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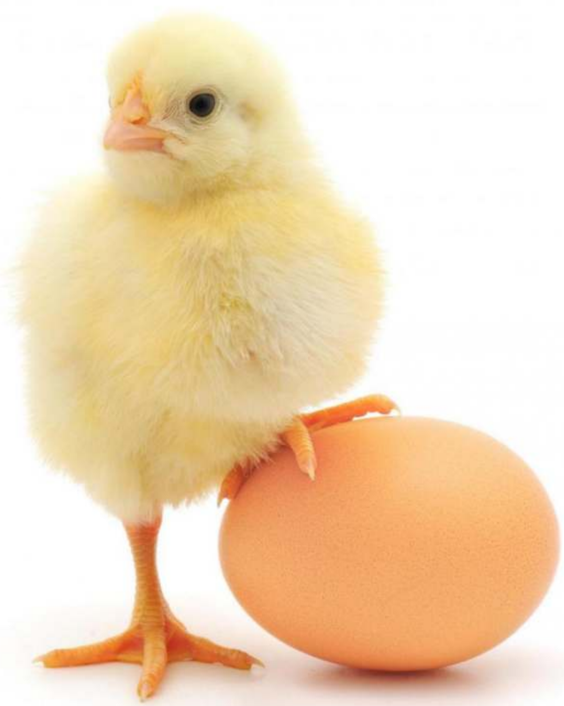

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# Morphology of Breast and Thigh Muscles of Red Jungle Fowl (*Gallus gallus spadiceus*), Malaysian Village Chicken (*Gallus gallus domesticus*) and Commercial Broiler Chicken

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**Abstract:** In most animals, myofibers number gets established before birth and postnatal growth is due to muscle hypertrophy, with very little contribution from muscle hyperplasia. The muscle myofibers exhibit different contractile, metabolic, physiological, chemical and morphological characteristics but the interaction between these factors and their outcome is not fully understood. Since, there is lack of literature regarding the skeletal muscle morphology of Red jungle fowl (RJ) and Malaysian village chicken (VC), therefore, the present study was undertaken with the aim to evaluate the morphology of skeletal muscle of Red jungle fowl (*Gallus gallus Spadiceus*) and Malaysian Village Chicken (*Gallus gallus Domesticus*) and commercial broiler chicken. A total of 150 unsexed birds consisting of 50 Red jungle fowl (RJ), 50 Malaysian village chicken (VC) and 50 commercial broiler (CB) were used in this study. *Pectoralis major* and *bicep femoris* were analyzed at the age of 1, 10, 20, 56 and 120 days post hatch. The number of muscle fibers in the breast and thigh muscles increased as the age advanced and the slow growing birds (RJ and VC) had higher Type I muscle fibers in breast and thigh muscles than fast growing CB. The percentage of Type 1 muscle fibers increased as the age advanced due to prolonged activity and also the type of muscle fibers changed throughout the age and activity. The importance of muscle fiber type changes is applicable to determine the future production of birds especially with regard to better quality meat of VC and RJ.

**Key words:** Myofibers, red jungle fowl, Malaysian village chicken, commercial broiler

## INTRODUCTION

In most animals, it is well known that myofibers number gets established before the birth and the growth of muscle mass during postnatal growth is due to muscle hypertrophy, with very little contribution from fiber hyperplasia (Rowe and Goldspink, 1969; Goldspink, 1970; Swatland, 1984; Remignon *et al*., 1995; Rehfeldt *et al*., 2004). However, Stickland (1995) reported that generally the number of fibers gets increased throughout the growth.

Generally, individual muscle fiber type exhibits different contractile, metabolic, physiological, chemical and morphological characteristics (Lee *et al*., 2010), however, the interaction between these factors and their outcome is not fully understood (Lawrie, 1985; Lawrie and Ledward, 2006). The composition of muscle fibers is related to intrinsic and extrinsic factors (Lawrie, 1985) such as selection, gender, age, breed, hormones and physical activity.

The Red jungle fowl and present Malaysian domestic or village chicken are known as slow growing birds (Azahan and Zahari, 1983; Petersen *et al*., 1991; Wall and Anthony, 1995) as against the fast growing commercial broiler chickens that have undergone tremendous genetic selection for increased growth, feed conversion and carcass quality (Schreiweis *et al*., 2005; Stevens, 1991). There is lack of literature regarding the skeletal muscle morphology of Red jungle fowl (RJ) and Malaysian village chicken (VC). Thus, the present study was undertaken with the aim to define the morphological structures of skeletal muscle of Red jungle fowl (*Gallus gallus Spadiceus*) and Malaysian Village Chicken (*Gallus gallus Domesticus*). In addition, an attempt was made to compare the results with the skeletal muscle morphology of commercial broiler chicken.

## MATERIALS AND METHODS

**Animals and experimental design:** A total of 150 unsexed birds consisting of 50 Red jungle fowl (RJ), 50 Malaysian village chicken (VC) and 50 commercial broiler (CB) were used in the study. The RJ and VC were identified and confirmed through phenotypic

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characteristics which include colour, head, comb and lappet, ear lobes, tail, body size, leg and vocal (Aini, 1990; Roberts, 2008; Amin Babjee, 2009). The eggs of RJ and VC were collected from Jenderam Hulu, Sepang, Selangor, Malaysia and were incubated and hatched in the University Putra Malaysia (UPM) laboratory. Day old chicks (DOC) of commercial line (Ross) were supplied by a private hatchery (Linggi Poultry farm Sdn. Bhd. CP lot 1354, Mukim Lubuk Tebrau, 33010, Kuala Kangsar, Perak, Malaysia). The bird were reared in the experimental house located at UPM (N 03. 00551°, E 101.70501°) in different cages according to their breed and age for a period of 120 days. The study protocol was approved by the Faculty Animal Ethics Committee. The feed and water were provided *ad libitum*. The feed consisted of standard commercial starter (201-P, Malayan Federal Flour Mill Sdn. Bhd.) from day 1 to day 21 post hatch and finisher (203-P, Malayan Federal Flour Mill Sdn. Bhd.) from day 22 to day 120 post hatch. All the birds were euthanized at different intervals by intravenous (cutaneous ulnar vein) administration of 80 mg/kg sodium pentobarbitone (Mitchell and Smith, 1991).

**Sample collections for histological examination:** Ten birds were randomly selected from each group at the age of 1, 10, 20, 56 and 120 days post hatch. *Pectoralis major* muscle was used as the representative of breast muscle and *bicep femoris* as representative of thigh muscle. All the muscles were consistently cut at the middle of the muscle groups and fixed in 10% buffered formalin. All the sampling procedures were standardized and consistently applied for all the three breeds evaluated. The histological evaluations were done in the Histology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia. The samples were subjected to standard tissue processing method for histology including dehydration, clearing, impregnation and embedding. Serial transverse sections of 5 μm thick were then stained with Haematoxylin and Eosin (Fig. 1). The muscle fibers counts per bundle were performed using a computerized image analyzer (Leica DM LB2, Germany). Seven muscle bundles were selected randomly for counting and the results were expressed as mean number of muscle fibers in a bundle. Samples for muscle fiber typing were collected together with histological sampling at aforementioned intervals except for day 1 due to insufficient sample. Approximately 1.5cm of muscle was cut at the middle of *pectoralis major* and *bicep femoris* muscles and immediately snap-frozen by using pre-cooled Isopentane (Sigma-USA, 19516) in liquid nitrogen (Malaysian Oxygen Bhd., Malaysia) to immobilize the constituent of living tissue. Serial tissue sections of 20 μm thickness were prepared by using a cryostat microtome (Leica, CM 1850, Germany) at -20°C. Cross-sectional tissue samples were stained for myosin ATPase according to the method of Brooke and Kaiser (1970) based on acids pre-incubation as described by Sazili *et al*. (2005) with some modifications. The incubation period in 2% $CoCl_2$ (Sigma, USA, C8661) was prolonged from 2 minutes to 30 minutes to produce better staining of the fiber. The slow-contracting Type I fibers stained dark in the sections pre-incubated under acidic conditions while the fast-contracting Type II fibers appeared light under light microscopy (Fig. 2) (Brooke and Kaiser, 1970). Fiber type counting was done using image analyzer (Leica DM LB2, Germany) and expressed as percentage fiber Type I or II of total fiber numbers counted per muscle fiber bundle. For each section, seven muscle bundles were selected at random.

**Statistical analysis:** The number of fibers was analyzed using one-way ANOVA. The percentage of Type I and II fibers was calculated using excel 2003 program. The Duncan's multiple range test was used to elucidate differing means (SPSS, 17.0).

## RESULTS

Number of muscle fibers per bundle of breast muscle: The number of muscle fibers of three breeds of chicken were not significantly different (p>0.05) on day 1 post hatch (Table 1). On days 10, 20 and 56 post hatch, the muscle fibers of RJ and VC were significantly less (p<0.05) compared to CB and there was no significant difference (p>0.05) between RJ and VC. However, on day 120 post hatch, the muscle fiber numbers in RJ were significantly (p<0.05) highest among the three breeds.

Within the breed, the muscle fiber numbers increased as the age of birds increased. In RJ and VC, the increment was significant (p>0.05) from days 10 to 20 post hatch and from days 56 to 120 post hatch. In CB, the increment was only significant from days 1 to 20 post hatch; thereafter, although the numbers of muscle fibers increased but the increment was not significant (p>0.05).

**Number of muscle fibers per bundle of thigh muscle:** The number of muscle fibers in RJ was consistently higher than the CB at all ages, but only showed significant difference (p>0.05) on day 1 and 120 post hatch (Table 2). The muscle fiber numbers were consistently non-significantly (p>0.05) higher in RJ as compared to VC; in VC, the numbers were also consistently higher than CB but with significance (p>0.05) only at day 1 post hatch.

Within the breed, the muscle fiber numbers increased as the age of birds increased. In RJ, the muscle fibers significantly increased (p<0.05) from days 10 to 20 and from days 56 to 120 post hatch. In VC, the muscle fibers significantly (p<0.05) increased from day 10 post hatch

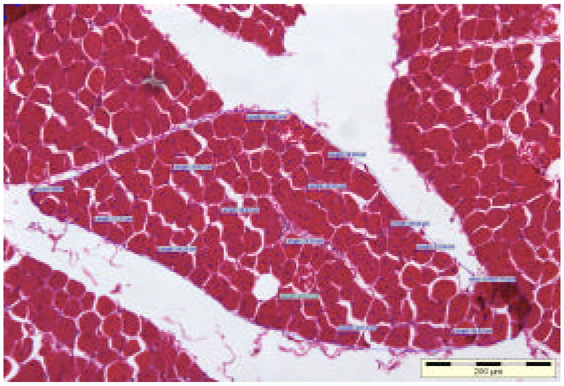

Fig. 1: H and E Staining for muscle fibers

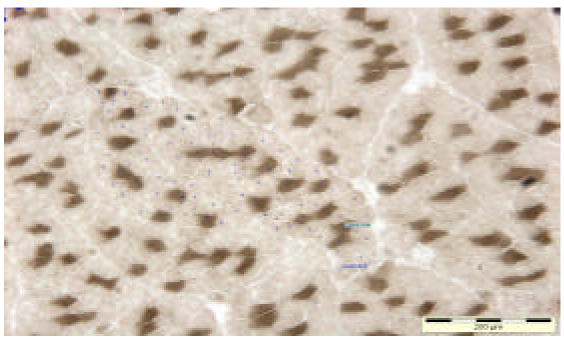

Fig. 2: Types of muscle fiber. Type I appears darker while Type II appears lighter

onwards, while as in CB, the muscle fibers significantly (p<0.05) increased from days 1 to 20 and from days 56 to 120 post hatch.

**Type I muscle fibers in breast muscle:** The percentage of Type I muscle fibers per muscle bundle was highest in RJ, followed by VC and then CB (Table 3). The Type I muscle fibers in RJ were significantly higher (p<0.05) at all ages in CB and at day 56 and 120 post hatch in VC. Within the breed, as the age increased, the percentage of Type I fibers in breast muscle of RJ, VC and CB showed increasing pattern.

**Type I muscle fibers in thigh muscle:** The percentage of Type I muscle fibers per muscle bundle was highest in RJ, followed by VC and then CB at all ages (Table 4). Within the breed, generally the percentage of Type I fibers in thigh muscle showed an increasing pattern as the age increased.

**Type II muscle fibers in breast muscle:** Type II muscle fibers in RJ were significantly lower (p<0.05) than CB at all ages, day 56 to 120 post hatch in VC (Table 5). Similarly, Type II muscle fibers were significantly lower

Table 1: Mean number of muscle fibers per bundle of breast muscle in RJ, VC and CB at different ages (mean±SE)

| | ------------------------------ Breeds | ------------------------------ | |
|---|---|---|---|
| Age | RJ | VC | CB |
| Day 1 | $66.88\pm8.52^{a,v}$ | $69.86\pm2.95^{a,v}$ | $58.14\pm3.79^{a,v}$ |
| Day 10 | $69.06\pm3.41^{a,v}$ | $72.76\pm6.19^{a,v}$ | $87.64\pm5.24^{b,w}$ |
| Day 20 | $81.60\pm6.68^{a,w}$ | $87.05\pm5.42^{a,w}$ | $101.83\pm5.06^{b,x}$ |
| Day 56 | $88.45\pm2.89^{a,w}$ | $88.02\pm4.15^{a,w}$ | $102.64\pm3.61^{b,x}$ |
| Day 120 | $162.74\pm9.10^{a,x}$ | $111.95\pm7.87^{b,x}$ | $110.71\pm4.66^{b,x}$ |

$^{abc}$Means within a row with difference in superscript are significantly different (p<0.05)

$^{vwxyz}$Means within a column with difference in superscript are significantly different (p<0.05)

Table 2: Mean number of fibers per bundle of thigh muscle in RJ, VC and CB at different ages (mean±SE)

| | ------------------------------ Breeds | ------------------------------ | |
|---|---|---|---|
| Age | RJ | VC | CB |
| Day 1 | $73.29\pm2.09^{a,v}$ | $69.01\pm2.73^{a,v}$ | $56.91\pm3.60^{b,v}$ |
| Day 10 | $75.02\pm2.61^{a,v}$ | $73.45\pm3.16^{a,v}$ | $71.67\pm1.15^{a,w}$ |
| Day 20 | $82.62\pm2.34^{a,w}$ | $82.43\pm5.53^{a,w}$ | $76.86\pm0.89^{a,w\ x}$ |
| Day 56 | $86.03\pm3.46^{a,w}$ | $85.26\pm2.22^{a,x}$ | $79.90\pm2.61^{a,x}$ |
| Day 120 | $109.56\pm2.18^{a,x}$ | $98.45\pm3.34^{b,y}$ | $91.8\pm3.82^{b,y}$ |

$^{abc}$Means within a row with difference in superscript are significantly different (p<0.05)

$^{vwxyz}$Means within a column with difference in superscript are significantly different (p<0.05)

Table 3: Mean percentage (%) of Type I fibers per muscle bundle of breast muscle in RJ, VC and CB at different ages (mean±SE)

| | ------------------------------ Breeds | ------------------------------ | |
|---|---|---|---|
| Ages | RJ | VC | CB |
| Day 10 | $9.51\pm0.17^{a,v}$ | $9.16\pm0.93^{a,v}$ | $4.60\pm0.55^{b,w}$ |
| Day 20 | $9.97\pm0.75^{a,v}$ | $9.77\pm0.73^{a,v}$ | $4.11\pm0.39^{b,v}$ |
| Day 56 | $13.57\pm0.96^{a,w}$ | $10.08\pm1.00^{b,v}$ | $4.76\pm0.51^{c,vw}$ |
| Day 120 | $15.07\pm1.70^{a,w}$ | $10.58\pm0.95^{b,v}$ | $5.45\pm0.82^{c,w}$ |

$^{abc}$Means within a row with difference in superscript are significantly different (p<0.05)

$^{vwxyz}$Means within a column with difference in superscript are significantly different (p<0.05)

(p<0.05) in VC than CB except for day 120 post hatch. Within all the breeds, in general, the percentage of Type II fibers showed decreasing pattern as the age increased but the results were not consistent among the three breeds.

**Type II muscle fibers in thigh muscle:** The mean percentage of Type II muscle fibers per muscle bundle of thigh muscle in RJ, VC and CB at different ages is shown in Table 6. The percentages of Type II muscle fibers per muscle bundle were significantly lowest in the RJ, followed by VC and highest in the CB at all ages.
Within the breed, in general, the percentage of Type II fibers showed a decreasing pattern as the age increased. The percentage of Type II fibers in RJ was lowest (p<0.05) on day 120 post hatch, while the VC and CB showed no significant difference (p>0.05) between various age intervals.

## DISCUSSION

**Mean numbers of breast and thigh muscle fiber in a bundle:** The results indicated that the number of muscle fibers per muscle bundle in the breast muscle for slow growing birds (RJ and VC) were lower than fast growing birds (CB). The number of fibers was related to the growth of muscle fibers (Hooper, 1978). The higher growth of muscle in genetically designed fast growing CB birds was the main reason for higher muscle fibers than the slow growing birds, thus confirming the results of Remignon *et al.* (1995). Interestingly in this study, it was observed that at an advanced age (day 120) the number of muscle fibers of slow grow birds (RJ and VC) were significantly higher than the fast growing bird (CB) and the number of muscle fibers per muscle bundle in the thigh muscle were significantly ($p>0.05$) higher in the slow growing birds. These findings are in contrast with the findings of Remignon *et al.* (1995) who reported that the number of fibers are higher in fast growing animals than slow growing ones). The contrasting results found in present study could be due to the different characteristics of muscle growth in different muscles, mode of activity and location of the muscle as describe by Forrest *et al.* (1975), Pearson and Young (1989) and Klosowska *et al.* (1993).

Although it is generally accepted by most researchers that the number of muscle fibers remains unchanged after birth in chicken (Smith, 1963; Moss and Lough, 1968; Mizuno and Hakami, 1971; Aberle and Steward, 1983), but in present study it was observed that within the breeds the mean number of fibers in the breast and thigh muscles increased with the advancement of age. The increased number of muscle fibers was likely from the contribution of satellite cells located beneath the basal lamina of the myofiber (Mauro, 1961). The increased activity especially in RJ might have activated the satellite cells, which in turn causes fiber degeneration for the formation of new fibers as described by Rehfeldt *et al.* (1999, 2004) in a hypothetical model for activity-induced muscle fibers. Similar results have been reported in guinea pigs where the fiber numbers in the cross section of the *Plantaris* muscle increased following daily training for endurance running on a treadmill after eight weeks (Faulkner *et al.*, 1972). At older age, the RJ and VC become more active than the CB as compared to the young age. The activities such as anti predation and search for foods and sometimes flying may be the main the reasons for the increased number of muscle fibers at an older age. The CB was observed to be less active as the age increased, thus the increase in fibers numbers was only observed at a young age.

**Type I muscle fiber in breast and thigh muscles:** The percentage of Type I muscle fibers per muscle bundle was highest in RJ, followed by VC and then CB in both breast and thigh muscles. The results are in agreement with most researchers who suggested that slow growing animals have higher Type I muscle fibers (Klosowska *et al.*, 1993). The results suggest that the different rate of activities of birds leads to the variation in the fiber type composition of the muscle. The RJ were more active than other breeds, although kept in the same environment and confined area. The birds did not lose their flying behavior and sometimes showed berserk behavior when disturbed. Heritability of survival behaviors also have been observed by Wall and Anthony (1995), Hakansson *et al.* (2007), Irshad (1999) and Amin Babjee (2009).

Prolonged physical activity in the RJ compared to VC and CB was the main reason for higher percentage of Type I muscle fibers in this breed. The results agree with Pette and Steron (2001) who stated that the composition of fiber type was closely related to their functional capabilities.

Table 4: Mean percentage (%) of Type 1 fibers per muscle bundle of thigh muscle in RJ, VC and CB at different ages (mean±SE)

| | Breeds | | |
|---|---|---|---|
| Ages | RJ | VC | CB |
| Day 10 | 19.70±0.39$^{a,v}$ | 14.24±0.59$^{b,v}$ | 7.07±0.67$^{c,v}$ |
| Day 20 | 20.82±0.70$^{a,v}$ | 14.22±0.81$^{b,v}$ | 9.13±0.54$^{c,w}$ |
| Day 56 | 20.81±0.22$^{a,v\,w}$ | 15.17±0.66$^{b,v}$ | 10.13±0.48$^{c,w}$ |
| Day 120 | 21.80±0.81$^{a,w}$ | 15.72±0.65$^{b,v}$ | 10.99±0.93$^{c,w}$ |

$^{abc}$Means within a row with difference in superscript are significantly different ($p<0.05$)

$^{vwxyz}$Means within a column with difference in superscript are significantly different ($p<0.05$)

Table 5: Mean percentage (%) of Type II fibers per muscle bundle of breast muscle in RJ, VC and CB at different ages (mean±SE)

| | Breeds | | |
|---|---|---|---|
| Age | RJ | VC | CB |
| Day 10 | 90.49±0.17$^{a,v}$ | 90.84±0.93$^{a,v}$ | 95.40±0.55$^{b,w}$ |
| Day 20 | 90.22±0.75$^{a,v}$ | 90.23±0.73$^{a,v}$ | 95.89±0.39$^{b,w}$ |
| Day 56 | 86.43±0.96$^{a,w}$ | 89.92±1.00$^{b,v}$ | 95.24±0.51$^{c,vw}$ |
| Day 120 | 84.93±1.70$^{a,w}$ | 89.42±0.95$^{b,v}$ | 94.55±0.82$^{b,v}$ |

$^{abc}$Means within a row with difference in superscript are significantly different ($p<0.05$)

$^{vwxyz}$Means within a column with difference in superscript are significantly different ($p<0.05$)

Table 6: Mean percentage (%) of Type II fibers per muscle bundle of thigh muscle in RJ, VC and CB at different ages (mean±SE)

| | Breeds | | |
|---|---|---|---|
| Age | RJ | VC | CB |
| Day 10 | 81.02±0.39$^{a,w}$ | 85.76±0.60$^{b,v}$ | 92.93±0.67$^{c,v}$ |
| Day 20 | 79.18±0.70$^{a,w}$ | 85.78±0.81$^{b,v}$ | 90.87±0.54$^{c,w}$ |
| Day 56 | 79.19±0.22$^{a,v\,w}$ | 84.83±0.48$^{b,v}$ | 89.87±0.46$^{c,w}$ |
| Day 120 | 78.20±0.81$^{a,v}$ | 84.28±0.65$^{b,v}$ | 89.01±0.93$^{c,w}$ |

$^{abc}$Means within a row with difference in superscript are significantly different ($p<0.05$)

$^{vwxyz}$Means within a column with difference in superscript are significantly different ($p<0.05$)

Genetical factor may also account for the higher percentage of Type I fibers in RJ. Reggiani and Mascarello (2004) described that a fiber type is the result of a specific profile of gene expression and fiber transformation occurring over a long run of mechanical load and hormonal stimulation. A higher percentage of Type I fiber was also observed in VC breast muscle as the breed is genetically close to RJ and thought to have been descended from RJ (Azahan, 1992).

Within the breed, RJ breast and thigh muscles showed an increasing pattern of Type I fibers and the increments were higher at the older ages. The increased mean percentage of Type I fibers could be explained by a prolonged muscle fiber activity throughout the period which may have I changed the Type II fibers to Type I fibers as described by Tamaki (1987). The change of muscle fibers types were reported to be associated with age (Remignon *et al.*, 1995; Alnaqeeb and Goldspink, 1986) and training (Andersen and Hendrikssen, 1977; Ingjer, 1979). The change of Type II to Type I fibers were also observed in CB.

**Type II muscle fiber in the breast and thigh muscles:** The composition of Type II fibers in slow growing birds was lower than the fast growing birds. Type II muscle fibers which have larger diameter, glycolytic, white in colour, fast tiring with easy fatigue movement were higher in the fast growing birds confirming the reports of Remignon *et al.* (1993) and Goldspink and Yang (1999). The fast fatigue movement in CB as compared to VC and RJ may also explain the occurrence of a higher proportion of Type II muscle fibers in CB breast muscle. Type II fibers of VC breast muscle showed a similar pattern as that of Type I muscle fibers with no significant difference ($p>0.05$) at all evaluated ages. The changes in the composition of fiber types observed in this study are in agreement with the reports of most workers in chickens (Ashmore and Doerr, 1971), pigs (Beerman *et al.*, 1978; Davies and Gunn, 1972; Swatland, 1975), sheep (White *et al.*, 1978) and rats (Brooke *et al.*, 1971). The significant decreased and increased pattern of Type II and Type I fibers respectively at an older age in RJ, VC and CB thigh muscle showed that change of muscle fiber type occurs due to increased physical activity, leading to proliferation of blood capillaries to supply the oxygen to muscle fibers and change the glycolytic muscle fibers to oxidative muscle fibers (Leisson *et al.*, 2008; Gollnick *et al.*, 1982).

**Conclusion:** In conclusion, the number of muscle fibers per muscle bundle in the breast muscle of slow growing birds is lower than fast growing birds. The muscle type, mode of activity and location of the muscle reflects the numbers of muscle fibers. The number of muscle fibers in the breast and thigh muscles increase as the age advanced and activity increased especially in RJ which activates the satellite cells and fiber degeneration for the formation of new fiber. Slow growing birds have higher Type I muscle fiber in breast and thigh muscles and the percentage increases as the age advances due to prolonged activity and also indicates that the type of muscle fibers change throughout the age. The importance of muscle fiber type changes is applicable to determine the future production of birds especially with regard to better quality meat of VC and RJ.

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