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Growth Dynamics of Flexor Muscle Fibers in Developing Male White Leghorn Chicks

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ABSTRACT

The study examines the relationship between fiber orientation, functional activity, and growth dynamics in the flexor muscle of a male white Leghorn chick. It tests three hypotheses: similar histochemical fiber typing in muscle mass, distribution patterns influenced by species' functional activities, and fiber growth dynamics related to somatic growth rate. The study confirmed the hypothesis that all three basic fiber types (red, pink, and white) grow exclusively through hypertrophy. True hyperplasia was not evident in any age group, possibly in the late embryonic stage. Some cases of pink and white fibers showed splitting into smaller ones. All three basic fiber types grew by hypertrophy, regardless of location or functional activity. Muscle fiber growth in this muscle mass was directly related to the chick's somatic growth rate.

Keywords : Muscle fibers, Growth dynamics; Chick, Flexor, Hypertrophy, Hyperplasia

I. INTRODUCTION

Muscle, a specialized post-mitotic tissue, is the primary source of body mass and size in vertebrates, composed of specialized fibers that form the structural basis of skeletal muscle (Hedrick *et. al.,*1994). The correlation between muscle fiber size, number, and composition is widely recognized (Ryu *et. al.,* 2004). Histochemical staining using SDH is used to identify these fiber types (Mankodi, 1988). Each muscle type's color corresponds to the amount of circulation it receives (Kundu *et. al.,* 1991a & b, Lojda *et. al.,*1979, Kundu, 1991). Fish muscles consist of distinct muscle masses based on different fiber types (Weatherly, 1990), but the muscles of mammals and birds are made up of a combination of several fiber types (Sokal & Rohlf, 1969). Muscle fibers undergo morphological and biochemical adaptations to meet functional needs, with age positively influencing hypertrophy growth and fiber types changing based on functional adaptability (D'Angelis, 2004). Atrophy of the muscles is caused by an increase in white fibers after a period of inactivity (Urso *et. al.,* 2006). Numerous bird species exhibit yearly cycles of muscular atrophy and reconstitution (Cheral *et. al.,* 1988). Research indicates that certain muscles atrophy due to inactivity and are catabolized for energy, while fish muscles grow through the recruitment of new or enlarged fibers (Weatherley, 1990). Fish develop their muscles from an early age and continue to do so for the duration of their lives (Templeton *et. al.,*1988). Genetics determines the maximum diameter of fibers, which then break into several tiny threads (Johnston *et. al.,*1997, Anastasia and Lowery, 1999). Myosatellite cells are the primary source of extra nuclei in higher vertebrates like aves, where post-embryonic muscle expansion is primarily due to hypertrophy (Johnston *et. al.,*2001). Nuclei accumulate and undergo hypertrophy in post-hatch birds to influence the growth of skeletal muscle. In avian species, hypertrophy, or a rise in fiber size, is linked to an increase in the number of nuclei per fiber and results in post-hatch muscle growth (Sokal and Rohlf 1969).

The study analyzed different muscle fiber types in the Flexor muscle, focusing on histochemical and histological characteristics, distribution patterns, and growth in relation to somatic development. The findings could be useful in biomedical, sports, and animal agriculture sciences for improving meat quality and quantity by understanding skeletal muscle growth strategies.

II. MATERIALS AND METHODS

A study was conducted on 32 male chicks, Gallus gallus, from a poultry farm in Rajkot, India. The chicks were kept in a hygienic iron cage, following ethical norms set by CPCSEA India. They were fed a poultry starter mash by Hindustan lever Ltd, and tap water was available. Muscles were frozen in cryostat at -18˚C to -20˚C, and histochemical staining of SDH was performed on tissue samples of 10-15μm size using a Cryostat Microtome. Muscle fibers were identified using Lojda's method (Lojda *et. al.,* 1979), with sections observed under a Carl Zeiss Axioscope – II microscope and digitally photographed. Large pink and white fibers were measured three times from three angles, and at least 100 fibers of each type were measured from each possible region. The collected data underwent statistical analysis, including regression and correlation coefficient analysis (Stein & Padykula, 1962).

III. RESULTS

1. Fiber identification: Muscle sections under a microscope showed color, shape, size, distribution, and orientation differences. Staining for the oxidative enzyme SDH identified three types of fibers: red, pink, and white. Red fibers were smaller and rounder than pink and white fibers. Different lipids had different colors, with phospholipids staining blue and neutral lipids as red droplets. Larger fibers were lightly stained, while smaller, rounder fibers were heavily stained. Nuclei were blue, and glycogen was a bright red color.

2. Fiber 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.	*Flexor digitorum longus*	29.02 ± 2.95	38.68 ± 0.79	34.32 ± 1.40

Distribution: The study examined the ratios of red, pink, and white fibers in a chick's chosen muscle. Results showed more pink fibers than white fibers, with the least amount of red fibers. Chickens have more type II pink fibers compared to other animals. White fibers were prevalent at the periphery, while red fibers were more concentrated in deeper areas near the bone. Histochemical experiments showed that the predominant components of the muscle mass were pink (38.68%) and white (34.32%) fibers, with a lower percentage of red fibers (29.02%).

3. Fiber's diameter variation and Growth:

Red fibers: The study found that red muscle fibers in chicks ranged in diameter from 12.08 µm to 30.55 µm in the lowest age class to 89.53 µm in the highest age class. The mean diameter was 21.14 ± 3.23 µm in the lowest age class and increased to 58.78 ± 10.68 µm in the highest age class. The red flexor muscle fibers showed signs of hypertrophy, and the mean fiber diameter increased as the animal grew from lower to higher age classes (Table-2). The results show positive correlation ($R^2 = 0.848$) between age and mean fiber diameter.

Table – 2. Mean diameter of red fibers in ***Flexor digitorum longus*** 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 day. Thereafter there is a decline in 49th day and again increase at 56 days.

Age	Min Diameter	Max Diameter	Mean Diameter ±SD
7th Day	12.08	30.55	21.14 ± 3.23
14th Day	11.82	34.77	23.08 ± 4.40
21st Day	22.53	52.41	34.40 ± 5.21
28th Day	21.08	51.56	33.17 ± 5.02
35th Day	22.82	52.94	33.39 ± 5.54
42nd Day	24.85	62.66	44.90 ± 7.52
49th Day	21.41	59.22	40.36 ± 6.43
56th Day	31.29	89.53	58.78 ± 10.68

Pink fibers: The study found a similar pattern in pink fibers, with younger age groups attracting tiny new fibers. The 31-50 µm modes showed the highest fiber frequency in almost every age group. The mean fiber diameter increases with age, but as people age, their frequency values decrease and move towards higher diameter modes. Despite a negative correlation between age and fiber frequency, there is a strong positive correlation ($R^2 = 0.8865$) between age and mean fiber diameter. The largest diameter mode was found at the highest age class, with extremely high frequency values in intermediate diameter modes (Table-3).

Table – 3. Mean diameter of pink fibers in ***Flexor digitorum longus*** muscle of Chick. Values expressed are in µm. The muscle is showing gradual increase in mean fiber diameter from the subsequent age classes upto 49th day. Thereafter there is a decline in 56th day.

Age	Min Diameter	Max Diameter	Mean Diameter ±SD
7th Day	14.78	36.07	23.95 ± 3.78
14th Day	16.42	57.23	30.06 ± 8.95
21st Day	19.15	47.67	35.64 ± 5.12
28th Day	19.09	64.44	36.21 ± 7.80
35th Day	25.25	58.81	38.69 ± 7.64
42nd Day	30.09	67.96	48.55 ± 6.49
49th Day	30.63	67.25	47.86 ± 9.53
56th Day	28.37	95.13	67.27 ± 14.68

White fibers: The study revealed a consistent growth pattern in white and pink fibers, with higher age classes exhibiting almost all diameter modes except for a few smaller ones, with a perfect correlation between age and growth ($R^2 = 0.9151$). From 35 days onward, fiber diameters increased and maintained with intermediate diameter modes. White fibers in the muscle contained fibers of highest diameter in they range of 29.60-119.23 µm, with high frequency values in moderate diameter modes of 31-60 µm (Table – 4).

Table – 4. Mean diameter of white fibers in ***Flexor digitorum longus*** muscle of Chick. Values expressed are in µm. The muscle is showing gradual increase in mean fiber diameter from the subsequent age classes upto 35 days. Thereafter there is a decline in 42nd day and again increase at 56 days.

Age	Min Diameter	Max Diameter	Mean Diameter ±SD
7th Day	14.49	36.48	24.72 ± 3.89
14th Day	13.44	43.83	29.91 ± 5.01
21st Day	21.19	63.36	40.48 ± 5.51
28th Day	25.50	65.14	40.81 ± 8.38
35th Day	33.56	89.66	58.76 ± 11.14
42nd Day	27.54	89.66	61.13 ± 11.39
49th Day	33.81	78.00	56.32 ± 8.30
56th Day	29.60	119.23	76.97 ± 18.31

DISCUSSION

1. Fiber identification:

The study analyzed lipid, glycogen, SDH, and LDH in chick muscle, revealing three basic fiber types: Red (Types I), Pink (Type II), and White (Type II). These findings align with previous research by Peter *et. al.,* (1972), who identified red, pink, and white fibers as the main types. Histochemical experiments revealed significant differences in the physiological nature of three fiber types: red fibers exhibit high oxidative enzyme activity, suggesting aerobic metabolism with high lipid content, and white fibers have low SDH activity and high LDH activity (Kundu & Mansuri, 1994). Red and pink fibers exhibit higher levels of oxidative enzymes, such as succinic dehydrogenase (Stickland, 1995), as found in fish myotomal muscles and other studies (Pandya *et, al.*, 2003).

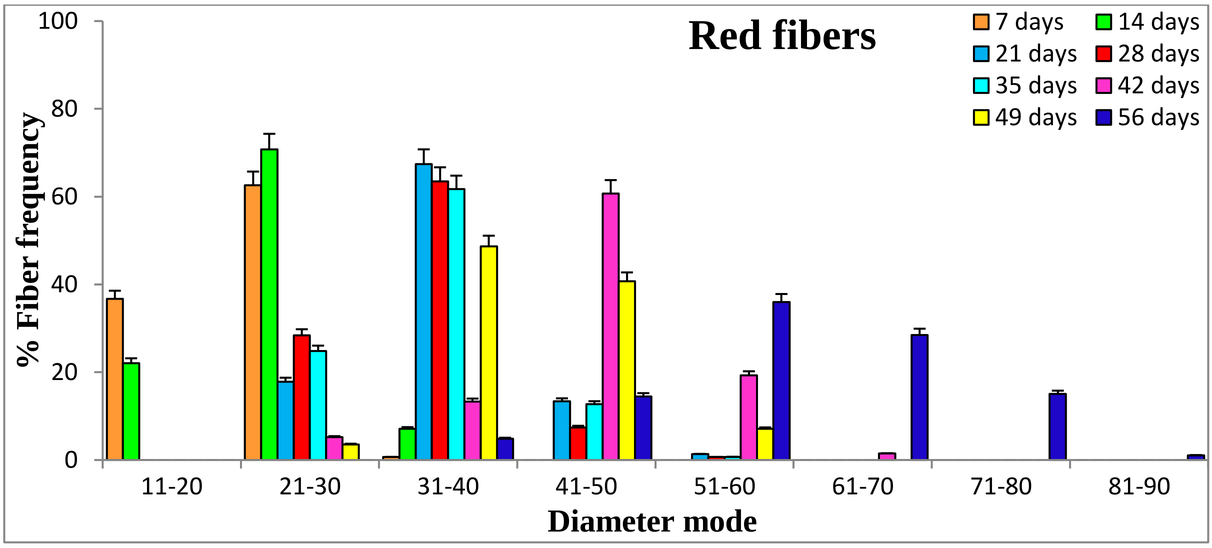

Fig. 1. Graphs showing percent fiber frequencies against diameter modes in red fibers of ***Flexor digitorum longus*** muscle of chick. Error bars represent the standard deviation.

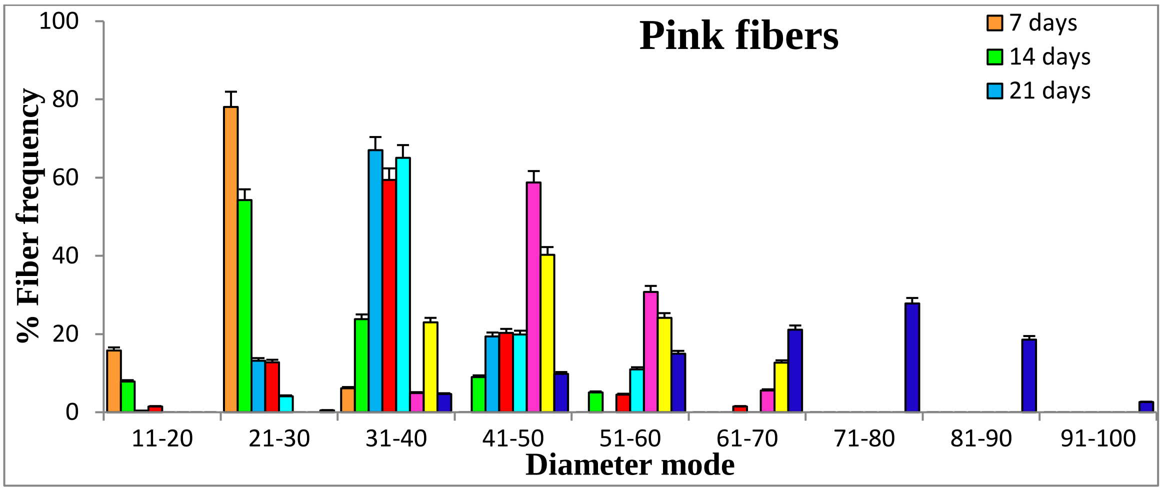

Fig. 2. Graphs showing percent fiber frequencies against diameter modes in pink fibers of ***Flexor digitorum longus*** muscle of chick. Error bars represent the standard deviation.

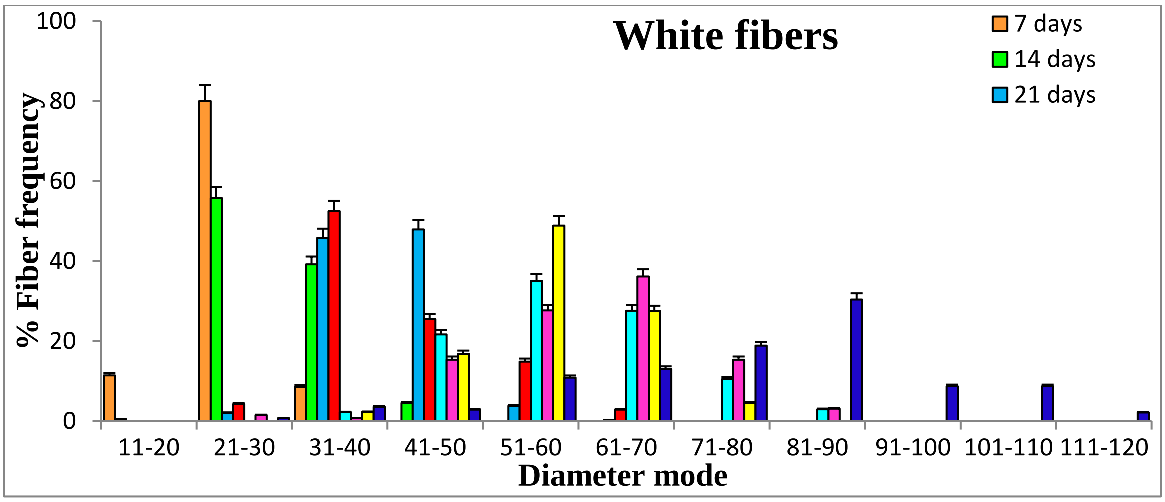

Fig. 3. Graphs showing percent fiber frequencies against diameter modes in white fibers of ***Flexor digitorum longus*** muscle of chick. Error bars represent the standard deviation.

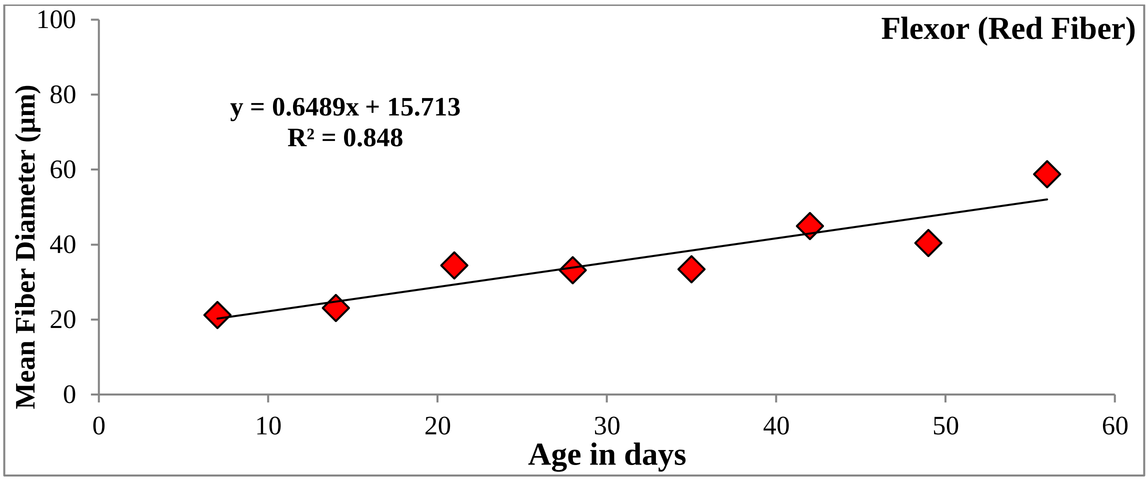

Fig. 4. Regression analysis of red fibers of ***Flexor digitorum longus*** muscle of developing chick. Regression equations and Correlation Coefficient values are given.

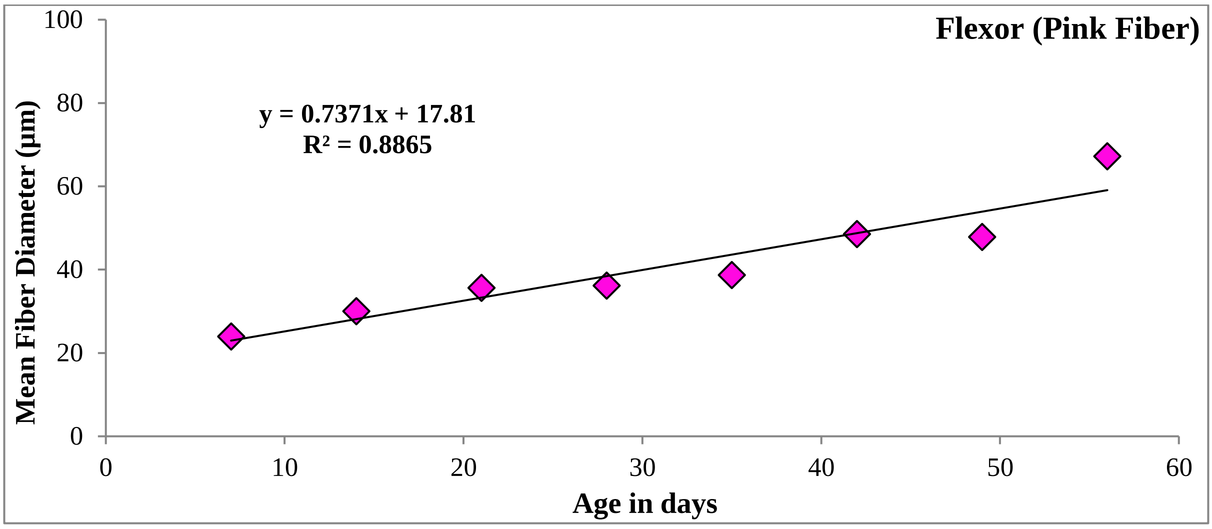

Fig. 5. Regression analysis of pink fibers of ***Flexor digitorum longus*** muscle of developing chick. Regression equations and Correlation Coefficient values are given.

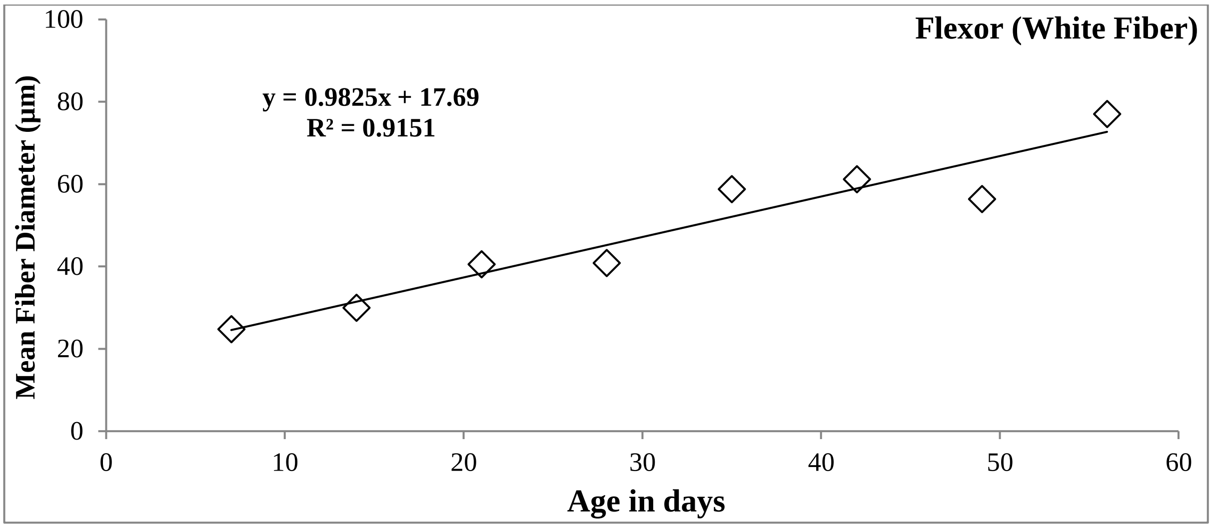

Fig. 6. Regression analysis of white fibers of ***Flexor digitorum longus*** muscle of developing chick. Regression equations and Correlation Coefficient values are given.

2. Fiber Distribution and Orientation:

The results showed that the fibers in the muscle under study had distinct orientation patterns. All three of the basic fiber types were found to be present in the chick's muscle mass, with the pink and white fibers predominating. Given that it can perform all of the movements that are represented by all of these fiber types, this suggests that the muscle plays a dynamic role in the animal's ability to move. Because it is believed that the size of a muscle corresponds to the size of the entire organism, the growth rate of the muscle serves as a proxy for the growth rate of the entire organism. Type II fibers are proportionately higher in chickens and turkeys. The presence and predominance of distinct fiber types in muscles determines their appearance; for example, muscles with a red appearance have more cytochrome and myoglobin (Chandra-Bose *et. al.,*1964); in contrast, muscles with a pale appearance are primarily made of white fibers with comparatively less myoglobin. In nearly every way, intermediate fibers are intermediate. However, no muscle in a bird has been reported to be completely composed of one or the other; rather, the majority of a bird's muscle consists of all three types of fiber (Chandra-Bose & George, 1965). The composition of different types of fibers varies depending on the animal species and the purpose of each individual muscle. Red fibers are primarily found in postural muscles because they are made for slow, continuous movements of the body. In contrast, the musculature of larger animals' forelimbs appears to be more involved in maintaining a standing position than in smaller animals.

3. Diameter variation and Growth:

The current study on the dynamics of muscle fiber growth in developing chicks revealed unique differences in every type of muscle fiber examined. In relation to the somatic growth of the species chosen for the study, the growth pattern was examined based on the types of muscle fibers.

Red fibers: A few tiny new fibers were recruited (hyperplasia) in the early stages, and as time went on, existing fibers in all age groups showed increases in diameter (hypertrophy). An inverse relationship was observed between age and the decline in fiber frequency values towards the higher diameter mode with increasing age Fig. 1). Age and fiber frequency values toward higher diameter modes were found to be negatively correlated. The regression analysis provides strong support for the outcome (Fig.4) with high positive correlation coefficient value ($R^2 = 0.848$). It is evident from the current study that hypertrophy is the primary mechanism of red fiber growth (Weatherley 1990; Kundu & Mansuri, 1994; Dimpal and Kundu, 2013).

Pink fibers: A gradual shifting of fiber frequency values towards next higher diameter modes (Fig.2) was evident in this muscle which is indicative of growth by hypertrophy (Table – 3). Hence, we can say that age and mean fiber diameter have strong positive correlation ($R^2 = 0.8865$) while fiber frequency and age have negative correlation (Fig.5). 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 (Kundu *et. al.,* 1990, Dimpal and Kundu, 2013).

White fibers: It appears from the results (Fig. 3); 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 ($R^2 = 0.9151$). The shifting of modal frequency values towards higher diameter modes was prominent in higher age groups (Fig. 6). It suggests an increase of medium-diameter fibers, which originate when giant fibers that have attained an optimal diameter split apart (Johnston *et. al.,* 1997, Kundu and Mansuri, 1992).

According to the present study, hypertrophy is the sole manner in which that red, pink, and white fibers expand. However, the frequencies of a few intermediate diameter modes were high in almost all age groups. The wide spectrum of fiber diameters observed in all three types of white fibers provides more proof that larger fibers are splitting into smaller ones (Mascarello *et. al.,* 1995). The addition of persistent myosatellite cells (Koumans *et. al.,* 1991, Higgins & Thorpe, 1990) also attributed towards the overall growth of muscle fibers in the selected muscle (Goldspink and Yang, 1999; Galloway *et. al.,* 1999). Current research suggests that hypertrophy is the only mechanism by which the three types of fibers in the flexor muscle increase (Pandya *et. al.,* 2003, Dimpal & Kundu, 2013). The recruitment of small new fiber is not at all evident.

It has previously been noted that physically active people have increased red and pink fiber area in both birds (Cheral *et. al.,*1988) and mammals (D'Angelis, 2004). Therefore, more muscle activity during the trial period—that is, greater contact between the broilers—may be linked to the enhanced hypertrophy of the fibers in the selected muscle of broilers starting at 28 days of age. The diameter of the red, pink, and white threads only increased until 42 days of age, according to our results. The three types of fibers showed no signs of hypertrophy between 42 and 56 days of age, suggesting that the muscle fibers attained their maximal growth at 42 days. However, the reason for the cessation of the hypertrophic growth of the muscle fibers during 42 days is not clear. The broiler chickens' Sartorius and Pectoralis major muscles showed comparable growth patterns. (Michaela *et. al.,* 2012) where growth of muscle fibers were assessed in response to enclosure sizes. There was no effect of enclosure sizes on growth of muscle fibers.

Broiler muscle fiber diameter increased until 42 days of age, then decreased from 42 to 49 days, indicating muscle atrophy, but a sudden, significant increase was observed. According to Urso *et. al., .* (2006), lack of physical activity causes decrease in the protein accretion in the muscle extracellular matrix. The study found that muscle atrophy in broilers aged 42-49 days was due to reduced extracellular connective tissue, suggesting that food and space competition in congested areas may have influenced performance and led to muscle atrophy. The study found that muscle atrophy in broilers aged 42-49 days was due to reduced extracellular connective tissue, suggesting that food and space competition in congested areas may have influenced performance and led to muscle atrophy (Chaplin *et. al.,*1971). The experiment indicates that the observed atrophy is a result of low physical activity, possibly due to the limited 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. As the selected animal is one of the major agricultural animals, the data can be used to contribute the knowledge in basic sciences as well as will be useful in applied sciences like in the fields of animal agriculture, sports medicine etc.

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