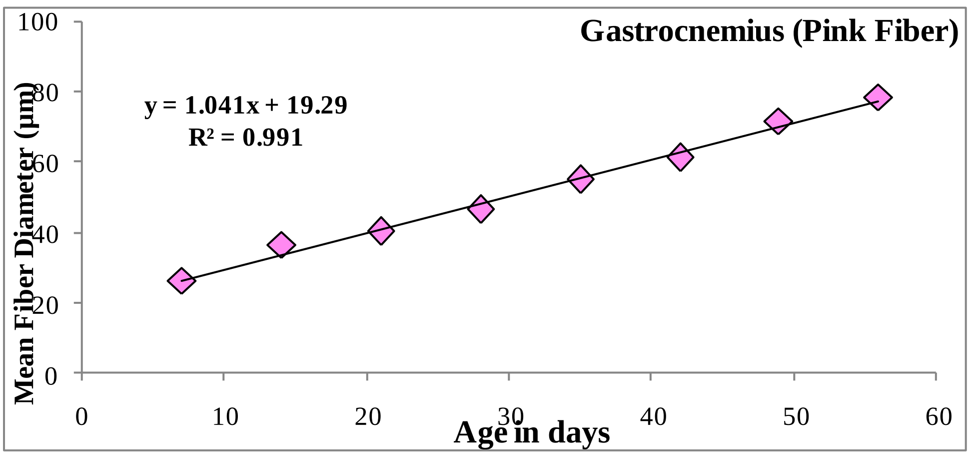


Figure 1. Graphs showing percent fiber frequencies against diameter modes in red, pink and white fibers of Gastrocnemius muscle of chick. Error bars represent the standard deviation.





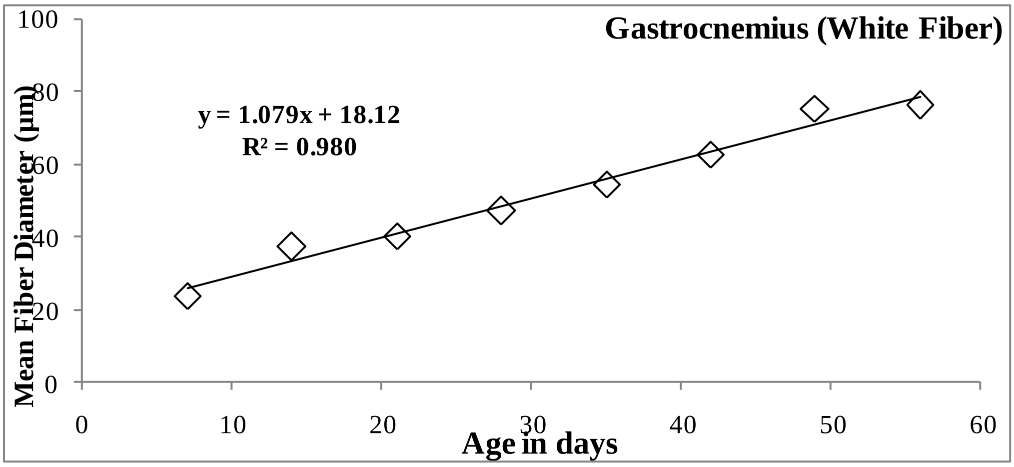


Figure 2. Regression analysis of red, pink and white fibers of Gastrocnemius muscle of developing chick. Regression equations and Correlation Coefficient values are given.

Present investigation revealed that the modes of growth of red, pink and white fibers are mostly by hypertrophy only. However, the frequencies of some intermediate diameter modes were high in almost all age classes. Moreover, the white fibers showed an extended array of fiber diameter with peak diameter modes in all three fiber types which is suggestive of the splitting of larger fibers into smaller ones [26]. The addition of persistent myoblasts or myosatellite cells [27, 28] also attributed towards the overall growth and development of muscle fibers in the selected muscle [29, 30]. As it appears from present investigation that the growth dynamics of all three fiber types in the Gastrocnemius muscle is by hypertrophy exclusively [20,23]. The recruitment of small new fiber is not at all evident. The growth dynamics of this muscle is typically by muscle fiber diameter only.

Increase in red and pink fiber area in physically active individuals has been reported earlier in mammals [10] and birds [12,31]. 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 period i.e. 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 patterns were observed in Pectoralis major and Sartorius muscles of broilers [32] where growth of muscle fibers was assessed in response to enclosure sizes. There was no effect of enclosure sizes on growth of muscle fibers.

It was 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*., [11], 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 used in this experiment is 42 days of age, the results showed that food and space competition in a congested place may have influenced the performance if the broilers were raised for a longer period. Also, due to this; lack of exercise might lead to muscle atrophy and vice versa [33]. The results of this experiment suggest that the atrophy seen is a result of the low physical activity that might be a consequence of the small space available for locomotion during that period.

CONCLUSIONS

Present study revealed the presence of all 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 poultry animal, the data can be used to improve the quantity as well as the quality of the meat meant for human consumption.

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AUTHOR'S CONTRIBUTION

The Corresponding author: Conceptualization of the research idea, planning and design, analyses of the data, final preparation of the manuscript. The Senior Author: Designing and execution of the experiments, data analyses, preparation of the manuscript.

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