Studies on Bovine Nitrogen II. Changes in Sarcoplasmic, Myofibrillar, Stroma and Lipid Protein and NPN During Growth

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Story in Brief

Experimental materials for this study consisted of muscle tissue acquired by live animal biopsy from the 11th and 13th rib and 2nd and 4th lumbar areas of the left and right longissimus muscle of six grade Angus steer calves. Nitrogen analysis was done by macrokjeldahl procedures and the results expressed as percent nitrogen on a wet tissue basis. Results showed no significant differences ($P>.05$) between period means in the sarcoplasmic protein subfraction although differences ($P<.05$) were observed in the sarcoplasmic NPN. Myofibrillar NPN displayed no significant changes ($P>.05$) with growth but differences ($P<.01$) were observed in the quantities of myofibrillar protein nitrogen. Neither the stroma protein nitrogen nor NPN showed apparent changes during growth ($P>.05$) although decreases were noted in the stroma protein subfraction. Both lipid protein and lipid extractable nitrogen reflected differences ($P<.01$) in their quantities during growth. No apparent changes ($P>.05$) were evident in the lipid NPN subfraction during the study.

Introduction

Cellular proliferation, be it in size or number, has been the object of intense research in mammalian muscle biology since the turn of the century. Gross studies on muscle tissue by such workers as Moulton (1923), Herrmann and Nicholas (1948) and Dickerson and Widdowson (1960) have shown increases in protein, minerals and nonprotein nitrogen with age. Such changes may be due to hyperplasia, an increase in cell numbers, which purportedly occurs prenatally in meat animals, or to hypertrophy, increases in cell size, which occurs postnatally. Assuming this to be true, animal growth can be indexed according to the fluctuations made by the cellular constituents, knowing that chemical growth postnatally infers cellular hypertrophy.

Animal scientists have exploited these "facts" in their use of calcium, potassium, total protein and gross carcass cutouts to predict or establish muscling potential or muscling in the meat animal. As-

sociated protein changes in the bovine have been described by Helander (1957) and Lawrie (1961), yet information is lacking on the complete quantitiation of all subfractions of muscle nitrogen which might occur from near birth to a constant market age in the bovine. This study was designed to obtain such information.

Materials and Methods

Muscle tissue acquired by live animal biopsy from the 11th and 13th rib and 2nd and 4th lumbar areas of the left and right longissimus muscle from six grade Angus steer calves served as the experimental materials for this study. Tissue was excised initially at an average age of 46 days and every 56 days thereafter until an average age of 428 days was attained. Upon removal, the muscle tissue was immersed immediately in liquid nitrogen, frozen solid, and then stored at —20°C until analyzed. Duplicate one gram muscle samples were partitioned into total sarcoplasmic and myofibrillar fractions by low ionic ($\tau/_2 = 0.048$) and high ionic ($\tau/_2 = 0.66$) KI-PO_4 buffers respectively, pH 7.4 and at 1.1°C. That tissue which remained after successive homogenation and centrifugation with the extracting buffers was referred to as the insoluble or stroma fraction. The "floating material" apparent after each centrifugation was separated and identified with its parent fraction.

After all fractions were prepared, 20 percent TCA was used to separate protein and nonprotein nitrogen subfractions. Thus, nitrogen analysis, performed by the macrokjeldahl method, was done on sarcoplasmic, myofibrillar, stroma and lipid protein and nonprotein nitrogen. The data were analyzed via a completely randomized design and the results expressed as a percent nitrogen on a wet tissue basis.

Results and Discussion

Sarcoplasmic protein nitrogen, Figure 1, showed consistant, nonsignificant ($P>.05$), slightly decreasing trends during growth. Data from this study appear to correlate favorably with some research and varies considerably from conclusions of others. In the literature there appears to be different patterns of change of sarcoplasmic proteins during growth and these differing patterns exist between and within species. Hence, inferences made from the changes in this fraction between animals within the same specie could be questionable due to differing age and environmental effects at time of sampling. Comparisons between species are valid only in establishing general trends of growth.

Sarcoplasmic NPN, Figure 1, showed variable responses between the preweaning and postweaning phases of the study. Such changes were

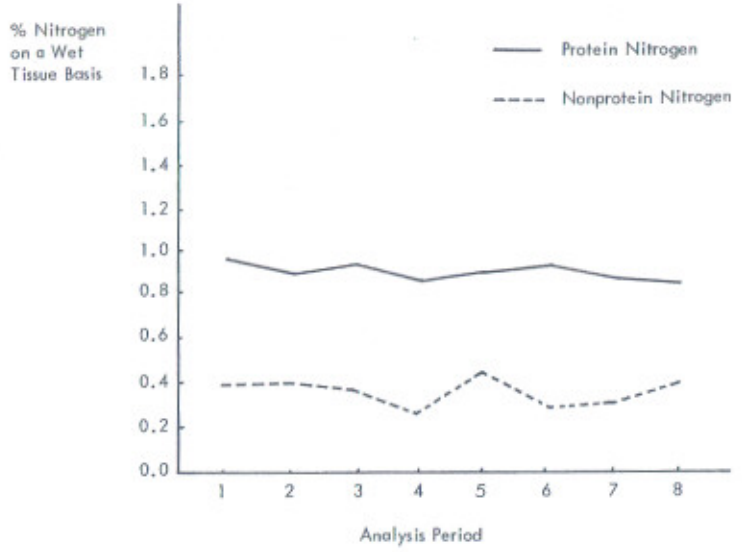

Figure 1. Changes in Sarcoplasmic Protein and Nonprotein Nitrogen During Growth

found to be significant ($P<.05$) as noted in Table 1. The general patterns of this fraction were similar to those reported by Robinson (1952) and Helander (1957). The inflections noted in periods five and eight were not expected and the causes of these inflections cannot be substantiated

Table 1. Period Mean Values[1] for Sarcoplasmic, Myofibrillar, Stroma and Lipid Protein and Nonprotein Nitrogen During Growth

Nitrogen Fraction	Nitrogen Subfraction	Analysis Period							
		1	2	3	4	5	6	7	8
Sarcoplasmic	Protein	0.97	0.90	0.94	0.86	0.90	0.93	0.89	0.88
	NPN*	0.38	0.39	0.36	0.31	0.44	0.31	0.32	0.40
Myofibrillar	Protein**	0.50	0.62	0.62	0.68	0.85	0.68	0.91	1.19
	NPN	0.02	0.01	0.01	0.01	0.08	0.01	0.09	0.02
Stroma	Protein	0.39	0.20	0.18	0.38	0.31	0.32	0.26	0.24
	NPN	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01
Lipid	Protein**	0.29	0.40	0.48	0.39	0.33	0.35	0.36	0.37
	Extractable**	0.48	0.47	0.34	0.17	0.11	0.10	0.09	0.08
	NPN	0.01	0.03	0.02	0.03	0.01	0.02	0.04	0.04

[1] All values expressed as percent nitrogen on a wet tissue basis.
* Values indicate significance ($P\leq.05$)
** Values indicate significance ($P\leq.01$)

at this time. It appears logical, however, that the change-over to the concentrate feeding regime may have resulted in nitrogen retention in the tissues. Also, in period eight, elevated protein synthesis may have caused an increase in amino acids and peptides which were too small in molecular weight to be precipitated by TCA and were included in the NPN fraction.

Myofibrillar protein nitrogen displayed increasing trends from the first to the final analysis period (Figure 2) with differences between period means being significant ($P<.01$). These conclusions correspond well with results of Helander (1957) and Dickerson and Widdowson (1960), yet the increases through periods seven and eight were not expected. Such continued inflections in protein deposition might be attributed to compensated growth resulting from nutritional restriction early in life.

Changes in myofibrillar NPN during growth, Figure 2, have resulted in no apparent changes ($P>.05$) between period means during the course of this study. The quantities which this subfraction comprised represented a small percentage of the total myofibrillar nitrogen with 4.0 percent in period one and a decrease to 1.7 percent in period eight.

Stroma protein and nonprotein nitrogen changes during growth are shown in Figure 3. No differences ($P>.05$) between period means were

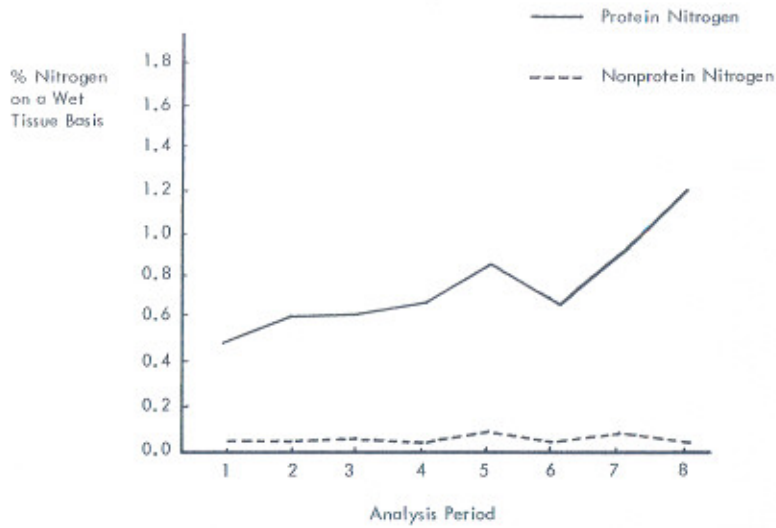

Figure 2. Changes in Myofibrillar Protein and Nonprotein Nitrogen During Growth

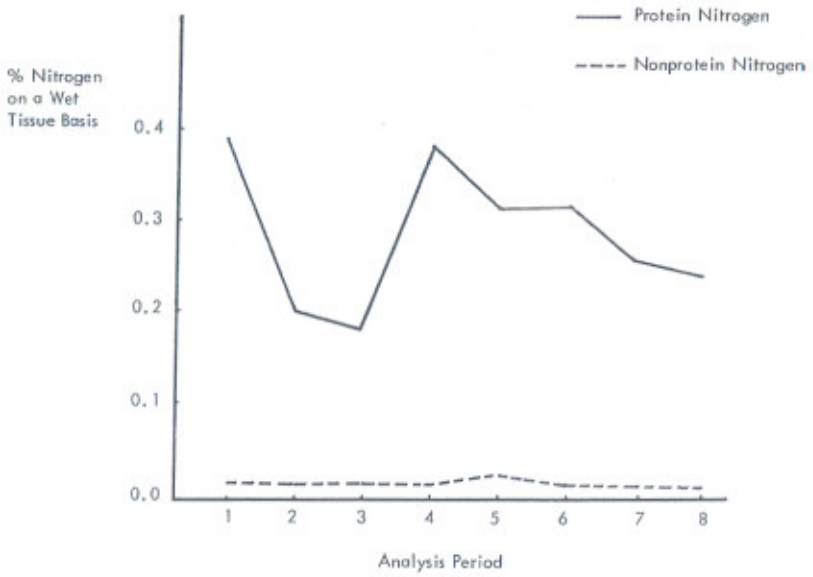

Figure 3. Changes in Total Stroma Protein and Nonprotein Nitrogen During Growth

noted in either of these fractions (Table 1). Moreover, only very small quantities were observed in the NPN subfraction. The protein component showed variable but decreasing trends from the first to the last test periods. Such decreasing trends have been reported by Lawrie (1961), Chiakulas *et al.* (1965) and Hunsley *et al.* (1971) and these reports agree that stroma nitrogen decreases in quantity during the growth process of animals.

Total lipid nitrogen, Figure 4, was partitioned into its protein and NPN subfractions and into a lipid extractable portion. This extractable subfraction was obtained by successive low and high ionic strength extractions of the lipid floating material to insure that no sarcoplasmic or myofibrillar nitrogen was retained in the lipid network prior to TCA separation of the protein and NPN. Lipid NPN displayed very slight fluctuations with no differences ($P>.05$) noted between period means. Lipid protein nitrogen increased from periods one to three and then decreased with age resulting in statistically significant ($P<.01$) changes. Lipid extractable nitrogen resulted in significant changes ($P<.01$) during growth with decreasing quantities preweaning and low, consistant values postweaning. Results of this nature are antagonistic to trends normally associated with lipid deposition in skeletal muscles. Hence, these sub-

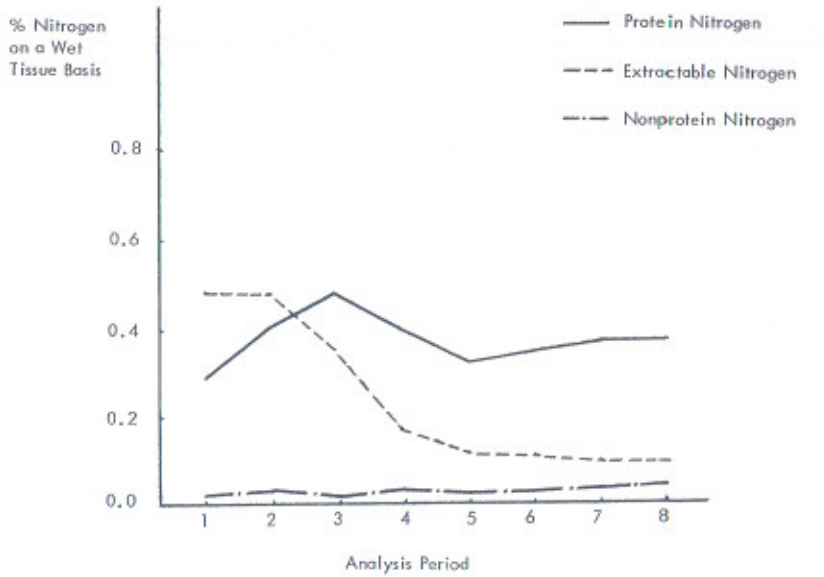

Figure 4. Changes in Lipid Protein, Nonprotein and Extractable Nitrogen During Growth.

fractions are not directly associated with depot fat deposition, but appear to coincide with plasma lipid concentrations.

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Use of K^{40} Net Count as a Monitor of Body Composition Changes in Growing and Fattening Swine

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Story in Brief

This research was initiated to investigate the possibility of using the K^{40} technique to monitor the body composition changes in muscling in swine at different ages and weights. A new detector arrangement was utilized in this series of whole-body counting studies with swine in an attempt to improve K^{40} counting efficiency over live weights ranging from 100 to 300 pounds. The new arrangement of detectors provided for more flexibility in the K^{40} counting of live pigs over a range of live weights than has been possible in the earlier phases of this work using plastic scintillation detectors.

One hundred barrows (70 Hampshires and 30 Yorkshires) were used in this study that involved ten replications of ten pigs each. Each replication consisted of ten feeder pigs that were randomly allotted to slaughter weight groups of 100, 150, 200, 250 and 300 pounds and placed on a growing-fattening ration. From each replication, two pigs were randomly assigned to each slaughter weight group, making a total of 20 pigs for each of five slaughter weights. Each pig was taken off feed and K^{40} evaluated at each weight interval, irrespective of final slaughter weight, and then was placed back on feed until it reached the pre-determined slaughter weight. The pigs were slaughtered at their pre-determined slaughter weight immediately following live K^{40} evaluation.