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THE INFLUENCE OF AGE AND DIET ON MUSCLE FIBRE TYPE IN CHIANINA BREED: 2ND NOTE

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INTRODUCTION

Many factors such age, diet and exercise have been shown to affect muscle fibre types and distribution Swanson *et al.* (1965), Beecher *et al.* (1968), Johnson and Beattle (1973), West (1974) and Nicastro *et al.* (1991) showed that the type of muscle as well as the location within a muscle can have a drastic effect on fibre type characteristics. The role that muscle fibre composition and differentiation contributes to our understanding of animal growth and development is by no means fully delineated. It is well established that muscle which differs in function often differs in its metabolism. Muscle that is tonically active (red skeletal muscle), has a considerably higher rate of oxidative metabolism that white skeletal muscle. However, most skeletal muscle contains both a red (higher-oxidative) and white (low-oxidative) portion. The bovine species transforms nitrogen from its feed into proteins. Better knowledge of the physiology of growth and muscle development will help to define methods for producing high quality meat with an optimal yield. The aim of this study was to investigate the effects of two diets and three different ages on animal growth and development in two skeletal muscles (*semimembranosus* and *triceps brachii*) in the Chianina breed.

MATERIALS AND METHODS

Twenty-four Chianina breed bulls of six months old were divided equally into two treatment groups. Group A received a diet with medium energy level (0.76 Meat FU kg/DM), while group B was given diet with higher energy level (0.94 Meat FU kg/DM), throughout the study. Four cattle from each group were slaughtered respectively at 8, 12 and 14 months old (Nicastro and Maiorano, 1993) after 60, 180 and 240 day feeding regimes. Carcasses were held at 4°C for seven days. After chilling for 24 hours at 4°C carcasses were evaluated for quality and yield grade (ASPA, 1988). Samples of *semimembranosus* (SM) and *triceps brachii* (TB) muscles were collected from all animals four hours after slaughter were immersed into liquid nitrogen and mounted on spindles before sectioning 12μm thick using a Reichert-Jung freezing microtome. Serial sections mounted on glass microscope slides were stained with NADH-Tr (Engel and Brooke, 1966) and myofibrillar ATPase reacted at alkaline pH (Guth *et al.*, 1970) to differentiate muscle fibre type according to their oxidative and glycolytic capability. Fibres were classified into ßRed, αRed and αWhite according to Ashmore and Doerr (1971). Sections were analyzed using an Image Analyzer Vidas by Zeiss to determine fibre diameter and fibre percentage type for each fibre type. Sections from previously mentioned muscles were stained with Oil-Red-O and hematoxylin according to Lillie (1965) in order to stain fat cells in the intercellular space. The same Image Analyzer as described for muscle fibre type was used to measure fat cell size. The muscles evaluated for histological study were used for determining total myofibrillar, sarcoplasmatic, soluble non proteic and stroma (connective tissue) nitrogens based on that described by Helander (1957) as modified by Lawrie (1961). Data were analyzed by least squares procedures (SAS, 1985), assuming a mathematical model that include diet and age.

RESULTS AND DISCUSSION

Muscle fibre characteristics for the *semimembranosus* are presented in Table 1. The least squares means of all three fibre types in this muscle revealed little variation ($P<0.05$) in muscle fibre size in cattle that were fed two different energy level for up to 240 days. For comparing the three different ages, bulls of 14 months showed larger α-white fibres (74.2μm; $P<0.05$) and bulls of 12 months presented smaller ß-red fibres (48.7μm; $P<0.05$). These results, are in agreement with Gauthier (1970), that reported ß-red fibres have the smallest and α-white the largest diameter. The percentage of α-red fibres decreased and the percentage of α-white fibres increased slightly with increasing age, thus an increase occurred in the conversion of α-red to α-white fibres. Our results support those of Dreyer *et al.* (1977) and Solomon *et al.* (1986) who studied the influence of different breed and age in bovine muscles.

Least squares means for size and population of muscle fibres in the *triceps brachii* are presented in Table 2. Bulls at 12 months showed in this muscle from the forequarter, a larger fibre type with statistical significance for α-red and α-white fibres ($P<$).05). Between 12 and 14 months of age we noted a significant reduction in diameter of α-white fibres, which was due to animal variation since, the study did not reflect a continuous (biopsy) sampling (longitudinal) procedure to observe muscle from the same animal over time. The percentage of α-red and α-white fibres followed same trend of the SM muscle, though only the α-red fibres showed a significant ($P<0.05$) decrease. This variation within a specific area provides evidence that fibre transformation may be influenced by chronological age. The most likely path of fibre conversion is the shift in α-red type toward α-white fibres.

No significant energy level differences were apparent for both muscles on morphological characteristics of fibre types and percentages, further the diet B showed a trend for larger fibre size.

Least squares means for intramuscular fat cell diameters for SM and TB are also given in Tables 1 and 2. Fat cell diameters increased with level of energy in both muscles (26.7 vs 33.5μm in SM and 31.6 vs 37.2 μm in TB), but the differences were not significant, although as dietary energy levels increased the body fat normally increases with a concomitant increase in fat cell size. Chronological age appears to have a consistent effect on fat cell diameters in the SM muscle. The morphological parameters of fat cells were higher ($P<0.05$) in bulls of fourteen months which probably were closer to physiological maturity and also fatter than younger cattle.

Age influenced total nitrogen content (Table 3) that was higher in the older animals and the sarcoplasmatic fraction that decreased from 12 to 14 months but started increasing again from 12 to 14 months. Higher nutritive level was reflected in higher total nitrogen content in the muscles but did not influenced the nitrogens fractions.

Muscles showed differences whether in total nitrogen content, higher in semimembranosus than in triceps brachii; in particular semimembranosus differed from the other muscle showing the highest stroma nitrogens and the lowest myofibrillar nitrogen content and the triceps brachii presented the lowest soluble non protein content.

CONCLUSIONS

The characteristics of fibre types from the muscles used in this study appeared to be susceptible to animal growth, while no definite conclusion can be drawn on muscle fibre size response to nutritional manipulation with energy level.

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Table 1. Least squares means for size and population of muscle fibres and size of fat cells in *semimembranosus* muscle.

	Age, months			Nutritive level	
	8	12	14	A	B
# bulls	8	8	8	12	12
Diameter of muscle fibres,µm					
ßR	59.4[b]	48.7[a]	56.2[b]	53.1	55.9
αR	63.0	64.2	65.5	61.1	64.0
αW	70.8	65.5	74.2[b]	72.7	70.2
Populations, % of total					
ßR	25.9	26.9	32.7	24.3	23.6
αR	46.8[b]	41.6	30.7[a]	41.7	43.9
αW	41.6	31.5	36.5[b]	34.0	32.5
Diameter, fat cell, µm	30.4	21.3[a]	38.1[b]	26.7	33.5

[a,b] Mean values in the same row bearing different letters differ (P<0.05).

Table 2. Least squares means for size and population of muscle fibres and size of fat cells in *triceps brachii* muscle.

	Age, months			Nutritive level	
	8	12	14	A	B
# bulls	8	8	8	12	12
Diameter of muscle fibres,µm					
ßR	55.4	58.7	52.6	52.2	55.8
αR	54.9[a]	65.6[b]	61.6	60.0	61.6
αW	65.6	73.8[a]	63.5[b]	69.9	72.2
Populations, % of total					
ßR	27.3	29.2	31.7	30.4	28.4
αR	38.3[a]	34.3	30.3[b]	35.2	40.4
αW	34.4	36.5	38.0	34.4	31.2
Diameter, fat cell, µm	33.3	38.2	32.9	31.6	37.2

[a,b] Mean values in the same row bearing different letters differ (P<0.05).

Table 3. Nitrogen fractions as influenced by age, nutritive level and muscles. Percentages on total nitrogen. Total nitrogen % on wet basis.

	Total N %	Soluble non-proteic N % tot N	Sacroplasmatic N % tot N	Myofibrillar N % tot N	Stroma N % tot N
Age, months					
8	3.39[a]	12.33	18.88[b]	35.72	33.07
12	3.46[b]	12.81	17.07[a]	34.41	35.75
14	3.52[c]	12.58	18.67[b]	34.14	34.80
Nutritive level					
A	3.43[a]	12.38	18.25	35.20	34.14
B	3.48[b]	12.78	18.16	34.31	34.94
Muscles					
SM	3.48[a]	12.90[a]	17.85	31.24[a]	37.98[a]
TB	3.35[b]	12.03[b]	18.00	36.84[b]	33.09[b]
Muscle x age	NS	NS	NS	NS	NS
RSD	0.08	0.99	1.52	3.91	4.76

[a,b] Mean values in the same column bearing different letters differ ($P<0.05$).