Studies in Bovine Nitrogen I. Changes in Total Nitrogen, Protein Nitrogen, NPN and Lipid Nitrogen During Growth

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Story in Brief

Muscle tissue excised from the longissimus dorsi muscle of six grade Angus steer calves was partitioned into total nitrogen, protein nitrogen, NPN and lipid nitrogen. Nitrogen determinations of muscle extracts were accomplished by kjeldahl analysis and the results were expressed as a percent nitrogen on a wet tissue basis. Total protein nitrogen showed increasing yet nonsignificant ($P>.05$) trends during growth with very evident increases noted at the final analysis periods. Total NPN showed relatively low but variable quantities with no significant ($P>.05$) changes during the study. These changes appear to be influenced directly by dietary effects and protein synthesis effects. Total lipid nitrogen showed a highly significant ($P<.01$) decreasing pattern during growth. This component appeared to be greatly affected by dietary changes between the maternal and feedlot phases of growth. Total muscle nitrogen showed little net increase in its quantities and resulted in no apparent ($P>.05$) changes during growth.

Introduction

Quantitative nitrogen analysis of mammalian muscle tissue has been used in clinical research to ascertain changes in protein deposition during growth and development. Age associated nitrogen changes were determined by Herrmann and Nicholas (1948) in their work with rat tissue from midgestation throughout the rat's life span. This work was followed by similar research on various species common to the laboratory. When trying to compare nitrogen changes with age between laboratory animals and animals used in "red meat" production the inferences obtained become very questionable.

In bovine muscle studies, Helander (1957) and Hunsley (1971) determined the changes in various nitrogen components with age and between breed types. Such researchers, however, failed to exhaustively extract and quantitate total muscle nitrogen with regards to its major components from immediately postpartum to a standard end point. Realizing that information obtained from such a bovine growth study would

allow the researcher to gain an understanding of protein deposition and its development patterns, investigations of this nature were initiated at this station.

Materials and Methods

Tissue obtained from the 11th and 13th thoracic and 2nd and 4th lumbar areas of the left and right longissimus muscle of six grade Angus steer calves served as the experimental materials. Live animal biopsies as described by Guenther (1972) were taken initially at an average age of 46 days and subsequently at 52 day intervals until an ultimate 428 day market age was attained. The calves remained with their dams until approximately 205 days of age and were then placed on feed. After the final analysis period, the steers were slaughtered by standard slaughter procedures.

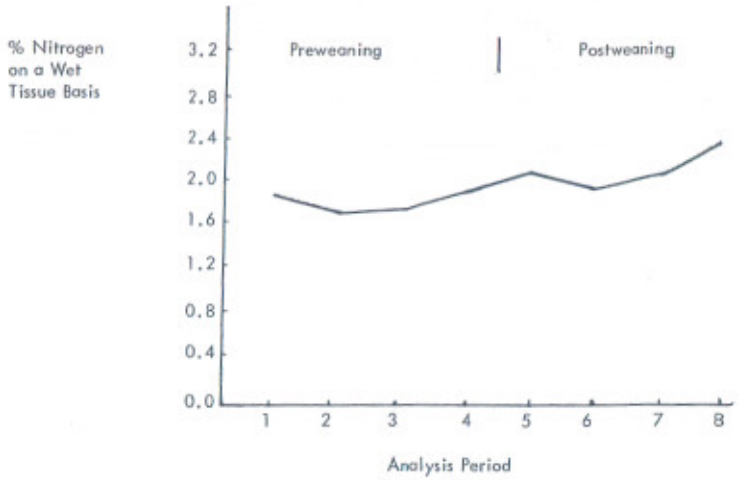
The tissue obtained from the live animal biopsies was frozen immediately in liquid nitrogen and stored at —20°C until extraction. Muscle tissue fractionation procedures utilized were modified techniques of several workers, especially Helander (1957) and Rickansrud (1969). The muscle tissue nitrogen was partitioned into its protein nitrogen, NPN and lipid nitrogen components according to their solubility characteristics in KI-PO_4 buffers or trichloroacetic acid (TCA). The lipid nitrogen was of special interest due to its flotation properties in the extracting buffers and to its total quantity of nitrogen noted in this study.

The nitrogen extracts obtained from tissue fractionation were subjected to the kjeldahl analysis and the data obtained expressed as percent nitrogen on a wet tissue basis. These data were analyzed through a completely randomized design (Snedecor and Cochran, 1967) using animals as blocks and age effects as treatment.

Results and Discussion

Data from the protein nitrogen fraction (Figure 1) showed decreasing trends from the first to the third period preweaning, and coincide favorably with data presented by Helander (1957). An increasing trend was evident from the third analysis period throughout the remainder of the study. Overall, a slight inflection was noted between the initial and the ultimate test periods yet there were no significant differences ($P>.05$) between the period means. These results indicated that protein deposition occurred during the entire 14.3 months growth span at variable but noticeable rates.

Total muscle NPN has been shown to constitute a decreasing quan-



igure 1. Changes in Total Muscle Protein Nitrogen During Growth

ity of total nitrogen in the animal from near birth to maturity (Hickerson and Widdowson, 1960 and Helander, 1957). Trends that were observed in the present work (Figure 2) are very similar to those described in the literature. However, points of inflection were observed in periods five, seven and eight, probably due to the abrupt dietary changes, as noted in period five, and to elevated protein deposition resulting in synthesis of tissue peptides too small in molecular weight to be precipitated by 20 percent TCA, as observed in periods seven and eight. The overall NPN changes were not statistically significant ($P>.05$) and averaged approximately 0.4 percent nitrogen during growth.

Muscle tissue fractionation procedures in the past have been instrumental in detecting quantitative differences in nitrogen components. When referring to such, the researcher must not only discuss protein and nonprotein isolates but also that nitrogen complexed with lipids in the sarcosol and fiber membranes. Lipid-nitrogen complexes have been isolated by centrifugation and chromatographic techniques and have been studied by several workers including Hanahan (1962). These complexes have been shown to possess unique densities upon which separation by centrifugation was based. In the present study, repeated centrifugation of muscle extracts produced a large amount of floating material which has been referred to by Emery (1969) as lipoproteins. This floating material was separated and retained for nitrogen analysis from all extractions. The results of the kjeldahl analysis (Table 1) indicated that a significant

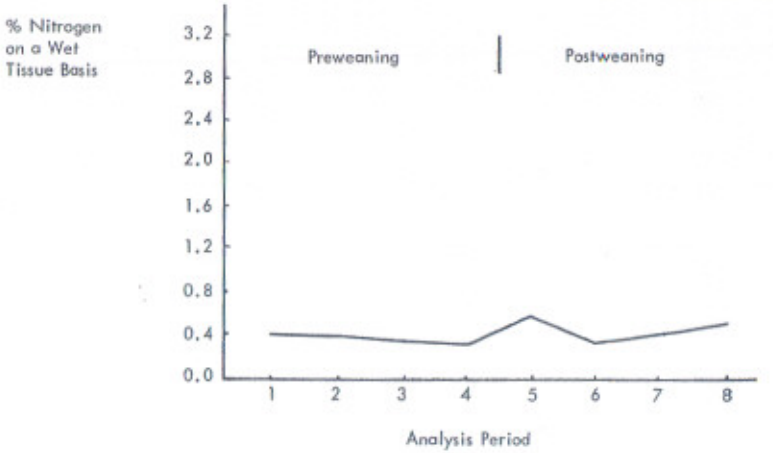


Figure 2. Changes in Total Muscle NPN During Growth

($P<.01$) decline in total lipid nitrogen was evident from the initial to the final analysis periods. The trend which this component followed (Figure 3) appears to parallel the fat intake of the animal. Preweaning, while the calves were depending primarily on a milk diet, the values averaged 0.78 percent nitrogen and postweaning, while protein and carbohydrate concentrates were the principal source of nutrition, the values averaged 0.45 percent nitrogen.

Figure 4 depicts the changes which occurred in all components and their relationship to total muscle nitrogen. Total nitrogen made no

Table 1. Period Mean Values for Total Muscle Nitrogen and Its Major Components During Growth[1]

Period	Total Nitrogen	Total Protein Nitrogen	Total NPN	Total[2] Lipid Nitrogen
1	3.05	1.86	0.41	0.78
2	3.02	1.72	0.40	0.90
3	2.93	1.73	0.37	0.83
4	2.84	1.92	0.33	0.59
5	3.03	2.05	0.54	0.45
6	2.72	1.92	0.32	0.48
7	2.97	2.07	0.41	0.49
8	3.13	2.31	0.43	0.39

[1] All values expressed as percent nitrogen on a wet tissue basis.
[2] Values of this subfraction displayed statistically significant ($P<.01$) changes during growth.

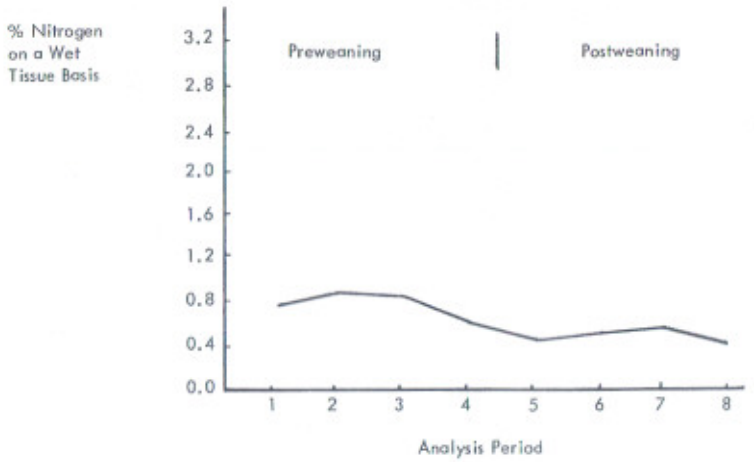


Figure 3. Changes in Total Muscle Lipid Nitrogen During Growth

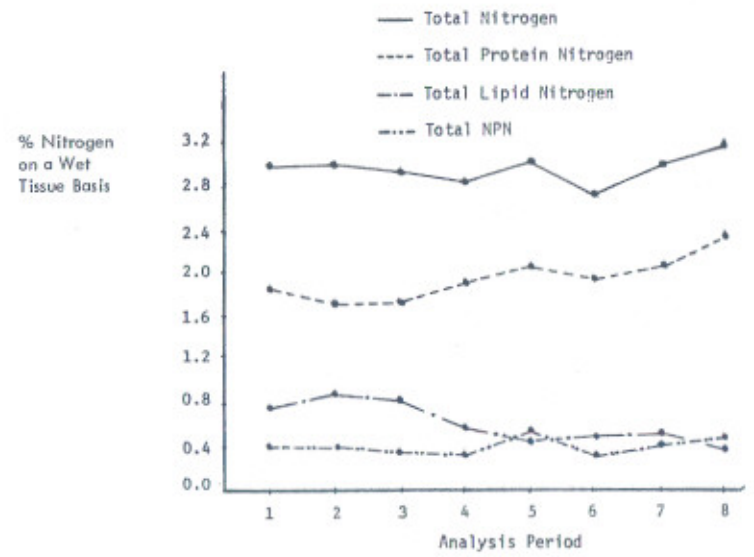


Figure 4. Changes in Total Muscle Nitrogen and Its Major Subfractions During Growth

significant ($P>.05$) changes (Table 1) during growth and only a small net increase was noted from the first to the final periods. This type of pattern does not follow those established by Helander (1957) or Dickerson and Widdowson (1960). However, these workers did not isolate and retain the lipid fraction as was done in this study. If this lipid fraction was eliminated by acetone extraction prior to partitioning and kjeldahl analysis, an increase from 2.28 to 2.74 percent nitrogen would be evident. The results obtained after such lipid nitrogen deletion would then follow, very closely, the trends established in the literature and lends some doubt as to the validity of certain "quantitative" nitrogen isolation procedures used in the past.

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