RELATIONSHIPS BETWEEN PERFORMANCE, MUSCLE AMINO ACID CONTENT, AND MUSCLE FIBER CHARACTERISTICS IN YEARLING BULLS

by

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INTRODUCTION

As the world population increases, the subsequent demand for a high quality protein source makes it imperative for the wide scale, efficient production of high-protein products. The main source of animal protein for the United States as well as several other countries is beef. Cattle that will grow to market weight with efficient utilization of feed are becoming more and more desirable. Ideally, it would be best to be able to predict the feed efficiency of cattle without costly feeding trials. Numerous authors have studied the effects of muscle fiber traits, age, and nutrition on the quality and quantity of muscle, but none have related these to the feed efficiency of the animal. In addition, feed efficiency has not been related to the amino acid content of muscle.

The purposes of this study were: 1) to determine the relationships between feed efficiency, various muscle fiber traits, and amino acid content in longissimus muscle, 2) to determine if feed efficiency can be predicted in bulls using amino acid content or muscle fiber traits from muscle taken by biopsies and 3) to calculate the correlations between fiber traits, amino acid content, and certain performance traits.

GENERAL REVIEW OF LITERATURE

General Histochemistry

Most mammalian muscle is composed of three fiber types (Stein and Padykula, 1962; Ogata and Mori, 1964; Moody and Cassens, 1968) commonly known as red, white, and intermediate, although various terms have been used to describe the different fiber types. Red fibers have been called granular, dark, slow, small, tonic, Type C, Type I, and βR; while white fibers have been called granular, pale, fast, large, tetanic, twitch, Type A, Type II, and αW. Intermediates have been known as medium, Type B, and αR. To prevent confusion due to the different terminology, the αW, αR, βR classification system (Ashmore and Doerr, 1971) will be used. The cited author's terminology will be given first with the synonym given in parenthesis after it.

Red (βR) fibers are described as being smaller in diameter, higher in mitochondria, (Dubowitz and Pearse, 1960), oxidative enzyme activity (Farrell and Fedde, 1969; Nachamas and Padykula, 1958; Dubowitz and Pearse, 1960), lipase activity (Piantelli and Rebollo, 1967), and triglycerides (Adams et al., 1962; Denney-Brown, 1929), and lower in glycolytic ATPase activity (George and Naik, 1958b) than white fibers.

White (αW) fibers are low in oxidative enzyme activity, high in glycolytic enzyme activity (George and Naik, 1958b), high in ATPase activity, high in phosphorylase activity, large in diameter (Farrell and Fedde, 1969; Engle, 1962; Dubowitz and Pearse, 1960) and have few mitochondria per unit area (Nachimas and Padykula, 1955; Ogata and Mori, 1964; Stein and Padykula, 1962; Moody and Cassens, 1968).

Intermediate (αR) fibers are described as having histochemical and physiological characteristics which are in between those for βR and αW fibers (Bullard, 1919; Nachimas and Padykula, 1958; Ogata and Mori, 1965; Cooper et al., 1970).

Ogata and Mori (1964) found different fiber distributions in different muscles. Some muscles (for example, gastrocnemius and longissimus dorsi are termed "white" muscles due to their high content of αW fibers). Others (for example, soleus and triceps) are termed "red" due to their high content of βR fibers. Muscles near the body surface are high in αW fibers while less superficial muscles tend to be high in βR and αR fibers. This distribution also applies within a muscle with more αW fibers being found in the more superficial part of the muscle and more βR in the middle (Ogata and Mori, 1964; Beecher et al., 1965, 1968; May et al., 1977; Hunt and Hedrick, 1977).

Muscle Development

Denney-Brown (1929) stated that muscle fibers change as the animal matures. Pre-natally the fibrils are packed with granules and are slow in contracting. Soon after birth the fibrils lose the granular appearance until the muscle becomes a mixture of dark and clear fibers.

Tomanek (1975) found both the red soleus and white plantaris muscles are homogeneous in the neonatal cat. In the soleus, the ATPase reaction changed by the second post-natal week: between the third and eighth week there was a marked decrease in (αW) fibers and by week eighteen and in adult cats, dark (βR) fibers were almost absent. In the plantaris,

there was a slight decrease in the number of dark (βR) fibers between three and six weeks, but dark (βR) fibers comprised 70% of the fiber population thereafter.

Cooper et al. (1970) found fiber types cannot be distinguished in day old pigs; however, bundles or groups of bundles often differed in reaction from neighboring bundles. This indicated that the entire muscle does not develop at the same rate, but white (αW) and intermediate (αR) fibers could not be distinguished. By four weeks of age red (βR), white (αW), and intermediate (αR) fibers could be clearly recognized.

Fenchel (1966) could not recognize the three fiber types in human skeletal muscle between five and eight weeks of age. Mitochondrial reactions were uniformly intense and total quantity of myofibrils were insufficient for conclusive ATPase testing. Between eight and ten weeks of age Type II (αW) fibers were greater in number and had a larger diameter than Type I (βR) fibers. Type II (αW) fibers remained larger in diameter than Type I (βR) fibers. By week twenty, the percentages of fiber types were equal; however, fiber size was reversed with Type I (βR) being the larger fiber.

Wirsen and Larsson (1964) and Levine and Hegarty (1977) reported that three fiber types developed as three separate populations, while Germino et al. (1965), Dubowitz (1965), and Ashmore et al. (1973) suggested all fibers were similar and variations took place within a pool of fibers. Dubowitz (1965a) stated there was a positive correlation between length of gestation, and differentiation and general maturity of muscle at birth.

Ashmore et al. (1973), working with fetal pig muscle, found fibers which were to become β (red) fibers appeared before α (white) fibers, or red preceded white in fiber development. The central location of red fibers in pig muscle can be explained by this pattern of fiber development in the muscle.

Late in chick embryonic development, Germino et al. (1965) found a few fibers that exhibited low SDH activity. These were thought to have developed from dark staining red fibers that existed in early development. The reaction to SDH decreased in intermediate cells as the animal matured until they became light staining, and so gave the three fiber types.

Staun (1963) examined fiber number and diameter in the M. longissimus muscle in swine, finding that pre-natal growth was due to an increase in the number of fibers, and post-natal growth was due to an increase in length and diameter of existing fibers.

On the other hand, Swatland (1976) took samples from the M. sartorius muscle of pigs that were fed to grow at the rate of .7 kg per day and from the same pigs when held on a maintenance diet. When growing, the apparent number of fibers increased by 300 fibers per day; however, fiber number stayed the same or decreased when the pigs were held at maintenance level. This would support the idea that muscle is a dynamic and not a static entity.

Bendall and Voyle (1967) studied growth of the M. longissimus and the M. semitendinosus muscles in eight Hereford and eight Holstein steers from 11 days to 24 months of age. Fiber diameter was 15 μm at 1 month and 45 μm at 24 months of age. For the first 12 months the

cross-sectional area of the fibers increased in proportion to the diameter of the muscle; but, from 12 to 24 months of age the cross-sectional area of the fibers increased more rapidly than the diameter of the muscle so that it seemed as if fibers were lost from the muscle.

Muscle Fibers and Production Traits

Jeremiah and Martin (1976) observed significant sex differences in fiber diameter of the M. longissimus within Simmental, Charolais, and Chianina sire groups of bulls, heifers, and steers. Samples were taken from carcasses weighing approximately 270 kg. In general, at one-day post-mortem, mean fiber diameter was larger in bulls than in heifers, while mean fiber diameter in steers was generally smaller than bulls and heifers in all breeds.

Johnson et al. (1975) studied the effects of breed and time on feed on muscle fiber types in Angus and Charolais steers. Cattle fed 233 days (long-fed) had significantly larger mean fiber diameters and a higher mean β fiber area than cattle fed 153 days (short-fed). Long-fed cattle had a significantly higher βR diameter associated with extreme hypertrophy. There was a slight increase in number of βR fibers with increased time on feed. Alpha W fibers in Charolais were proportionally larger than in Angus. Breed itself did not have a constant effect on fiber distribution among αW, αR and βR fibers. In the group fed 153 days, Charolais had more αR fibers, while the Angus had more, large αR fibers at 233 days than Charolais steers. Charolais steers had a higher mean fiber diameter for all fiber types compared with Angus steers. Johnson et al. (1976) also found significant breed effects on fiber

diameters. Lewis et al. (1977) found fibers of crossbred steers from Angus dams had larger cross-sectional areas than steers from Charolais dams; breed of sire had no effect on the fiber areas.

Swatland and Cassens (1972) examined a high-gain and a low-gain line of rats for muscle hypertrophy. The dark (βR) muscles of the high-gain line were 19% larger in area than in the low-gain line; light (αW) fibers were 40% larger in area in the high-gain line. This would indicate that muscle fiber hypertrophy played a major role in the enlargement of the muscle. Alpha W fibers are larger in diameter than βR (Farrell and Fedde, 1969; Engle, 1962; Dubowitz and Pearse, 1960); therefore, an increase in (αW) fiber population contributes more to the cross-sectional area than a proportional increase in red (βR) fibers. On the other hand, Hooper and McCarthy (1976) and Aberle and Doolittle (1976) found mean muscle weight between lines remained the same, but the high-gain lines had more fibers; or, the muscle experienced hyperplasia instead of hypertrophy.

Hanrahan et al. (1976) studied the effect of selection for high and low body weights at five and ten weeks of age in mice. Selection for increased body weight resulted in higher muscle weights and fiber numbers. Fiber diameter increased only when selecting for weight gain at five weeks. Muscle weights, fiber numbers, and fiber diameters all declined when selection was for decreased body weight. Therefore, it seems that changes in muscle weight can be achieved in different combinations of fiber number and fiber size. Sex had no effect on fiber number; the effect of sex on fiber diameter appears to be dependent on the particular muscle and age at which the animals were sampled.

Conforth et al. (1973) slaughtered one steer and one heifer from the Holstein and Hereford breeds at six different weights (91, 182, 273, 364, 455, and 546 kg). Samples were taken to correspond to the round, loin, rib and chuck. There was a two-fold increase in fiber size over the weight range regardless of fiber type, age, sex or location of the sample. As weight increased, the percentage of white (αW) fibers increased, especially in the round, loin, and rib, while percentage of red (βR and αR) fibers decreased. Intermediate (αR) fibers remained about constant. Differences in fiber type or size at a given weight were non-significant, regardless of the sex or breed. Holsteins and heifers tended to have greater percentages of red fibers in the round, loin, and rib than Herefords and steers, especially at lower weights.

Fiber Types and Meat Quality

Selection for certain fiber types may cause deleterious effects on the quality of muscle as food (Ashmore et al., 1974). Double muscled cattle have fibers with larger diameters; more of these fibers are α with a larger percentage of those being αW (Holmes and Ashmore, 1972). These cattle are also more susceptible to stressful conditions, and have a more excitable temperament than normal cattle (Holmes et al., 1972; Holmes et al., 1973). With a higher proportion of glycolytic fibers utilizing more muscle glycogen, double muscled cattle under stress may produce meat of abnormal quality such as pale, soft, and exudative or dark cutting carcasses. The decreased fat deposition in the carcass would result in lower quality grades (Kidwell et al., 1952; Pomery and Williams, 1962; West et al., 1973).

The pale, soft, and exudative (PSE) condition in pork has been attributed to an increase in the number of αW fibers. Didley (1970) found heavily muscled pigs that developed the PSE condition had higher αW:βR and αR ratios than pigs with normal muscle. PSE pigs also had red (βR) fibers with smaller diameters than normal pigs.

Fiber Typing and Carcass Traits

If cattle were to be selected for performance traits based on fiber typing, other factors which are correlated to fiber types, diameter, and area would have to be taken into consideration. Fiber types have been shown to be related to carcass traits and palatability (Melton, 1975), double muscling (Holmes and Ashmore, 1972), maturity (Ramamohanarao, 1968), and live weight (Waldman, 1967). Fiber diameter has been correlated with meatiness ($r = .56$, Tuma et al., 1962), tenderness ($r = .43$, Tuma et al., 1962; $r = .47$, Cooper et al., 1968; $r = .82$, Herring et al., 1967), and have been shown to be significantly different between levels of maturity ($P < .05$, Romans et al., 1965). Individual cross-sectional fiber area was found to be significantly correlated to live weight ($r = .59$), hot carcass weight ($r = .64$), fat thickness at the 12-13th rib ($r = .75$), and yield grade ($r = .67$) (Melton, 1975).

Amino Acid Content

Amino acids are contained in about the same relative quantities in all muscles; variations within and between muscles are small (Block and Weiss, 1956).

Several methods have been used for amino acid determination. Microbial analysis used microorganisms which utilize a specific amino acid, and create various acid by-products in proportion to the amount of amino acid present. The acid is then titrated with base and the concentration of amino acid can be determined from a standard curve.

Column chromatography utilizes a column packed with a solid phase. The amino acids are eluded with a liquid phase, collected and analyzed. The amino acid analyzer uses this method with three liquid phases at three different pH's. The elucidant is mixed with ninhydrin at the end of the column and analyzed by colorimetry at 570 nm and 440 nm.

Values for various amino acids in beef obtained by other researchers are given in table 1.

TABLE 1. PERCENTAGE OF AMINO ACIDS AND AMMONIA IN BEEF AND VEAL MUSCLE OBTAINED BY VARIOUS RESEARCHERS

Sample	Lys	His	Arg	Thr	Ser	Glu	Pro	Gly	Ala
Beef	8.11	6.25	6.91	4.57	5.43				
Beef									
Veal									
Round									
Rib									
Chuck	8.31	3.18	7.01	4.13	4.34	14.5	5.31	6.26	
Flank	7.53	3.12	6.61	3.98	4.17		5.85	7.06	
Neck	8.09	3.12	6.56	4.05	4.04	14.9	5.59	7.75	
Plate	9.17	2.91	6.42	3.75	4.08	15.4	6.12	8.51	
Rib	8.83	3.22	7.17	4.18	3.79	15.3	5.41	7.00	
Rump	9.19	3.39	6.54	4.00	3.80	14.3	5.44	7.39	
Mean	8.52	3.16	6.72	4.02	4.04	14.88	5.59	7.34	
Loin	9.07	3.74	6.22	4.50					
Brisket	8.79	4.10	6.50	4.46					
Round	9.22	3.68	6.07	4.55					
Round	8.73	2.46	6.90	4.56	4.42	15.07	4.79	6.49	6.22
Round					4.5			4.5	6.2
Shoulder					4.6			4.6	6.4
Beef	8.90	3.41		6.32	4.59	4.03	15.28	3.78	4.86
Beef	8.2	3.2	4.1	5.2	5.3	14.1	4.0	8.2	9.0
Veal	8.1	3.2	5.2	5.5	5.3	13.8	4.2	7.7	8.8
Beef	8.4	3.4	4.7	5.2	5.2	14.0	4.0	7.5	8.9

TABLE 1. (Cont'd.) PERCENTAGE OF AMINO ACIDS AND AMMONIA IN BEEF AND VEAL MUSCLE OBTAINED BY VARIOUS RESEARCHERS

Val	Met	Ile	Leu	Tyr	Phe	Asp	NH_3	References
	3.17			4.30	4.94			Beach et al., 1943
5.0			7.7					Schweigert et al., 1944
5.3			7.3					Schweigert et al., 1944
5.2		5.9	7.9					Schweigert et al., 1945
4.9		5.8	7.6					Schweigert et al., 1945
5.79	2.44	5.13	6.15	3.09	4.08	8.78		Greenwood et al., 1951
5.75	2.25	5.07	9.23	3.19	4.03	8.82		Greenwood et al., 1951
5.82	2.20	5.81	8.55	3.36	4.25	8.73		Greenwood et al., 1951
6.50	2.29	5.06	7.75	3.15	4.58	8.58		Greenwood et al., 1951
5.48	2.40	5.27	8.48	3.17	4.02	8.84		Greenwood et al., 1951
6.41	2.41	5.11	7.98	3.63	3.85	8.80		Greenwood et al., 1951
5.91	2.29	5.24	8.35	3.33	4.14	8.80		Greenwood et al., 1951
5.29	2.47	5.20	8.60		4.23			Lyman & Kuiken, 1949
5.27	2.47	4.97	8.61		4.19			Lyman & Kuiken, 1949
5.49	2.50	5.18	8.89		4.34			Lyman & Kuiken, 1949
4.51	2.93	4.38	7.98	3.37	4.50	8.23	1.40	Tsen & Johnson, 1959 Alexander et al., 1953
4.84	5.01	2.71	4.82	8.11	3.60	8.99		FAO, 1970
5.6	2.5	5.1	8.2	2.8	3.5	9.6		Bigwood et al., 1953
5.6	2.3	4.9	8.2	2.7	3.7	9.4		Bigwood et al., 1953
5.5	2.4	4.6	8.4	2.6	3.4	9.7		Bigwood, E.J., 1963

MATERIALS AND METHODS

Biopsies were taken from 103 Polled Hereford bulls between 12 and 14 months of age and weighing between 354 and 409 kg. Cattle in the selection herd (n = 66) were selected for feed efficiency for 10 years while those from the control herd (n = 37) had not been selected (Blum, 1976). Both herds were of similar background. Bulls from the 1975 and 1976 calf crop were used. Age and pedigree were known on all bulls.

Prior to biopsying, the bulls were individually fed on a 140 day post-weaning feeding trial. The ration that was fed is given in table 2. Weights were taken every 28 days and feed consumption was recorded for each bull.

Biopsy Technique

The biopsies were taken following the procedure described by Melton (1975). The bulls were injected with a tranquilizer (Rompun^{R}) intramuscularly and with a local anesthetic (Xylocaine^{R}) along an incision site parallel to the mid-line and 3/5th of the way medial to the lateral edge of the longissimus muscle at the 12th rib. An incision approximately 15 cm long was made, the skin and fat retracted, and two parallel incisions approximately 13 cm long and 2 cm apart were made. A pair of Kelly forceps attached to a brace were inserted approximately 7 cm into the incision and clamped. Incisions were made on either side of the clamps and across the bottom of the clamps to remove the 13 cm x 7 cm x 2 cm sample. The site was closed with interrupted sutures and sprayed with Furacin powder.

TABLE 2. RATION FOR TEST BULLS.[a]

Feedstuff	International Reference Number	Percent Contribution to the Ration			
		Percent	TDN (%)	Crude Protein (%)	Digestible Protein (%)
Prairie Hay	1-07-956	25.0	12.50	2.13	1.03
Corn	4-02-931	43.0	39.13	4.30	3.23
Dehydrated Alfalfa	1-00-023	15.0	9.30	2.88	2.25
Soybean Meal	5-04-6000	12.5	10.13	6.44	5.48
Molasses	4-00-668	4.0	2.16	.51	.20
Salt		.5	.00	.00	.00
Total		100.0	73.22	16.26	12.19

[a]Values determined on as-fed basis.

Histological Sample Preparation

The muscle sample was immediately soaked in .05 M sodium pyrophosphate until contractions no longer occurred in the muscle when irritated with a razor blade. Samples 1 cm^3 were cut with a sharp razor blade, attached to a wet cork gasket material, and frozen in 2-methyl butane pre-cooled in liquid nitrogen. Samples were placed on dry ice to evaporate excess 2-methyl butane before tissue was sealed in plastic bags. Samples were stored in an ultra-low freezer (-81 C). Sections 10 μm thick were cut on a cryostat-microtome maintained at -20 C, mounted on coverslips, and stained for adenosine triphosphatase (ATPase) by a procedure (Appendix A) modified from Padykula and Herman (1955). In place of the sodium barbital buffer, 221 Buffer (2-amino 2 methyl 1 propyl solution, Sigma Chemical Co., St. Louis, Missouri) was used. The samples were washed in .04 M calcium chloride solution prior to preincubation to leach out excess sodium pyrophosphate. A serial section was stained for succinic dehydrogenase (SDH) using a procedure (Appendix B) modified from Nachlas (1957). Photomicrographs of both ATPase and SDH slides were taken on a Leitz Dialux microscope. Photographs were enlarged 200X and processed through a Spirotome print processor. Fiber size (least diameter, Brooke and Engle, 1969) was measured with a Zeiss Particle Size Analyzer. At least 400 fibers were counted for each bull. Beta fibers were those staining negative for ATPase and strongly positive for SDH (Ashmore and Doerr, 1971). Alpha fibers were those staining positive for ATPase and negative or intermediate for SDH. The mean percentage of β fibers for SDH slides was 38.64% vs. 36.06% for ATPase; the mean percentage of α fibers was 61.36% and 63.94% for SDH and ATPase, respectively.

Amino Acid Sample Preparation

Tissue not used for histological analysis was trimmed of excess fat and connective tissue, frozen on dry ice, and stored in an ultra-low freezer (-81 C). Frozen samples were placed in liquid nitrogen and pulverized in a commercial Waring Blender using the procedure of Borchert and Briskey (1965). The pulverized samples were freeze dried (Appendix C) for 24 to 28 hrs. in 250 or 500 ml round bottom flasks. Samples were then hydrolyzed and analyzed for amino acid content on a Beckman Model 119 Auto Amino Acid Analyzer.

Statistical Analysis

Least Squares analysis of variance was used to find the effect of year, sire, line (selection line vs. control line), and line by year interaction. Simple correlation coefficients were calculated among and between muscle fiber traits, amino acid content and live animal traits. Variables included birth weight, feed consumed, feed efficiency, adjusted feed efficiency (Blum, 1976), age, average daily gain, total number of β and α fibers, total area of β and α fibers, percentage of β and α fibers, percentage area of β and α fibers, β:α ratio, percentage of seventeen amino acids and percentage of protein in the longissimus muscle. Methods used to obtain the above variables are given below:

Feed Efficiency:

$$\frac{\text{Pounds of feed consumed}}{\text{Total pounds gained in 140 days}}$$

Average Daily Gain:

$$\frac{\text{Total pounds gained in 140 days}}{\text{140 days}}$$

Total Number of β and α Fibers:

This was obtained by individually counting the number of α and β fibers in a 4" x 6" area of photomicrographs.

Total Area of β and α Fibers:

The Zeiss Partical Size Analyzer was used to measure the diameter of each β and α fiber (at least 400 fibers per bull). This value was used to calculate individual fiber areas which were then added for total β or α fiber area.

Percentage of β and α Fibers:

$$\text{e.g. } \frac{\text{Total Number of } \beta \text{ Fibers}}{\text{Total number of } \beta \text{ fibers + total number of } \alpha \text{ fibers}}$$

Percentage Area of β and α Fibers:

$$\text{e.g. } \frac{\text{Total Area of } \beta \text{ Fibers}}{\text{Total area of } \beta \text{ fibers + total area of } \alpha \text{ fibers}}$$

β to α Ratio:

$$\frac{\text{Total number of } \beta \text{ fibers}}{\text{Total number of } \alpha \text{ fibers}}$$

Only bulls from 1976 (n = 53) were used in the muscle fiber analysis.

Partial correlations were run between certain production traits and certain amino acids.

RESULTS AND DISCUSSION

Ten years of selecting for feed efficiency has resulted in selection line bulls requiring .25 kg less feed per kg gain ($P < .05$) than the control line bulls. Selection line bulls gained an average of .90 kg per day on post-weaning test while control bulls gained an average of .87 kg per day ($P < .05$, table 3).

The means of muscle fiber characteristics were not significantly different between the two lines (table 4). Percentage of β fibers, as well as percentage of α fibers showed a non-significant 2.78% difference between the selection and control lines. The percentage of β fibers (35.21%) was larger than the 24.1% found by Johnston et al., (1975); 22.3% found by West (1974); 27.5% found by Hunt (1973); or 24.5% found by Reddy (1975). Romamohanarao (1968) reported 32.56% β fibers in the longissimus muscle from cattle of A maturity.

The selection line had 6.12% arginine in the longissimus muscle compared with 6.49% in the control line ($P < .05$, table 5). None of the other amino acid values were significantly different.

Muscle Fiber Correlation

Simple correlations (table 6) calculated between fiber characteristics showed percentage and area within each fiber type to be correlated ($P < .05$). The β:α ratio was correlated ($P < .05$) with the percentage of β fibers and the percentage area of β fibers ($r = .54$ and .59, respectively). The correlations between β:α ratio and α fiber

TABLE 3. LEAST SQUARES ± STANDARD ERROR OF PRODUCTION TRAITS OF SELECTION AND CONTROL BULLS.[a]

Variable	Selection Bulls Mean ± S.E.	Control Bulls Mean ± S.E.	Total Mean ± S.E.
Number	66	37	103
Birth Weight (kg)	34.13±.60	35.63±.78	34.88±.75
Feed Efficiency (kg feed/kg gain)	5.66±.06	5.91±.08*	5.79±.05
Average Daily Gain (kg/day)	.90±.01	.87±.01**	.89±.01
Adjusted Feed Efficiency[a]	5.64±.08	5.81±.11	5.72±.05

[a]Feed efficiency adjusted for $(\text{mid-weight})^{3/4}$ and age.

*Selection and control means differ ($P < .05$).

**Selection and control means differ ($P < .01$).

TABLE 4. LEAST SQUARES MEANS + STANDARD ERROR OF MUSCLE FIBER CHARACTERISTICS OF LONGISSIMUS MUSCLE IN SELECTION AND CONTROL BULLS.[a,b]

Variable	Selection Bulls Mean ± S.E.	Control Bulls Mean ± S.E.	Total Mean ± S.E.
Number	37	16	53
Total Number of β Fibers	180.77±14.96	166.24±20.02	171.51±10.10
Total Area of β Fibers (μm^2)	235,897.00±18.57	220,567.40±24.85	228,232.21±12.53
Percentage of β Fibers	36.60± 1.78	33.82± 2.38	35.21± 1.20
Percentage Area of β Fibers	31.15± 1.69	30.04± 2.26	30.59± 1.14
β:α Ratio	.56	.55	.56
Total Number of α Fibers	323.39±25.22	301.44±33.75	312.42±17.03
Total Area of α Fibers (μm^2)	537,161.13±39.00	500,141.36±52.19	518,651.37±26.33
Percentage of α Fibers	63.40± 1.78	66.18± 2.38	64.79± 1.20
Percentage Area of α Fibers	68.85± 1.69	69.96± 2.26	69.41± 1.14
Diameter of β Fibers (μm)	40.60± 1.37	41.81± 1.84	41.21± .93
Diameter of α Fibers (μm)	46.02± 1.53	45.79± 2.05	45.91± 1.03

[a]Model includes age, end-of-test weight, line and sire/line.

[b]Means between selection and control lines did not differ significantly ($P < .05$).

TABLE 5. LEAST SQUARES MEANS ± STANDARD ERROR OF PERCENTAGE OF AMINO ACIDS, AMMONIA, AND PROTEIN IN THE LONGISSIMUS MUSCLE IN SELECTION AND CONTROL BULLS.[a]

Variable	Selection Bulls Mean ± S.E.	Control Bulls Mean ± S.E.	Total Mean ± S.E.
Number	66	35	101
Lysine	9.08± .05	9.11± .08	9.09±.04
Histidine	4.20± .08	4.01± .10	4.11±.05
Arginine[b]	6.12± .09	6.49± .12	6.31±.06
Aspartic Acid	10.90± .83	10.25±1.14	10.57±.61
Threonine	4.80± .03	4.83± .04	4.81±.02
Serine	4.35± .05	4.38± .07	4.37±.04
Glutamic Acid	17.60± .14	17.50± .20	17.55±.11
Proline	4.19± .03	4.27± .07	4.23±.04
Glycine	4.56± .04	4.52± .06	4.54±.03
Alanine	6.34± .05	6.22± .07	6.28±.04
Valine	4.81± .06	4.63± .09	4.72±.05
Methionine	2.74± .05	2.80± .06	2.77±.03
Isoleucine	4.33± .03	4.29± .04	4.31±.02
Leucine	8.76± .07	8.65± .10	8.71±.05
Tyrosine	3.57± .05	3.71± .07	3.64±.04
Phenylalanine	4.27± .03	4.33± .04	4.31±.02
Ammonia	1.55± .08	1.61± .11	1.58±.06
Protein	87.02±1.19	84.79±1.64	85.91±.87

[a]Model includes line, line X year interaction, sire/line, end-of-test weight, and age.

[b]Selection and control means differ ($P < .05$).

percentages were the same in magnitude, but of opposite sign. Percentage of β fibers and percentage area of β fibers were both negatively correlated ($P < .01$) to all α fiber values. Percentage and percentage area of α fibers are negatively correlated to all β fiber values.

Birth weight was negatively correlated with percentage β fibers and percentage area of β fibers, and positively correlated with percentage and percentage area of α fibers ($r = .38$ and .34, respectively, table 7). Since the muscle fiber values are taken at a year of age, the meaningfulness of this relationship may be questioned. It does, however, support the hypothesis that α fibers develop from a primary β population (Germino et al., 1968; Cooper et al., 1970; Ashmore et al., 1975; Levine and Hegarty, 1977). In this case, as birth weight increases the percentage and percentage area of β fibers decrease and the percentage and percentage area of α fibers increase. This would indicate these individuals would tend to be heavier muscled at birth, and earlier maturing. Age was correlated ($P < .05$) with total area of α fibers. These were the only significant relationships between performance traits and fiber characteristics.

In the analysis of muscle fiber characteristics and the seventeen selected amino acids (table 8), percentage area of β fibers was correlated with phenalanine ($r = .28$) and lysine ($r = .26$). Percentage area of α fibers had negative correlations of equal magnitude with these amino acids. Isoleucine and β:α ratio were also correlated ($r = .29$). The lack of correlation between percentage protein and fiber percentages agrees with results found by Ramamohanarao (1968).

TABLE 6. CORRELATIONS AMONG MUSCLE FIBER CHARACTERISTICS OF THE LONGISSIMUS MUSCLE IN BULLS.[a]

Total Number of β Fibers	1.00				
Total Area of β Fibers	.89**	1.00			
Percentage of β Fibers	.49**	.40**	1.00		
Percentage Area of β Fibers	.49**	.41**	.93**	1.00	
β:α Ratio	.41**	.26	.54**	.59**	1.00
Total Number of α Fibers	.60**	.61**	- .36*	-.29*	- .14
Total Area of α Fibers	.48**	.66**	-.35*	-.38**	-.21
Percentage of α Fibers	-.49**	-.40**	-1.00**	-.92**	-.54**
Percentage Area of α Fibers	-.49**	-.41**	-.93**	-1.00**	- .59*
	Total Number of β Fibers	Total Area of β Fibers	Percentage of β Fibers	Percentage Area of β Fibers	β:α Ratio

[a]N = 53
*P <.05
**P <.01

TABLE 6. (Cont'd.) CORRELATIONS AMONG MUSCLE FIBER CHARACTERISTICS OF THE LONGISSIMUS MUSCLE IN BULLS.[a]

	Total Number of α Fibers	Total Area of α Fibers	Percentage of α Fibers	Percentage Area of α Fibers
Total Number of α Fibers	1.00			
Total Area of α Fibers	.88**	1.00		
Percentage of α Fibers	.36**	.35*	1.00	
Percentage Area of α Fibers	.29*	.38**	.93**	1.00

[a]N = 53
*P <.05
**P <.01

TABLE 7. CORRELATIONS BETWEEN MUSCLE FIBER CHARACTERISTICS AND PERFORMANCE TRAITS IN BULLS.

	Birth Weight	Feed Consumed	Feed Efficiency	Average Daily Gain	Adjusted Feed Efficiency	Age
Total Number of β Fibers	-.04	-.21	-.18	-.14	-.13	-.13
Total Area of β Fibers	-.06	-.07	-.13	.02	-.11	-.20
Percentage of β Fibers	-.38**	-.16	-.15	-.12	-.13	.08
Percentage Area of β Fibers	-.34*	-.21	-.15	-.21	-.10	.11
β:α Ratio	-.09	-.19	-.08	-.21	-.05	.08
Total Number of α Fibers	.31*	-.01	-.04	-.00	-.01	-.20
Total Area of α Fibers	.23	.13	.00	.18	.00	-.28*
Percentage of α Fibers	.38**	.16	.15	.12	.13	-.08
Percentage Area of α Fibers	.34*	.21	.15	.21	.10	-.11

*P <.05
**P <.01

TABLE 8. CORRELATIONS BETWEEN MUSCLE FIBER CHARACTERISTICS AND MUSCLE AMINO ACID; PERCENTAGE IN THE LONGISSIMUS MUSCLE IN SELECTION AND CONTROL BULLS.

	Total Number of β Fibers	Total Area of β Fibers	Percentage β Fibers	Percentage Area β Fibers	β:α Ratio	Total Number of α Fibers	Total Area of α Fibers	Percent α Fibers	Percentage Area α Fibers
Lys	-.03	.01	.18	.26*	.14	-.12	-.14	-.18	-.26*
His	.08	.07	-.02	-.04	-.00	.10	.09	.02	.03
Arg	.03	.05	.13	.14	.10	-.03	-.02	-.13	-.14
Asp	-.02	-.04	.05	.01	-.02	-.06	-.06	-.05	-.01
Thr	.00	-.02	-.14	-.13	.03	.06	.03	.14	.13
Ser	.05	.01	-.15	-.20	.08	.09	.10	.15	.20
Glu	.07	.08	-.20	-.20	-.13	.19	.19	.20	.20
Pro	-.10	-.02	-.05	.05	.00	-.04	-.04	.05	-.05
Gly	-.04	.00	.12	.04	.11	-.10	-.02	-.12	-.04
Ala	-.14	-.11	.19	.15	-.02	-.18	-.12	-.19	-.15
Val	.13	.20	.14	.16	.04	.03	.07	-.14	-.16
Met	.03	.03	.08	.03	.06	-.02	.01	-.08	-.03
Ile	-.03	-.08	.12	.17	.29*	-.14	-.21	-.12	-.17
Leu	-.03	-.02	.10	.18	.06	-.07	-.13	-.10	-.18
Tyr	.04	.02	.24	.21	.12	-.13	-.10	-.25	-.21
Phe	.16	.21	.17	.28*	-.04	.16	.11	-.17	-.28*
NH_3	.01	.01	.08	.12	-.07	-.06	-.09	-.08	-.12
Protein	.05	.12	-.21	-.21	.19	.19	.22	-.21	.21

*P <.05

Amino Acid Correlations

Tyrosine was negatively correlated with feed consumption ($r = -.26$) and average daily gain ($r = -.26$), and positively correlated with adjusted feed efficiency ($r = .26$) and birth weight ($r = .31$) (table 9). Age was positively (non-significant) correlated to tyrosine. Methionine was correlated ($P < .05$) with birth weight ($r = .27$). Tyrosine is active in thyroid hormone production which is involved in the regulation of metabolic rate. The relationship between tyrosine and production traits may reflect differences in individual metabolic rates.

Partial correlations were calculated between the production traits feed efficiency, feed consumption, and average daily gain; and the amino acids tyrosine and methionine (table 10) for the selection and control lines, and for the two lines pooled. Correlations for feed consumption with tyrosine and methionine were negative ($P < .01$) for the selection line, near zero for the control line, and negative ($P < .05$) for the pooled line values. Feed efficiency was positively correlated with tyrosine and methionine in the control line and pooled line ($P < .01$), but not in the selection line. Average daily gain was positively correlated to both amino acids ($P < .01$) in all cases. From simple correlation (table 9) it is generally noted that as the percentage of methionine and tyrosine increased in the longissimus muscle the animals consumed less, gained more slowly, and are less efficient in feed conversion. The partial correlations between average daily gain and tyrosine and methionine are of opposite sign from the simple correlations.

TABLE 9. CORRELATIONS BETWEEN PERCENTAGE AMINO ACID, AMMONIA AND PROTEIN IN THE LONGISSIMUS MUSCLE; AND PERFORMANCE TRAITS IN BULLS.[a]

	Birth Weight	Feed Consumed	Feed Efficiency	Average Daily Gain	Adjusted Feed Efficiency	Age
Lysine	-.02	-.02	.05	-.01	.03	-.00
Histidine	-.08	-.03	.04	-.02	.04	.03
Arginine	.04	-.00	.12	.00	.04	-.01
Aspartic Acid	-.01	-.03	.00	-.02	.02	.02
Threonine	-.07	-.08	.17	-.05	.09	.06
Serine	.12	-.15	.21	-.12	.16	.09
Glutamic Acid	-.21	.11	-.13	.11	-.13	-.16
Proline	-.07	-.12	.13	-.09	.11	.01
Glycine	-.13	-.10	.09	-.08	.09	.11
Alanine	.01	-.08	.11	-.06	.08	.08
Valine	.03	.15	-.09	.12	-.13	-.03
Methionine	.27*	-.15	.03	-.15	.10	.00
Isoleucine	-.01	.16	-.14	.13	-.15	-.04
Leucine	.04	-.05	.08	-.05	.05	.11
Tyrosine	.31*	-.26*	.22	-.26*	.26*	.17
Phenylalanine	-.08	-.01	.00	-.00	.00	-.04
Ammonia	.04	.10	-.16	.07	-.13	-.07
Protein	.03	.01	-.00	.01	-.01	-.02

[a] N = 103

*P <.05

TABLE 10. PARTIAL CORRELATIONS[a] OF TYROSINE AND METHIONINE WITH FEED CONSUMPTION, FEED EFFICIENCY, AND AVERAGE DAILY GAIN.

Trait	Selection Bulls		Control Bulls		Total	
	Tyrosine	Methionine	Tyrosine	Methionine	Tyrosine	Methionine
Number	68		36		104	
Feed Consumption	-.33**	-.37**	.07	.08	-.22*	-.22*
Feed Efficiency	.24	.09	.34**	.36*	.31**	.21*
Average Daily Gain	.44**	.31**	.37**	.34*	.38**	.29*

[a]Weight and age at end-of-test were included in the model
*P <.05
**P <.01

The biochemical and physiological relationships between muscle fiber characteristics, muscle amino acid content, and production traits are not understood. Although the means of the fiber percentages were not significantly different, the selection line tended to have a greater percentage of smaller β fibers in the longissimus muscle. This would indicate a slower conversion rate from β to α. Fiber content has been related to muscularity and physiological maturity. There was little difference between the amino acid content of α and β fibers and the relationship between muscle amino acids and performance traits is confusing. Since gene action is expressed through protein synthesis, different amino acid content would be caused by different genotypes. However, at this time there is little knowledge of these metabolic pathways and the exact cause and effect relationships are not known.

SUMMARY

Selecting for feed efficiency has differentiated the selection line from the control line with the selection line requiring .25 kg less feed per kg of gain than the control line. Birth weight, weaning weight and yearling weight were lower in the selection line; however, average daily gain on 140 day post-weaning test was higher in the selection line. There were no differences in the means of muscle fiber characteristics between the two lines. The percentage of arginine in the longissimus muscle was significantly lower in the selection line.

The percentage and percentage area within each fiber type were highly correlated ($P < .05$). Birth weight was negatively correlated to percentage and percentage area of β fibers ($r = -.38$ and $-.34$, respectively) and positively correlated with percentage and percentage area α fibers. Percentage area β and α fibers were correlated with phenalanine ($r = .28$ and $-.28$, respectively) and lysine ($r = .26$ and $-.26$, respectively). Isoleucine and β:α ratio were also correlated ($r = .29$). The lack of correlation between percentage protein and fiber percentages agrees with results found by Ramamohanarao (1968).

Tyrosine was negatively correlated with feed consumption and average daily gain, and positively correlated with adjusted feed efficiency and birth weight. Methionine was significantly correlated with birth weight. Partial correlations of tyrosine and methionine with feed consumption were negative ($P < .05$) but positive ($P < .05$) with average daily gain and feed efficiency using the pooled lines.

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APPENDIX

APPENDIX A

ATPase Staining Procedure (Modified from Padykula and Herman, 1955).

Procedure

1. Freeze tissue in 2-methyl butane cooled in liquid nitrogen. Store in an ultra-low freezer or on dry ice.

2. Cut sections on a cryostat maintained at -20 C. Air dry 30 minutes.

3. Fix in cold (-5 C) 10% formal-saline solution (75 ml 40% formaldehyde, 3.75 gm NaCl, 425 ml distilled water) 5 minutes at pH 7.0 ± .2.

4. Wash in 4 changes of distilled water for a total of 1 minute with continuous agitation.

5. Soak tissue in .04 M calcium chloride solution 15 minutes.

6. Pre-incubate 15 minutes in a solution of:

221 Buffer (Sigma)	5.0 ml
.18 M calcium chloride	5.0 ml
Distilled water	40.0 ml

   Adjust pH to 10.6

7. Wash in 4 changes of distilled water for a total of 2 minutes.

8. Incubate 30 minutes at 35 C in a solution of:

221 Buffer (Sigma)	5.0 ml
.18 M calcium chloride	5.0 ml
Distilled water	40.0 ml
ATP	.07480 gm

   Adjust pH to 9.6.

9. Rinse in 4 changes of 1% calcium chloride for 1 minute with continuous agitation.

10. Place tissue in 2% cobalt chloride for 3-5 minutes.

11. Rinse well in distilled water.

12. Place tissue in 1% light ammonium sulfide solution for 3 minutes.

13. Rinse in running tap water 5 minutes.

14. Dehydrate in decreasing ethanol series ending in ½ toluene-½ ethanol and 2 changes of toluene.

15. Mount in Histoclad.

APPENDIX B

SDH Staining Procedure (Modified from Nachlas et al., 1957).

Procedure

1. Section frozen tissue on cryostat at -20 C. Do not allow sections to dry.

2. Incubate 3-5 hours in the following medium:

.2% Nitro Blue Tetrazolium*	5.0 ml
.2% Sodium phosphate buffer	2.0 ml
1.626% sodium succinate*	2.0 ml
Mammalian ringers solution	1.0 ml

*Will keep for months when stored at 2-4 C.

3. Rinse in cold distilled water for 3 minutes.

4. Place tissue in 10% formalin for 30 minutes.

5. Rinse in distilled water 4 times for a total of 1 minute.

6. Place in 30% ethanol for 5 minutes.

7. Wash in distilled water for 3 minutes.

8. Mount in PVP (polyvinylpyrrolidone) prepared as follows:

   Dissolve 50.0 gm polyvinylpyrrolidone in 50.0 ml of distilled water. Let stand over night. Add 2.0 ml glycerol and stir well. A crystal of thymol may be added as a preservative.

APPENDIX C

Procedure for Freeze Drying.

1. Immerse frozen samples in liquid nitrogen. Break samples into smaller pieces.

2. Cool blender container with liquid nitrogen. Run it to make sure the blades are not frozen.

3. Put sample in blender and blend. Sample will be a fine powder. Store in freezer until ready to use.

4. Put dry ice and methanol in the center of the freeze dryer. Start vacuum pump.

5. Place pulverized sample in 250-500 ml round bottom flasks. Keep sample frozen.

6. Set flask in the dry ice-methanol mixture in the freeze dryer to cool.

7. Attach flask to freeze dryer attachment and turn valve to pull a vacuum. Bottom of flask should frost up and remain frosted. If flask should not frost or if it should melt, re-immerse flask in the dry ice-methanol mixture.

8. Freeze dry until sample crumbles to a powder when rolled between fingers and no moisture is apparent.

RELATIONSHIPS BETWEEN PERFORMANCE, MUSCLE AMINO ACID CONTENT, AND MUSCLE FIBER CHARACTERISTICS IN YEARLING BULLS

by

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ABSTRACT

Biopsies were taken from 103 Polled Hereford bulls between 12 and 14 months of age and weighing between 354 and 409 kg. One line of bulls (n = 66) came from a herd in which selection for feed efficiency had taken place for 10 years, while the other line (n = 37) came from a herd which had no selection. The biopsies were frozen, serially sectioned, and stained for SDH and ATPase.

Bulls from the selection line had a better average daily gain ($P < .01$) and were significantly more efficient in converting feed to gain ($P < .05$) than bulls from the control line. Percentage arginine in the longissimus muscle was found to be lower ($P < .05$) in the selection bulls.

Percentage and percentage area within each fiber type was found to be highly correlated ($P < .05$). Birth weight was correlated to percentage and percentage area of β fibers ($r = -.38$ and $-.34$, respectively). Percentage area β and α fibers were correlated with phenalanine ($r = .28$ and $-.28$, respectively) and lysine ($r = .26$ and $-.26$, respectively). Isoleucine and $\beta:\alpha$ ratio were also significantly correlated ($r = .29$). The lack of correlation between percentage protein and fiber percentages agrees with results found by Ramamohanarao (1968).

Tyrosine was negatively correlated with feed consumption and average daily gain, and positively correlated with adjusted feed efficiency and birth weight. Methionine was significantly correlated with birth weight ($r = .27$). Partial correlations were calculated

between the significant performance traits and methionine and tyrosine. The selection line bulls had significant partial correlations for feed consumption and average daily gain with both tyrosine and methionine. Control bulls had significant correlations for feed efficiency and average daily gain with both methionine and tyrosine. Partial correlations for the pooled values were all significant. From the simple correlations it was generally noted that as the percentage of methionine and tyrosine increased in the longissimus muscle average daily gain decreased, feed consumption decreased and the animal is becoming less efficient at converting feed into gain.

None of the correlations found in this study could be used to predict the feed efficiency of an animal due to their low values.