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### SUMMARY

The purpose of this study was to determine if compensatory growth affected the histochemistry of muscles differing in growth impetus. Steers were fed three levels of dry matter intake: restricted, nonrestricted, restricted/realimented. Fiber traits differed more by muscle type than nutrition level, and interactions occurred. Realimentation affected fiber area more than fiber type. Ratios of  $\alpha$ -white fiber areas were highest in restricted animals and lowest in realimented animals. All fiber traits, especially percentage fiber area, differed by muscle. The percentages of  $\beta$ -red,  $\alpha$ -red, and  $\alpha$ -white fiber types by individual muscle were ECR (33.1, 30.3, 36.6); L (17.7, 42.1, 40.2); L (23.1, 30.6, 46.3); and ST (17.4, 30.2, 52.6), respectively. General ranking of muscles for redness of fiber was ECR = G > L = ST, whereas anaerobically ( $\alpha/\beta$  ratio), they ranked ST = G > L > ECR. Interactions for diet and impetus were observed that ST and L, both high/average impetus, followed a compensatory growth pattern;  $\alpha$ -white fibers were largest from animals on R diets, smallest from those on R diets, and intermediate from those on NR diets. The  $\alpha$ -red and  $\alpha$ -white fiber areas of the ECR, L and ST impetus muscle, increased as DMI increased. The G, an average impetus muscle, followed no definite pattern across fiber type and diet. We conclude that 1) diet affects muscles with different impetus classifications to different degrees, 2) generalizations of fiber type based on one muscle can be erroneous, and 3) muscle cell hypertrophy occurs to a greater extent in animals undergoing compensatory growth than in animals gaining at a more constant rate.

### INTRODUCTION

Nutrition influences muscle growth and distribution and size of muscle fiber types. Johnston et al. (1981) determined that as energy level in the diet increased, percentage of intermediate fibers decreased, while percentage of white fibers increased. Additionally, Berg et al. (1980) suggested that higher energy diets caused a physiological shift from intermediate fibers to white fibers.

During weight loss, muscle regions that are most necessary for survival are least affected, whereas those least necessary are affected the most (Berg and Butterfield, 1976). However, if nutrition becomes limiting for a period of time and then is returned to an adequate level, compensatory growth may result. Compensatory growth is a phenomenon whereby dietary restricted animals enter an accelerated phase of regrowth upon realimentation to a higher plane of nutrition, resulting in a greater than normal growth rate (Osborne and Mendel, 1915; Fox et al., 1972) and increased rates of gain and feed efficiency. The degree of compensatory growth is affected by the animal's growth impetus and the animal's stage of growth. Muscles of low impetus (distal limbs) develop early and constitute a higher percentage of the total muscle at birth than at maturity. Conversely, high impetus muscles (support muscles) constitute a lower percentage of total muscle at birth and develop faster later in the growth cycle (Berg and Butterfield, 1976). Because a better understanding of factors affecting muscle growth impetus will enable more efficient muscle food production, the primary objectives of this study were to determine the effects of dry matter intake on histological and histochemical characteristics of muscles differing in growth impetus.

### MATERIALS AND METHODS

Crossbred steers (n=18, 234-284 kg) were fed a supplemented corn silage diet and randomly assigned to one of three nutritional treatments differing in dry matter intake (DMI): restricted (R), fed at 1% of body weight for 120 d; non-restricted (NR), fed at 2.5% of body weight for 120 d; and restricted/realimented (RR), fed at 1% and then at 2.5% of body weight for two consecutive 60 d periods. Four individually penned animals were weighed weekly, and diets adjusted after a 12-h restriction of feed and water. Four muscles, selected by growth impetus, were sampled 4 to 6 h postmortem. The extensor carpi radialis (ECR), a low impetus

muscle, was sampled from its midpoint on the cranial surface of the radius. The gracilis (G), an average impetus muscle, was sampled at its midpoint between the stifle and the pelvic bone. The longissimus (L), a high/average impetus muscle, was sampled at the geometric center of the 9-10<sup>th</sup> thoracic vertebrae region. The lateral portion of the semitendinosus (ST), also a high/average impetus muscle, was sampled one-fourth the distance between the origin of the muscle and the achilles tendon. Samples (4 to 7) were cut (1 cm<sup>3</sup>) perpendicular to fiber alignment, mounted on cork, and frozen in liquid nitrogen. Transverse sections (10 μm) were cut using an IEC Microtome (1988). Fibers were classified by the β-red, α-red, and α-white system of Ashmore and Doerr (1971). Stained sections were analyzed morphometrically to determine: fiber area, fiber percentage type, and percentage-area for each fiber type. In addition, red/white α/β fiber ratios (Hunt and Hedrick, 1977) were calculated as  $[(\beta\text{-red} + \alpha\text{-red}) / \alpha\text{-white}]$  and  $[(\alpha\text{-red} + \alpha\text{-white}) / \beta\text{-red}]$ , respectively. Data were analyzed using a split-plot analysis with diet regimen as the whole plot and muscle as the sub-plot. Least significant difference procedures for split-plot analysis with unbalanced data were used to separate least squares means.

## RESULTS AND DISCUSSION

Diet affected only fiber areas, whereas significant differences occurred among muscles for all fiber traits. Interactions between diet and muscle were significant for several traits and will be discussed after main effects. Animals in the RR group responded as expected during the last 60 d when they received greater DMI; they outgained (ADG=3.0) the NR group (ADG=2.5). Observations also indicated more muscle development in both live animals and carcasses of the RR and NR groups than the R group. Throughout our data, there was a reoccurring "compensatory growth pattern" (CGP) whereby the R group was lowest (or highest) for a trait, the RR group was opposite for that trait, and the NR group was intermediate between them.

### DIET REGIMEN

**Fiber Area.** Ratios of red/white fiber areas (Table 1) were highest from animals on R regimens, smallest from those on RR regimens, and intermediate from those on NR regimens, which demonstrates the CGP discussed earlier. As a result of the animals' need to make efficient use of limited nutrients, muscles from those on R regimens were more aerobic than muscles from those on RR and NR regimens. Our results support Moody et al. (1980), who postulated that high energy diets caused a physiological shift from intermediate (α-red) to white (α-white) type fibers.

### MUSCLE

**Fiber Area.** Ratios of α/β fiber areas for ST and L were higher than those for G and ECR (Table 1). Ratios of red/white fiber areas of G and ECR (Table 1) were not different, but both were higher ( $P < .05$ ) than ratios of ST and L. Additionally, L had the lowest ratio of red/white area because of small β-red and α-red fibers. Hunt and Hedrick (1977) also reported that the ST and L were relatively white muscles. However, L fiber areas in this study were smaller than those reported by Hunt and Hedrick (1977). The ECR fiber areas are not well characterized in the literature, so no comparison could be made.

**Fiber Type.** Percentages of β-red, α-red, and α-white fiber types, respectively, by individual muscle were ECR (33.1, 36.6, 30.3); G (17.7, 42.1, 40.2); L (23.1, 30.6, 46.3); and ST (17.4, 30.2, 52.6). Consequently, ECR, G, and ST had the highest percentages of β-red, α-red, and α-white fibers, respectively. The percentages of β-red fibers in descending order were ECR > L > G = ST, whereas the percentages of α-white fibers in descending order were ST > L > G = ECR. Hunt and Hedrick (1977) reported the outside portion of the semitendinosus to be the whitest muscle they studied, having a smaller percentage β-red and a larger percentage α-white fibers than the longissimus. Fiber type percentages of the ECR and G were unavailable, so no comparisons could be made. Orders for ratios of α/β percentages and ratios of red/white percentages (Table 1) were ST = G > L > ECR and ECR = G > L > ST, respectively. Although these two ratios are somewhat opposite, the large percentage of α-red fibers in G was responsible for 1) ratios of α/β fiber percentages being larger in G than in L and 2) ratios of red/white fiber percentages in G being similar to ratios of ECR and L.

**Fiber Percentage-area.** This measurement may be the best indicator of muscle metabolism potential, because it accounts for fiber size and type. The ranking for percentage-area of  $\beta$ -red fibers was ECR > G = L > ST (Table 1). ECR had the largest percentage-area of  $\beta$ -red fibers. The ST was the whitest muscle in this study and had the lowest percentage-area of  $\beta$ -red fibers. The G had a larger  $\alpha$ -red percentage-area than all other muscles, because it had the highest percentage  $\alpha$ -red fibers. The ST and L had larger  $\alpha$ -white percentage-areas than the G and ECR. The ranking for ratio of  $\alpha/\beta$  fiber percentage-areas was ST > L = G > ECR. The ST had greater anaerobic potential than the L, because it had the largest and most  $\alpha$ -white fibers, as well as a smaller percentage of  $\beta$ -red fibers. Based on fiber percentage-areas, the ST was the most anaerobic muscle, the L and G were intermediate, and ECR was the most aerobic muscle tested. Ranking the muscles by increasing growth impetus (ECR > G > L = ST) resulted in a similar to that for aerobic fiber characteristics. This suggests that impetus may be due to inherit differences in fiber composition of the muscles.

#### DIET REGIMEN BY MUSCLE INTERACTION

**Fiber Area.** Areas (Table 2) of  $\beta$ -red fibers in ECR (low impetus) from animals on NR regimens were larger than those from animals on R and RR regimens. Diet regimen had no effect on the area of  $\beta$ -red fibers from L, ST, and G muscles. However, numerically, a CGP occurred in the ST and L. The area of  $\alpha$ -red fibers in ECR increased with increasing DMI (Table 2). Although fiber areas in G, L, and ST were not affected by diet, a numerical CGP was found in  $\alpha$ -red fibers from high/average impetus muscles (ST and L). Area of ECR  $\alpha$ -white fibers increased with DMI. The  $\alpha$ -white fibers from G were largest from animals on RR regimens, smallest from those on NR diets, and intermediate from those on R diets. The L showed a significant CGP, whereas the ST  $\alpha$ -white fiber area increased with increased DMI, but also showed a similar numerical CGP.

In general, fiber areas of low impetus muscle (ECR) increased as DMI increased, whereas average impetus muscle (G) was stable and no clear pattern was evident. The dynamic nature of intermediate fibers ( $\alpha$ -red) has been reported (Suzuki and Cassens, 1983). The large percentage of these fibers in G may explain why no pattern was evident. Although only significant for  $\alpha$ -white fiber area, the high impetus muscles (ST and L) exhibited a CGP for area of all three fiber types.

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Table 1. Least square means of fiber characteristics<sup>d</sup> by diet regimen<sup>e</sup> and muscle.<sup>f</sup>

Fiber area, $\mu\text{m}^2$	Diet regimen			Muscle: Impetus classification (n=15)			ST:High/Avg.
	R (n=4)	RR (n=6)	NR (n=5)	ECR:Low	G:Avg.	L:High/Avg.	
$\beta$ -red <sup>g</sup>	1619.6	1730.3	1788.5	2208.1	1600.0	1283.6	1759.4
$\alpha$ -red <sup>g</sup>	1815.3	2121.5	2021.0	2440.9	1700.6	1558.2	2444.1
$\alpha$ -white <sup>g</sup>	2258.8	2979.5	2797.9	2766.2	1949.2	2646.6	3353.0
$\alpha/\beta$ ratio	2.6	3.1	2.8	2.3 <sup>b</sup>	2.3 <sup>b</sup>	3.3 <sup>a</sup>	3.4 <sup>a</sup>
Red/white ratio	1.6 <sup>a</sup>	1.3 <sup>b</sup>	1.4 <sup>ab</sup>	1.7 <sup>a</sup>	1.7 <sup>a</sup>	1.1 <sup>c</sup>	1.3 <sup>b</sup>
<b>Fiber type, %</b>							
$\beta$ -red	23.5	24.1	20.9	33.1 <sup>a</sup>	17.7 <sup>c</sup>	23.1 <sup>b</sup>	17.4 <sup>c</sup>
$\alpha$ -red <sup>g</sup>	32.8	31.7	35.4	30.3	42.1	30.6	30.2
$\alpha$ -white	43.7	44.2	43.7	36.6 <sup>c</sup>	40.2 <sup>c</sup>	46.3 <sup>b</sup>	52.1 <sup>b</sup>
$\alpha/\beta$ ratio	3.6	3.7	4.5	2.2 <sup>c</sup>	4.8 <sup>a</sup>	3.4 <sup>b</sup>	5.2 <sup>b</sup>
Red/white ratio	1.5	1.3	1.4	1.9 <sup>a</sup>	1.6 <sup>a</sup>	1.2 <sup>b</sup>	0.9 <sup>b</sup>
<b>Percentage-area, %</b>							
$\beta$ -red	20.4	17.6	16.5	30.7 <sup>a</sup>	16.1 <sup>b</sup>	14.9 <sup>b</sup>	10.8 <sup>c</sup>
$\alpha$ -red	29.7	27.9	31.0	27.8 <sup>b</sup>	40.2 <sup>a</sup>	23.7 <sup>b</sup>	26.5 <sup>b</sup>
$\alpha$ -white	50.0	54.5	52.5	41.5 <sup>b</sup>	43.7 <sup>b</sup>	61.4 <sup>a</sup>	62.7 <sup>a</sup>
$\alpha/\beta$ ratio	5.0	5.8	6.5	2.5 <sup>c</sup>	5.7 <sup>b</sup>	5.9 <sup>b</sup>	8.9 <sup>b</sup>
Red/white ratio <sup>g</sup>	1.2	0.9	1.1	1.6	1.4	0.6	0.6

<sup>abc</sup> Means bearing different letters within a row and fiber trait are different  $P < .05$ .

<sup>d</sup>  $\alpha/\beta$  ratio was calculated using  $(\alpha\text{-white} + \alpha\text{-red})/\beta\text{-red}$  and indicates anaerobic potential. Red/white ratio was calculated using  $(\beta\text{-red} + \alpha\text{-red})/\alpha\text{-white}$  and indicates muscle redness.

<sup>e</sup> Diet regimen: R = Restricted, 120 day feeding at 1% body wt.; RR = Restricted/realimented, 60 day feeding at 1% body wt. 60 day feeding at 2.5% of body wt.; NR = Non-restricted, 120 day feeding at 2.5 % of body wt.

<sup>f</sup> Muscles: ECR = extensor carpi radialis, G = gracilis, L = longissimus thoracis, ST = semitendinosus).

<sup>g</sup> Interaction of diet and muscle was significant.

Table 2. Least square means of fiber area  $\mu\text{m}^2$  for significant interactions of diet regimen<sup>d</sup> and muscle<sup>e</sup>

Fiber type/muscle <sup>e</sup>	Diet regimen <sup>d</sup>		
	R (n=4)	RR (n=6)	NR (n=5)
<b><math>\beta</math>-red</b>			
ECR	2000.6 <sup>ay</sup>	2038.7 <sup>ay</sup>	2585.1 <sup>ax</sup>
G	1720.1 <sup>a</sup>	1556.2 <sup>bc</sup>	1523.8 <sup>bc</sup>
L	1158.4 <sup>b</sup>	1441.2 <sup>c</sup>	1251.3 <sup>c</sup>
ST	1599.3 <sup>a</sup>	1885.0 <sup>ab</sup>	1793.9 <sup>b</sup>
<b><math>\alpha</math>-red</b>			
ECR	1624.6 <sup>bz</sup>	2293.3 <sup>ay</sup>	2804.8 <sup>ax</sup>
G	1781.9 <sup>b</sup>	1808.0 <sup>b</sup>	1511.9 <sup>c</sup>
L	1405.4 <sup>b</sup>	1761.9 <sup>b</sup>	1507.1 <sup>c</sup>
ST	2449.2 <sup>a</sup>	2622.9 <sup>a</sup>	2260.2 <sup>b</sup>
<b><math>\alpha</math>-white</b>			
ECR	1869.6 <sup>by</sup>	2998.7 <sup>bx</sup>	3430.3 <sup>ax</sup>
G	1920.4 <sup>bxy</sup>	2228.2 <sup>cx</sup>	1699.0 <sup>cy</sup>
L	2327.0 <sup>by</sup>	3022.5 <sup>bx</sup>	2590.4 <sup>bxy</sup>
ST	2918.3 <sup>ay</sup>	3668.5 <sup>ax</sup>	3472.0 <sup>ax</sup>

<sup>abc</sup> Means bearing different letters within a column and fiber trait are different  $P < .05$ .

<sup>d</sup> Diet regimen: R = Restricted, 120 day feeding at 1% body wt.; RR = Restricted/realimented, 60 day feeding at 1% body wt/ 60 day feeding at 2.5% of body wt.; NR = Non-restricted, 120 day feeding at 2.5 % of body wt.

<sup>e</sup> Muscles: ECR = extensor carpi radialis, G = gracilis, L = longissimus thoracis, ST = semitendinosus.

<sup>xyz</sup> Means bearing different letters within a row and fiber trait are different  $P < .05$ .