Determination of the Proportions of Muscle Fibre Types from Selected Muscles of the Forelimb: A Comparative Study of Cattle (*Bos taurus indicus*) and One-humped Camel (*Camelus dromedaries*)

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Abstract

A total number of fifty forelimbs comprising Twenty-five forelimbs male dromedary camels (*camelus dromedaries*) and those of male cattle, Zebu type, (*Bos taurus indicus*) within the ages of 6 months, 1 year, 3 years, 5 years and 7 years, were purchased from Sokoto Municipal Modern abattoir. Selected muscles sampled (1 cm^2) from the forelimbs of both cattle and camel were taken from the middle part of the muscle bellies (from the biceps brachii, triceps brachii and deltoideus), fixed in Bouin's solution and prepared for histochemical analyses.

The photomicrographs obtained from the biceps brachii, deltoideus and triceps brachii among both cattle and camel demonstrated the morphology of the different fibre types (type I, type IIA and type IIB). Muscle fibre types showed different proportions. Even though the overall mean proportion of muscle fibre type I, type IIA and type IIB could not present any significant ($p>0.05$) differences between the camel and the cattle looking at it holistically. As revealed in this work, the effects of the interactions of species versus age, species versus muscle and muscle versus age on the proportion of muscle fibre type I revealed that the deltoideus muscle of 1 year old camel had significantly ($p<0.05$) higher proportion. Type IIA muscle fibre obtained from the deltoideus muscle of 5-year-old camel showed a significantly highest value, while muscle fibre type IIB showed significantly higher value from the triceps brachii of cattle aged 1 year. The importance of the knowledge of microscopic characteristics in the determination of myofibre types could be of help in advancing the knowledge on muscle morphology (anatomy) and as well be of help in meat science industry.

Keywords: Muscle Fibre, Proportion, Triceps Brachii, Biceps Brachii, Deltoideus, Cattle; Camel.

Introduction

The phenotypic differences among skeletal muscle cells, termed fiber types, their potential for adaptation and underlying mechanisms have been a topic of study for several decades. In mammals, skeletal muscles are composed of a mixed population of red, intermediate and white muscle fibres (Close, 1972). Most mammalian skeletal muscles are composed of varying proportions of the three major types (I, IIA and IIB) of fibers, while certain muscles such as the rat soleus consist predominantly of type I fibers (Dimov and Dimov, 2007).

Skeletal muscles are composed mainly of three myofibre types that differ in histochemical properties, which reflect physiological and functional aspects of myofibres (Suzuki *et al*., 1999). Myofibres are classified into types I, IIA, and IIB by differences in reactivity for myosin ATPase and dehydrogenases. Type I myofibres correspond to slow-twitch/oxidative myofibres, type IIA myofibres to fast-twitch/ oxidative/glycolytic myofibres, and type IIB to fast-twitch / glycolytic myofibres (Handel and Stickland, 1987). The distinctive physiological properties of different skeletal muscle fibre types allow the muscle to respond to various mechanical (speed and endurance) and metabolic (anaerobic or aerobic) demands (Pette, 2001).

Paucity of information particularly on the muscle histochemical profile in camel and cattle is a driving force in this present research, considering the utility of these animal species in different fields. Similarly, observation of the proportion of the muscle fibre types in these animal species may help at elucidating general principles of musculoskeletal function and design, which could find application in Anatomy and Meat Science Industries.

Materials and Methods

Twenty-five forelimbs of male one-humped camels (*Camelus dromedaries*), and twenty-five of male cattle ,Zebu type, (*Bos taurus indicus*), all within the ages of 6 months, 1, 3, 5 and 7 years, were purchased from

Sokoto Municipal Modern abattoir. The age of each animal was determined using the method of Wilson (1984) and Dyce *et al.* (2010) to determine their ages, while evaluation of the animals to exclude any animal with musculoskeletal deformity or diseases was done through physical examination. The live body weights of the animals were estimated using linear body measurement based on the formula used by Yagil, (1994).

The samples (forelimbs) obtained were wrapped in clean sterile polyethylene bags and transported in a clean cool box containing ice cubes to the laboratory of the Department of Veterinary Anatomy, Usmanu Danfodiyo University, Sokoto-Nigeria, where the triceps brachii, biceps brachii and the deltoideus muscles were all carefully dissected out using the methods of Chibuzo (2006) as slighly modified by Sonfada (2008), noting the origin and insertion of every muscle before further different processing and analyses followed.

After the dissection and isolation of the muscles of interest, scalpel blade was used to excise portions of muscle samples measuring about 1 cm^2 (measurements were obtained with the aid of a tape rule, placed across the muscle section) from the forelimbs of both cattle and camel were taken from the middle part of the muscle bellies (from the biceps brachii, triceps brachii and deltoideus), fixed in Bouin's solution and prepared for histochemical analyses using the method of Dubowitz (1985) as a guide. This procedure helped in demonstrating the specific muscle fibre types from the selected muscles. After histological preparation of the slides, the prepared slides were viewed using a microscope (Olympus® CH 23, Germany) at different magnifications (x40, x100, x400). Photomicrographs were then obtained and transferred into a computer (Compac® Laptop, HDM, Presario CQ60) for further evaluation and detailed studies. The area and number of muscle fibres were calculated from 5 randomly selected fields. The proportions of muscle fibre types were calculated by dividing the number of each muscle fibre types by the total number of muscle fibre types as indicated by Fuentes *et al*. (1998).

Numerical data obtained were reported as mean±SD (Standard deviation) and presented in form of tables and charts. Data generated from the study were analyzed following a completely randomized design with a factorial arrangement of treatments using the general linear model (GLM) of SPSS (Version 16.0, 2007). Statistical significance of experimental observations was set at $P<0.01$ and $P<0.05$ where appropriate. All statistical analyses were done using SPSS (Version 16.0, 2007).

Results

The photomicrographs obtained from the biceps brachii, deltoideus and triceps brachii from both cattle and camel demonstrated the morphology of the different fibre types (type I, type IIA and type IIB) as presented in Plates (1) and (2).

Even though the overall mean proportion of muscle fibre type I, type IIA and type IIB could not present any significant ($p>0.05$) differences between the camel and the cattle looking at it holistically (Table 2). As revealed in this work, the effects of the interactions of species versus age, species versus muscle and muscle versus age on the proportion of muscle fibre type I revealed that the deltoideus muscle of 1 year old camel had significantly ($p<0.05$) higher proportion (Table 1, Figures 1, 2 and 3). Type IIA muscle fibre obtained from the deltoideus muscle of 5-year-old camel showed a significantly highest value (Table 1; Figure 4), while muscle fibre type IIB showed significantly higher value from the triceps brachii of cattle aged 1 year (Table 1; Figures 5 and 6).

Discussion

Although the overall mean proportion of muscle fibre types I, IIA and IIB could not present any significant ($p>0.05$) differences between the camel and the cattle in this work, the effects of the interactions of species versus age, species versus muscle and muscle versus age on the proportion of muscle fibre type I revealed that the deltoideus muscle of 1 year old camel had significantly ($p<0.05$) higher proportion. The observed larger proportion of muscle fibre type I in triceps brachii irrespective of the species, is indicative that this muscle is larger and heavier than other muscle types. This observation is consistent with the report of Fuentes *et al*. (1998) that who furthermore also observed that type I fibre is designed for slow body movements and are significantly present in postural muscles. *Muscle fiber types differ between individuals, but they also differ between muscles. Some muscle groups are very slow twitched (they have a high type I muscle fiber proportion) and other muscle groups are very fast twitched (they have a high type II muscle fiber proportion), although most muscle groups display an even mixture of both fiber types.* The musculature of the forelimbs in larger animals appears to be more involved with maintaining a posture and as well aiding in propulsive movements, even though in this present research the individual roles of the limbs/muscles were not assessed.

All effects of interactions on type II A muscle fibre proportions indicated that the deltoideus muscle of 5 years old camel, triceps brachii of 3 years old camel and deltoideus of 3 years old cattle all had higher significant ($p<0.05$) values. All interactions on the proportion of muscle fibre type IIB indicated that 1 year old camel and cattle both had higher proportions ($p<0.05$), with the biceps brachii of camel having the highest value which was closely followed by the triceps brachii. This is consistent with the findings of Sonfada (2008) in camel, who reported that biceps brachii muscle showed the presence of fast twitch (white fibres) predominating the red fibres. That is possibly why camels can walk for long distance with sustained activitity and sometimes even being used in racing. In the same vein, it has been reported by Gonyea *et al*. (1981) that the proportion and regional distribution of muscle fibre types within a muscle appear to be related to the degree of functional complexity.

In agreement to the findings of Kadim *et al*. (2009) on their work on camel and cattle, and to the work of Brandstetter *et al.* (1998) on fibre characteristics in growing bull, this present work indicated that muscle fibre type IIB had the highest mean proportion followed by muscle fibre type IIA and type I from both the camel and cattle studied. For both species studied in this work, higher proportion of type IIB fibres is in agreement with Wegner *et al.* (2000), although with different distribution pattern for the other fibres types (types I and IIA).

The fact that muscle fibre type IIB (white fibres) predominate in the biceps brachii and triceps brachii muscles may be due to the fact that these muscles are under constant tension due to their structural, supportive and propulsive roles in these animal species thus depleting them of their glycogen content. Another possible reason could be due to the likely conversion of muscle fibres between types IIA and IIB as was reported by Brandstetter *et al.* (1998), with emphasis being laid on the preference of type IIB fibres even at an early stage after birth (Picard, *et al.*, 1995). Kassem *et al.* (2004) also reported that in camel muscles, type IIA fibres had the highest percentage followed by type IIB and type I respectively. The differences between the finding of the current study and that of Kassem *et al.* (2004) might be attributed to variations between the camels due to the heterogeneity of dromedary camels, as there are no established pure camel breeds as obtainable in other species, and this could also be probably due to differences in muscle type as they used longissimus thoracis muscle in their study.

A myofibre's indigenous physiological and metabolic properties contribute to the effectiveness and efficiency of the functioning of the skeletal muscle with respect to the support and movement of the body. The most comprehensive system of nomenclature for myofibre 'typing' gives a full description of these physiological and metabolic capacities, based on the relative contraction speed of the fibre and its propensity for oxidative and glycolytic metabolism (Handel and Stickland, 1987).

In general, the proportion of muscle fibre is related to changes throughout growth, and fast-growing farm animals were known to have more muscle fibres than slower growing ones. Within strain, the fibre number may increase with increasing average daily gain and gain: feed ratio (Stickland, 1995).

The importance of the knowledge of microscopic characteristics in the determination of myofibre types has been stated by several works, especially the parameters concerning meat quality (Lawrie, 1974). According to this author, meat quality is largely influenced by the changes in the morphology and muscle fibre composition during growth. The adoption of morphological and histochemical methods was also useful in the study of changes in myofibre size, distribution, frequency and connective tissue of different breeds of lambs (White *et al*., 1978; Sivachelvan and Davies, 1986; Suzuki and Tamate, 1988). Thus, this present work could be of help in advancing the knowledge on muscle morphology and as well be of help in meat science industry.

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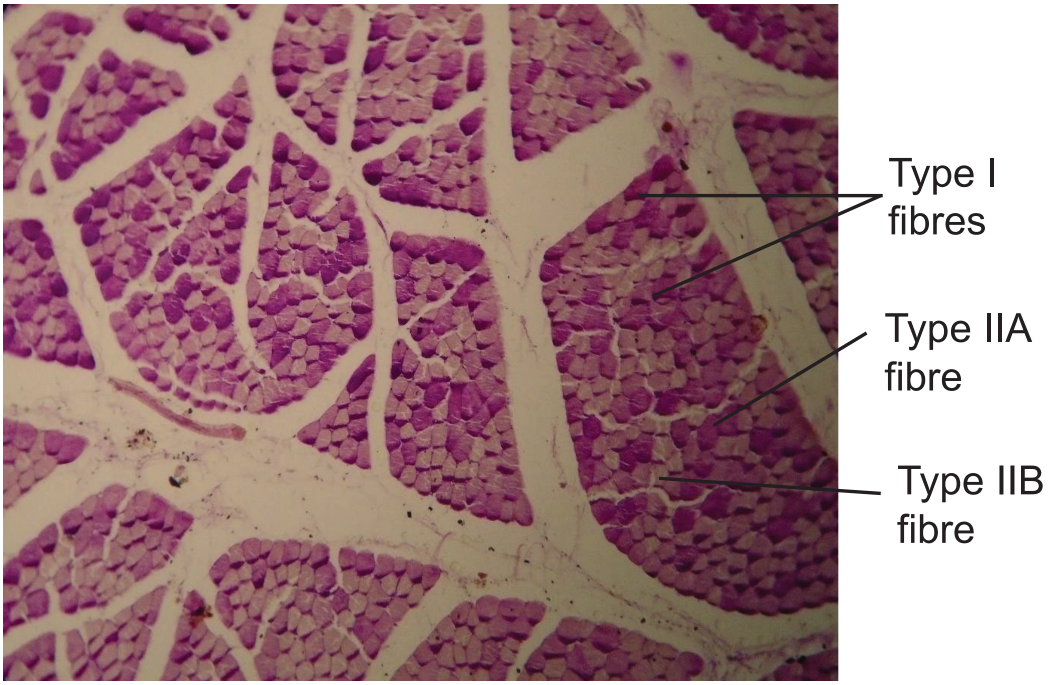

Plate (1): Photomicrograph of camel's deltodeus muscle demonstrating the fibre types (PAS x100).

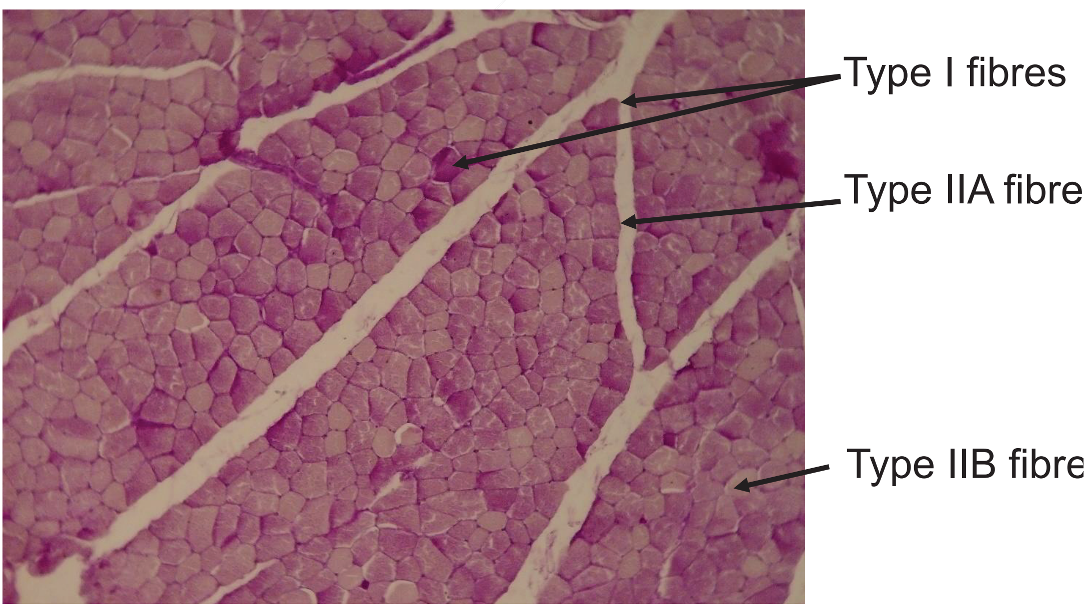

Plate (2): Photomicrograph of cattle's deltoideus muscle demonstrating the different fibre types (PAS x100)

Table (1): Proportions of Muscle fibre types in camel and cattle

Factors	Camel Type I	Cattle Type I	Camel Type IIA	Cattle Type IIA	Camel Type IIB	Cattle Type IIB
6 MO						
BB	17±1.30 ^{b}	32.2±1.59 ^{a}	32.2±2.76 ^{a}	17.6±1.21 ^{b}	48.8±1.36	49.2±1.56
D	40.6±1.20 ^{a}	34.2±1.77 ^{b}	30±1.00	25±2.17	29.4±1.57 ^{b}	40.8±2.56 ^{a}
TB	18.2±1.46 ^{b}	39.6±3.09 ^{a}	30.2±2.82	37.2±2.22	51.6±3.44 ^{a}	23.2±2.81 ^{b}
1 YO						
BB	20.2±3.14 ^{b}	35.6±2.16 ^{a}	22.2±1.98 ^{a}	14.4±1.86 ^{b}	54.6±3.54 ^{a}	50±2.70 ^{b}
D	53.4±5.46 ^{a}	36.8±1.56 ^{b}	23.8±2.48 ^{b}	29.8±0.86 ^{a}	22.8±3.48 ^{b}	33.4±1.50 ^{a}
TB	52.3±2.08 ^{a}	29.8±5.05 ^{b}	14.8±1.43	15.6±2.20	32±1.58 ^{b}	56.6±2.36 ^{a}
3 YO						
BB	31±1.22 ^{a}	20.2±2.59 ^{b}	21±2.00	27.8±2.42	48±1.67	50.4±3.09
D	33.4±3.85	35±3.19	35±4.59	39.4±3.07	31.6±3.91	25.6±3.53
TB	33.6±3.88	32.8±2.59	38.2±3.56	36±2.41	27.8±6.77	31.2±2.82
5 YO						
BB	33±2.12	26.2±2.35	30.2±1.16	30.4±0.51	36.8±3.04	43.4±1.96
D	24.6±4.90	33.4±2.50	40.6±3.19 ^{a}	27.6±4.94 ^{b}	34.8±5.23	39±5.51
TB	50.6±2.58	46.2±3.97	17±1.52 ^{b}	29.8±2.67 ^{a}	32.4±1.89	24±4.37
7 YO						
BB	29.4±3.41	23.6±3.34	22±1.58 ^{b}	29.4±2.16 ^{a}	48.2±3.51	47±3.88
D	24.6±4.13	38±4.70	36.2±4.69	31.6±3.81	37.4±3.29	30.4±5.39
TB	44.8±5.58	44.2±5.23	28.6±3.83	31.8±5.39	26.6±5.33	24±3.78

Key: *MO = Months Old, YO = Year Old, BB = Biceps brachii, D = Deltoideus, TB = Triceps brachii*

^{ab} Means bearing different superscript in the same row within a subclass differ ($p<0.05$)

Table 2: Overall mean proportions of muscle fibre types and their interactions in camel and cattle muscles

Factor	Percentages of muscle fibre types		
	Muscle fibre type I (%)	**Muscle fibre type IIA (%)**	**Muscle fibre type IIB (%)**
Species			
Camel	33.96 [a]	28.13 [a]	37.59 [a]
Cattle	33.92 [a]	28.23 [a]	37.88 [a]
SEM	1.07	0.82	1.07
Age			
6 months	30.47 [c]	28.70 [b]	40.83 [a]
1Year	38.17 [a]	20.10 [c]	41.40 [a]
3 Years	31.00 [bc]	32.90 [a]	35.77 [b]
5 Years	35.67 [ab]	29.27 [ab]	35.07 [b]
7 Years	34.40 [abc]	29.93 [ab]	35.60 [b]
SEM	1.69	1.29	1.67
Muscle			
Biceps	26.94 [c]	24.72 [c]	47.74 [a]
Deltoid	35.58 [b]	31.90 [a]	32.72 [b]
Triceps	39.30 [a]	27.92 [b]	32.74 [b]
SEM	1.31	1.00	1.31
Interactions			
S x A	**	NS	NS
S x M	*	NS	*
A x M	**	NS	**

[abcd] Means bearing different superscripts along the same column within a subclass differ ($p<0.05$)

Key: S = Species; M = Muscle; A = Age; SEM = Standard Error of the mean, NS = Not Significant; * $p<0.05$; ** $p<0.01$

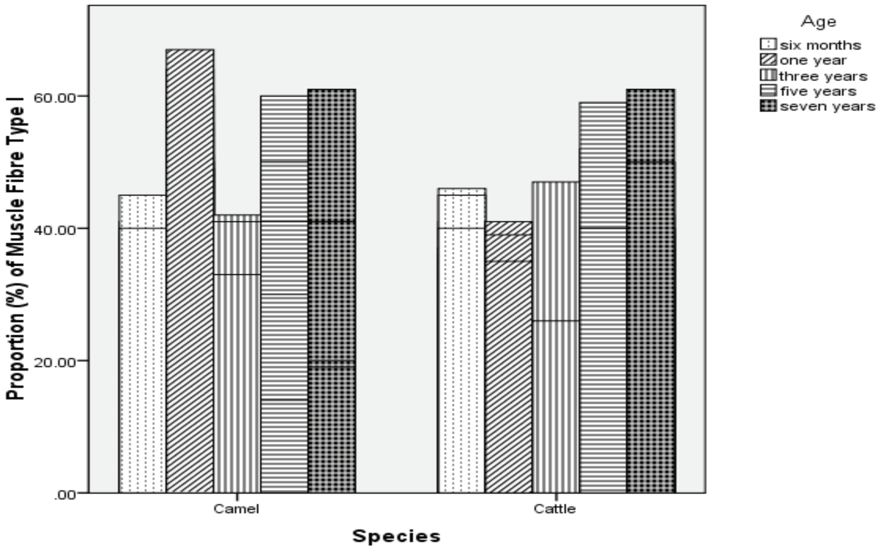

Fig (1): Effect of age versus species interaction on proportion of muscle fibre type I

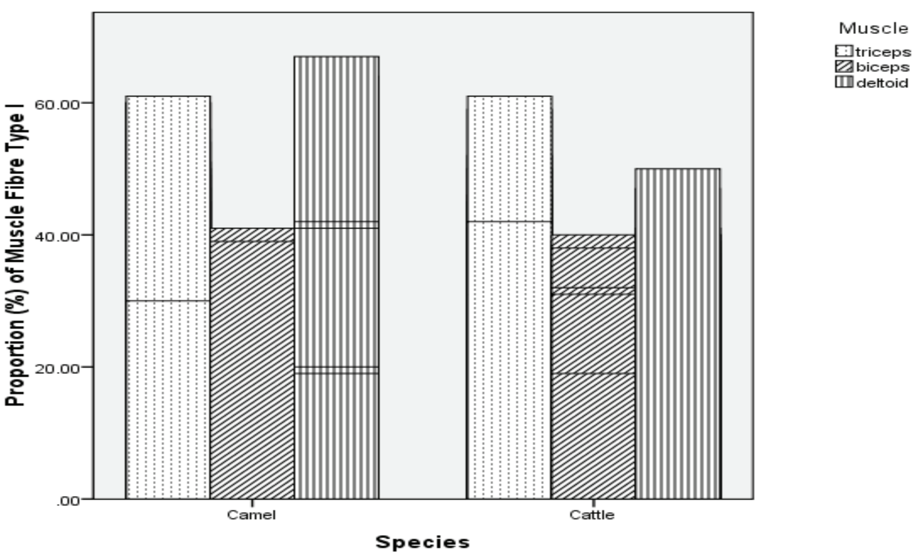

Fig (2): Effect of muscle versus species interaction on proportion of muscle fibre type I

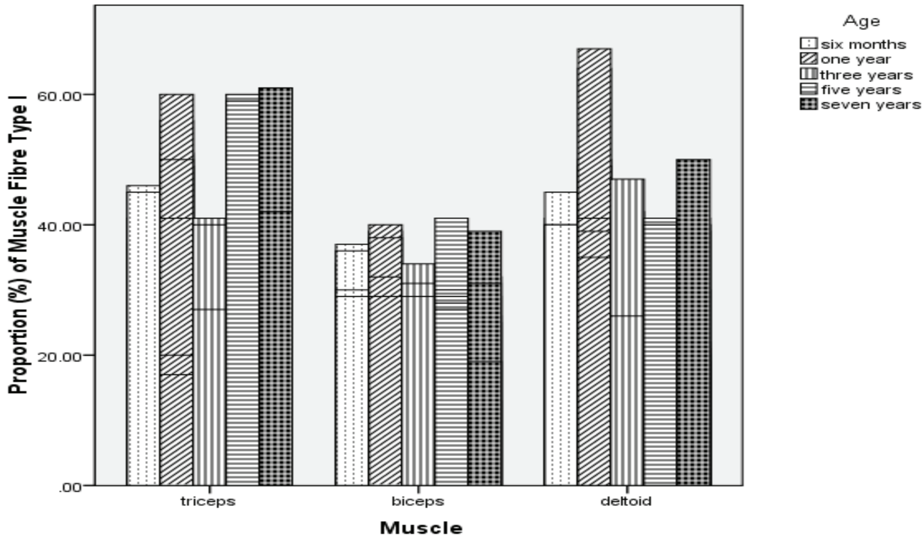

Fig (3): Effect of age versus muscle interaction on proportion of muscle fibre type I

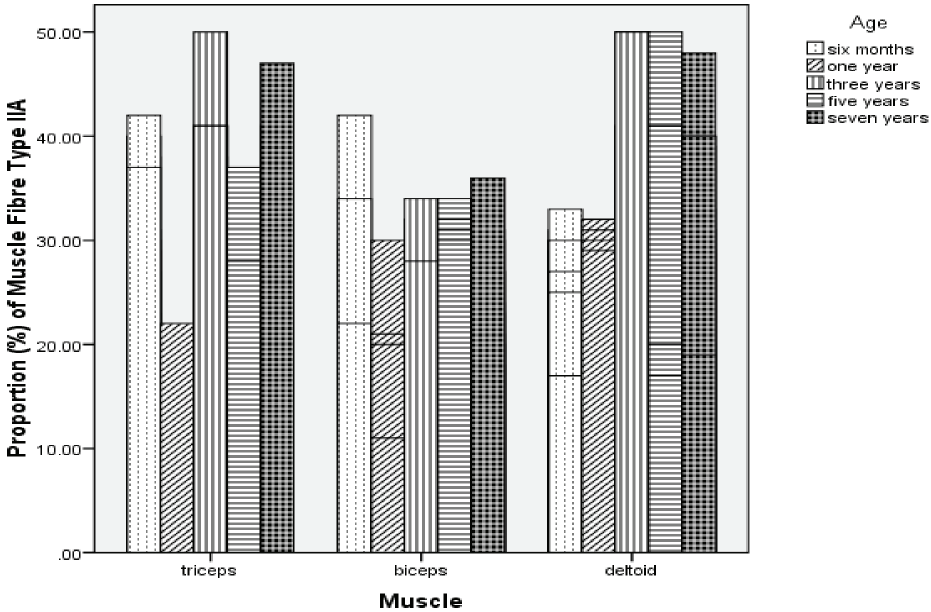

Fig (4): Effect of age versus muscle interaction on proportion of muscle fibre type IIA

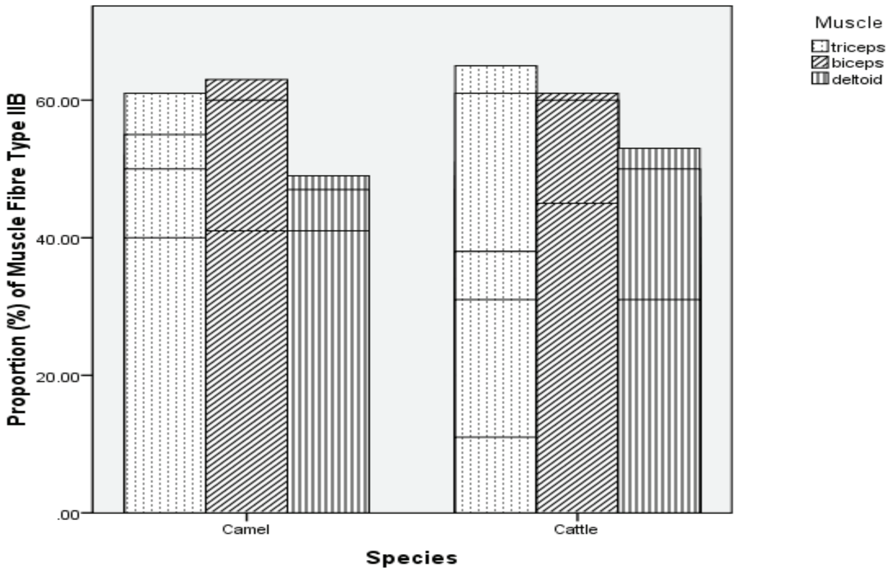

Fig (5): Effect of muscle versus species interaction on proportion of muscle fibre type IIB

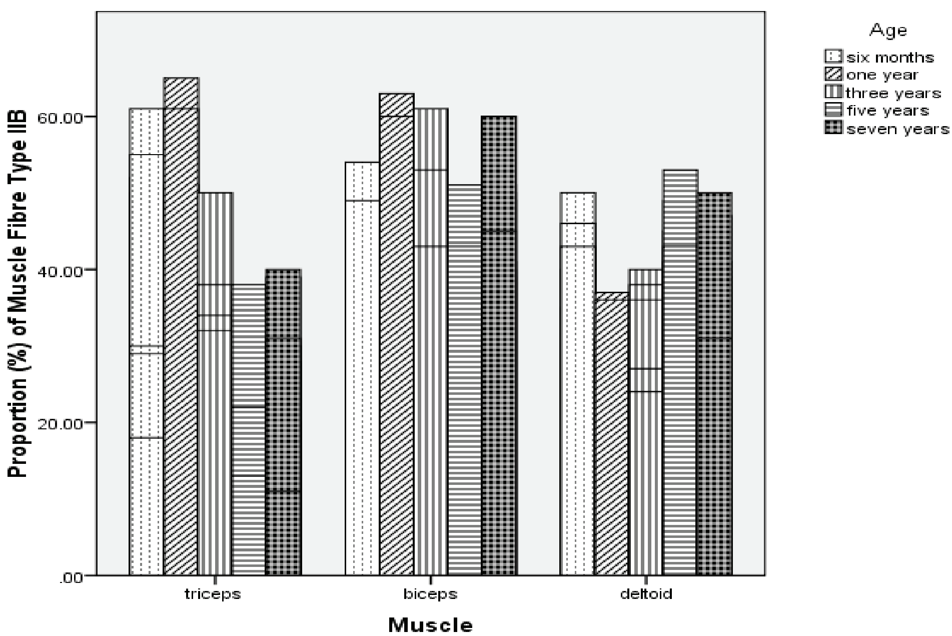

Fig (6): Effect of age versus muscle interaction on proportion of muscle fibre type IIB