

AN ABSTRACT OF THE THESIS OF

Bryan Edward Brokaw for the degree of Doctor of Philosophy
in Genetics presented on August 12, 1975

Title: SERUM T₄ AND LIVER NAD AND NADP AS PERFORMANCE
INDICATORS IN BEEF CATTLE

Abstract approved: **Redacted for privacy**
Ralph Bogart

The relationships of serum T₄ (thyroxine) levels and liver nicotinamide nucleotide coenzyme (NAD and NADP) levels with production of traits of beef cattle were investigated at both weaning (182 kg) and following performance testing (363 kg). In addition, the value of these parameters as selection criteria for improved performance was determined. Blood and liver samples were collected from 83 bulls and 41 heifers of the Hereford and Angus breeds in the years 1972 and 1973.

The levels of T₄ present in the serum were determined by the Tetrasorb-125 method (Abbott Laboratories, North Chicago, Illinois). T₄ levels at weaning were found to be positively associated with weaning age, while T₄ levels determined following performance testing were negatively associated with final age. This was due to the opposite influence of T₄ upon growth at the two different weights. Although T₄ level at 182 kg was found to be a significant predictor of

subsequent rate and efficiency of gains, the relationship of T_4 to rate of gain was of small magnitude and its biological importance was negligible. In the case of feed per unit gain, an increase of one unit in T_4 level at 182 kg was found to result in a decrease of 0.098 kg of feed required per kg of gain.

Fluorometric determination of the liver concentrations ($\mu\text{g/g}$ of frozen liver tissue) of nicotinamide nucleotide coenzymes showed that NAD^+ and NADPH were present in excess over NADH and NADP^+ respectively. No significant differences in coenzyme concentrations due to age were detected within the sampling weight groups. Coenzyme levels were significantly higher at 363 kg than at 182 kg. The oxidized forms (NAD^+ and NADP^+) were found to positively influence rate and efficiency of gains, while these traits were influenced negatively by the reduced forms (NADH and NADPH). No biologically important predictive relationships between coenzyme levels at 182 kg and production traits were found that would have value in selecting for improved performance in beef cattle.

Serum T₄ and Liver NAD and NADP as Performance
Indicators in Beef Cattle

by

Bryan Edward Brokaw

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed August 1975

Commencement June 1976

APPROVED:

Redacted for privacy

Professor Emeritus of Animal Genetics
in charge of major

Redacted for privacy

Head of Department of Animal Science

Redacted for privacy

Dean of Graduate School

Date thesis is presented August 12, 1975

Typed by Mary Jo Stratton for Bryan Edward Brokaw

ACKNOWLEDGEMENTS

The author would like to express his sincere appreciation to Dr. Ralph Bogart, his major professor, for the guidance given throughout the course of graduate study and in preparation of this dissertation. Thanks are also expressed to Drs. Hisaw, Kronstad, Swanson, Stamp and Harper for serving as members of the graduate committee.

Special thanks go to my wife, Jan, for her patient endurance of many hardships during the course of this study; and to my daughters, Lori and Lisa, who have endured their father's long absences.

My colleagues in the basement receive special acknowledgement for their helpful, cooperative and mutually encouraging habits. The help received from all members of the Animal Science staff is also greatly appreciated.

The assistantship received from the experiment station, without which this study would not have been possible, is also appreciated.

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SERUM T₄ AND LIVER NAD AND NADP AS PERFORMANCE INDICATORS IN BEEF CATTLE

INTRODUCTION

Although it is probably not the specific goal of the commercial livestock producer, the primary reason for the production of livestock products is to supply a source of quality nutrients for human consumption. In light of the current food shortages in the world, which due to an ever increasing population are likely to worsen if food supplies remain constant, the practice of feeding potential human food sources to livestock has come under increasing attack as an unnecessary and inefficient link in the food chain. These attacks have been combated by pointing out that livestock utilize many energy sources that are not suitable for human consumption, thereby harvesting otherwise nonutilizable natural resources and converting them into products which increase the human food supply. This is especially true of ruminants which consume large quantities of roughages. The future of the livestock industry will probably depend upon how well the livestock involved are able to make use of these resources and fill this niche in the human food chain. If livestock products are to maintain their importance in the human diet, the livestock must be able to utilize these resources efficiently. To achieve the goal of producing food to meet ever increasing demands it will be necessary

to continue to improve the efficiency with which livestock products are produced by genetic improvement of the livestock involved.

With these objectives in mind, the role of the animal breeder in the field of animal science is to select animals with the genetic constitution to meet the goals for food production of increasing rate and efficiency of growth and the economic goals of the commercial livestock producer. One means of approaching this problem is to discover physiological mechanisms that are associated with inherited differences in rate and efficiency of gains. If such mechanisms are discovered they can be used to select animals with genotypes which result in the type of metabolism that is associated with superior efficiency of production. Another use of such relationships is the possibility of selecting for these traits at earlier ages, thus helping to increase the rate at which genetic improvement can be made.

This type of research in relation to rate and efficiency of gains in beef cattle has been pursued at Oregon State University for a number of years (Bogart, 1975). Several of these studies have shown that the metabolism of rapidly and economically gaining animals resembles that of young calves, while the metabolism of poor doing animals resembles that of mature beef cattle (Bogart, 1975; Bogart et al., 1963; Clark et al., 1963; Price et al., 1956).

It is known that hormone balance influences the nature and amount of growth and in some instances is able to regulate protein

synthesis. Hormones are able to do this by controlling the activity of genes (Davidson, 1965). The thyroid hormones are regarded as the primary regulators of metabolic rate. More rapidly gaining animals have been shown to have a higher content of thyrotropic hormones in their pituitaries than less rapidly gaining animals (Burriss and Bogart, 1953; Burriss et al., 1953; Krueger et al., 1954); this finding suggests a possible relationship between blood thyroid hormone levels and rate of gain. This has been substantiated in beef heifers by a recent report by Gashaw et al. (1974). The research at Oregon State University also indicates a relationship between thyroid weights and growth rate (Anwar-Afghan, 1967; Bogart, 1975; Mason et al., 1956). This research, except that of Gashaw et al. (1974), has been conducted with slaughter animals. To make these relationships more useful in enhancing genetic improvement by means of selection, the determination of these relationships at younger ages is desirable.

The metabolic processes involve a series of oxidation-reduction reactions catalyzed by enzymes. In recent years, it has become quite clear that the synthesis of enzymes as well as their relationship to biochemical reactions and metabolism is under genetic control (Changeux, 1965; Jacob and Monod, 1961; Watson, 1970). Profiles of blood chemistry in cattle show evidence of individuality which in part is under genetic control (Rowlands et al., 1973). It is suggested that this might be useful for selecting superior stock because in the

appropriate environment, constituents with high heritability also appear to have a high correlation with growth rate. Most notable of these is high blood glucose concentrations. Blood glucose concentrations are the result of the metabolic state of the individual which is controlled by the activity of enzymes in the oxidation-reduction reactions. Vital to the functioning of these enzymes in oxidation-reduction reactions and thereby to metabolism are the coenzymes NAD and NADP. Therefore, the endogenous levels of these coenzymes would be expected to influence the general metabolic state of an organism. These nicotinamide nucleotide coenzymes have been found by Anwar-Afghan (1967) and also reported by Bogart (1975) to be particularly involved in fat metabolism of beef cattle. These authors found the levels of these coenzymes in liver tissue, when measured at slaughter, showed definite relationships with production traits. If these relationships were found to be true at younger ages also, they could prove to be useful selection criteria for the genetic improvement of beef cattle.

As part of the continuing effort at this institution to determine the relationships of physiological mechanisms to inherited differences in rates and efficiency of gains in beef cattle, the present study was undertaken to determine whether the relationships of thyroid activity and liver nicotinamide nucleotide coenzymes discussed above held true in younger calves. The relationships of serum thyroxine (T_4)

and liver nicotinamide nucleotide coenzymes were determined and their usefulness in selecting, at younger ages, animals with the ability to gain rapidly and efficiently was assessed.

REVIEW OF LITERATURE

Performance Testing

Relationships between Production Traits

One of the tools used by animal breeders in selection of superior breeding cattle is performance testing; the measurement of the ability of the animals to produce beef economically. The relative influence of genetic and environmental factors on the production traits of beef cattle has been reported (Clark et al., 1963). This report on the cooperative beef cattle breeding research in the western region, as well as one by Bogart (1975), document the interrelationships among performance traits. Some of the general relationships indicated are as follows: birth weight in some cases is positively associated with rate of gain and efficiency of gain on test, but is not a reliable selection tool; suckling gains are not consistently related to postweaning gains; age on test may or may not be related to test gains depending upon the influence of preweaning genetic and environmental factors; feed efficiency is closely related to rate of postweaning gain and the factors that are related to rate of gain are generally also related to feed efficiency; also, older calves at any given weight are less efficient; therefore much of the variation in feed efficiency for weight to weight tests can be accounted for by birth weight,

average daily gain, and weight per day of age at time when test starts; there are significant differences in average daily gain and efficiency of gain between bulls and heifers (i. e. bulls gain faster and are more efficient); and finally, scores for type and conformation, and body measurements show little relationship to rate of gain.

Adjustments for Interrelationships among Traits

Due to the interrelationships among traits, some investigators have suggested that adjustments for them be made when analyzing data for effects in related traits. Swiger et al. (1970) found sex to be a significant source of variation for all performance traits and recommended adjustment of data for sex influences. Marlowe et al. (1965), however, stated that adjusting average daily gain and grade for differences in age does not appear to be appropriate.

Serum Thyroxine

Factors Affecting Levels of Thyroid Hormones

Before using thyroxine levels as a predictor of future performance it is necessary to have an understanding of the factors which affect the levels of thyroid hormones present in bovine serum. There is considerable evidence that thyroid activity is increased in animals exposed to cold environmental temperatures and decreased by

exposure to high environmental temperatures. This is true in rats and mice (Dempsey and Astwood, 1943; Hurst and Turner, 1948) and also in dairy cattle (Blincoe and Brody, 1955b; Lewis and Ralston, 1953). However, under controlled conditions, Blincoe et al. (1951) found that the PBI (protein bound iodine) levels of dairy cattle did not appear to change in the temperature range -14°C to 40°C . The influence of temperature and feed intake on thyroid activity in cattle has also been reported by several other investigators (Armstrong and Hansel, 1956; Johnson and Kibler, 1963; Thompson et al., 1963; Lundgren and Johnson, 1964; Yousef and Johnson, 1965). Johnson and Yousef (1966) determined that thyroid function is dependent mainly on environmental temperature and not on feed intake, as increasing temperature depresses thyroid activity. These authors hypothesize that this direct effect of temperature may occur via the cold and warmth receptors of the skin that stimulate the hypothalamothermo-sensitive area of the brain, which activates the thyrotropin releasing hormone which in turn influences the anterior pituitary which influences thyroid activity.

In spite of these temperature effects, Pipes et al. (1963) found no significant difference in the thyroxine secretion rate (TSR) of beef cattle among months of the year. Even between summer months and winter months no significant difference was found. This differed, however, from the response of dairy cattle in which the TSR was

much higher in the winter (0.54 mg/100 lb body wt) than in the summer (0.20 mg/100 lb body wt). Pipes et al. (1959) reported earlier that seasonal variation in temperature did not influence the turnover rate of L-thyroxine in the blood of dairy cows. The turnover rate was found to be 28.4% per day. The data indicated a build up of thyroxine in the blood and total body space which is significantly higher than the daily TSR. These authors also reported that there were no significant differences in turnover rate between breeds. Other researchers (Blincoe and Brody, 1955a; Johnson and Ragsdale, 1960), also using dairy cattle, found significant differences among breeds in the response of thyroid activity and TSR to environmental temperature changes. Both found Jerseys to differ from Holsteins and Brown Swiss in that lower temperatures caused higher thyroid activity or TSR in the Jerseys. Comparison of responses of euthyroid, hyper- and hypo-thyroid beef steers to exogenous thyroxine indicated that the thyroid activity of Angus calves at all thyroid classifications was significantly greater than that of Hereford calves (Hatch et al., 1972). Goret et al. (1974) reported differences in T_4 (thyroxine) levels among six breeds of cattle. The breeds were Zebu, Scottish Highlanders, Angus, Hereford, Holstein and Guernsey. Zebu had the highest levels (127.6 ng/ml) while Guernseys had the lowest levels (47.0 ng/ml). Cowley et al. (1971), in comparing thyroid hormone levels of Hereford and Brahman cattle, also found

that Herefords had a significantly lower level of circulating thyroid hormones. In the same report these authors state that significant differences due to sex were also found, in that heifers had significantly higher levels of circulating thyroid hormones than bulls. Hernandez et al. (1972) also reported that serum T_4 levels for females were significantly higher than levels for male prenatal and neonatal calves.

The effect of age on thyroid hormone levels is not clear. Some investigators (Irvin and Trenkle, 1971) have found no significant age effects in cattle, while others give conflicting reports of age effects. Secretion rate decreases with age in growing rats (Monroe and Turner, 1946). Long et al. (1952) report that PBI levels in dairy cattle decrease with advancing age, while Reece and Man (1952) indicate that PBI levels are lower in calves than in mature cows. Blincoe (1958) reports that thyroid secretory activity remains relatively constant in Shorthorn, Santa Gertrudis and Brahman calves until about seven months of age and then declines until about one year of age when it becomes relatively constant again.

Relationship of Thyroid Activity to Production Traits

The determination of the relationship of thyroid activity with growth and production traits has been pursued by several researchers.

Yousef and Johnson (1966) found that injections of L-thyroxine at both 18 and 32°C increased metabolism, pulse rate, and lactation in dairy cattle. These authors suggest the possibility of predicting future performance from metabolic rates in young animals since the higher metabolic rate at both temperatures was followed by an increase in milk production.

In male rats, the thyroid weight was observed to increase in proportion to body weight at about the same ratio as did the thyroid secretion rate (Monroe and Turner, 1946). This agrees with earlier reports (Brody and Kibler, 1941; Kibler et al., 1943) that in growing animals the thyroid weight increases with the increase in body weight. Mason et al. (1956), working with four strains of mice and all possible crosses, indicated that both feed efficiency and rate of gain are related to thyroid activity. Growth rates were the fastest when thyroid weights were intermediate. This conflicts with earlier reports with cattle indicating a negative correlation between blood PBI and rate of gain (Gawienowski and Mayer, 1953; Kunkel et al., 1953). They also indicated, however, that individuals with intermediate levels have the highest rates of gain. Lucas et al. (1950) found that slaughter weight, daily gains and wither height had a high positive correlation with thyroid weight in beef cattle. Anwar-Afghan (1967) and Bogart (1975) have found that thyroid weights in beef cattle are positively correlated with daily gains. In addition, thyroid weights

were found to be positively correlated with percent lean in the carcass.

Anderson et al. (1973) suggest that the reason for differences found between TSR levels in Jerseys and Holsteins is due to their growth rate. The TSR of larger animals may be related to their growth rate, and since the Holsteins grow faster than Jerseys, a higher TSR is needed to synergize with growth hormone and other hormones required for optimal growth. Gashaw et al. (1974) reported that animals with higher circulating thyroid hormones were found to have higher yield grades, presumably the result of having mobilized fat stores and distributed it more to the periphery of the carcasses. In the same report, T_3 levels determined at the initiation of feed test were found to be useful in predicting subsequent gain performance in similar weight cattle. Higher T_3 index values, which indicate a hypothyroid state, were found to be negatively correlated with gains. In other words, gains were positively correlated with the level of circulating thyroid hormones.

Nicotinamide Nucleotide Coenzymes

Role in General Metabolism

The importance of nicotinamide nucleotide coenzymes in metabolism is apparent from the evidence which indicates that the

major metabolic sources of energy are channeled into a few mechanisms (i. e. the electron transport system and oxidative phosphorylation) in which these coenzymes play a determining role (Dickens, 1961). The multitude of metabolic reactions in which they play a role is reviewed by Racker (1955). Evidence has been presented that nicotinamide nucleotides are rate limiting factors in the interaction of substrates with dehydrogenases (Dixon, 1949; Krebs, 1958). NAD and NADP are important in mediating changes in the metabolic flux of a cell under varying conditions (Clark and Pinder, 1969). By virtue of their redox state in particular cell compartments, NAD and NADP may influence the direction of metabolism of certain intermediates in competing pathways (e. g. glycolysis and gluconeogenesis) (Krebs, 1967). NAD has also been implicated in the control of cell division (Morton, 1958). The role of NAD and NADP in metabolism is summarized in most textbooks of biochemistry and molecular biology (Lehninger, 1970; Watson, 1970).

Role in Fatty Acid Synthesis

Many investigators have indicated that there is a relationship between fatty acid synthesis and nicotinamide nucleotide coenzymes. Longdon (1957), in his work with rat liver homogenates, concluded that the fatty acids are synthesized in the cytoplasm and that NADPH serves as the electron donor for the reduction of the α and β

unsaturated acyl Co-A to their saturated counterparts.

Several early authors have indicated that an active oxidation pentose phosphate shunt provides a large supply of NADPH for fat synthesis (Dickens, 1959, 1961; McLean, 1958); more recent research, however, suggests that this source is not sufficiently rapid to account for the large amounts of NADPH required for lipogenesis (Flatt and Ball, 1964).

Several investigators have recently shed light on the role of NADP in fat synthesis in adipose tissue, the primary site of fatty acid synthesis in ruminants (Ingle et al., 1971). The rate of glucose catabolism via the pentose phosphate cycle in adipocytes appears to be determined by requirements for NADPH in lipogenesis; the amount of NADPH thus produced, however, is not sufficient to provide all the NADPH needed for fatty acid synthesis (Kather et al., 1972). Malic enzyme, a NADP-dependent malate dehydrogenase, has been shown to be associated with extra-mitochondrial lipogenesis in bovine tissue. It has been suggested that it is an important source of NADPH which is necessary for fat synthesis (Young et al., 1953; Young et al., 1969). Yang and Baldwin (1973) studied the role of NADPH in metabolism of acetate in fatty acid synthesis of isolated bovine adipose tissue cells. These authors propose an isocitrate- α ketoglutarate shuttle for the transfer of reducing equivalents from

the mitochondria to the cytoplasm, as the malic system is very low in bovine adipose tissue.

Relationship to Growth and Production Traits

Schwartz and Passonneau (1974) reported that in normal cultured fibroblasts, the NAD^+/NADH ratio increases as the cells cease growth. Total coenzyme concentrations in the liver increase with growth in the developing rat (Burch and Dippe, 1964). NAD increased 55% and NADP 188% during growth from five days before birth to 64 days following birth. Roux (1962) reported the same type of results with rabbits. There were significant increases in all four forms of the nicotinamide nucleotide coenzymes in the liver of the 28-day-old fetus over the 19-day-old fetus. NADH and NADPH increased in the liver tissue of guinea pigs from fetal to adult stages (Raiha, 1961). Caiger et al. (1962) and Filsell et al. (1963) assayed liver tissue of both young and adult sheep for NAD and NADP. They concluded that the concentration of total nicotinamide nucleotides increases during normal growth and that maximum levels are reached when growth ceases. These authors also indicated that low concentrations of the coenzymes are a characteristic of rapidly growing animals. Further work with ruminants has been reported by Anwar-Afghan (1967) and Bogart (1975). Working with 55 beef bulls of the Hereford and Angus

breeds, they found that the liver levels of all nicotinamide nucleotide coenzymes increase with growth. Total coenzyme, NAD^+ and NADPH levels were found to be positively associated with percent fat in the carcasses. In addition, lines and line crosses that gained more rapidly and were more efficient in feed conversion possessed lower levels of liver coenzymes associated with fat metabolism while the opposite was true in the case of lines and linecrosses that gained at slower rates and were less efficient.

SECTION I: THE RELATIONSHIP OF SERUM T_4 TO PRODUCTION TRAITS

Materials and Methods

Blood samples were collected via jugular vein puncture from 63 beef animals in 1972 and 60 in 1973. Samples were taken when each individual finished performance testing (363 kg) in both 1972 and 1973. In addition, samples were collected when each individual was weaned (182 kg) in the 1973 group. All samples were taken at a standard time to reduce any variation in T_4 level which might result from effects due to time of day, feeding, and daylight.

The individuals sampled were from two lines of Herefords and one line of Angus beef cattle. The Hereford line 1^S is a composite created by the crossing of three inbred lines, namely Lionheart, Prince and David, after which the line was closed. Thirty-four bulls (18 in 1972 and 16 in 1973) and 11 heifers (8 in 1972 and 3 in 1973) were sampled from this line. Hereford line 2^S is the result of crossing linecross 1 x 2, 1 x 3, and 2 x 3 Oregon cows with linecross 1 x 4, 1 x 10, and 4 x 10 U.S. Range Livestock Station bulls after which the line was closed. Twenty-nine bulls (9 in 1972 and 20 in 1973) and 9 heifers (6 in 1972 and 3 in 1973) were sampled from this line. Nineteen bulls (8 in 1972 and 11 in 1973) and 21 heifers (14 in 1972 and 7 in 1973) were sampled from the Angus line which has been

closed to outside breeding since 1955. These individuals represented 13 sire groups (6 in 1972 and 7 in 1973).

The blood samples were analyzed for serum T_4 (thyroxine) levels by use of the Tetrasorb-125, T_4 Diagnostic Kit obtained from Abbott Laboratories (1973).

All animals were performance tested from 196 kg to 363 kg. Individual records were obtained for each calf tested. These records included birth weight, age at weaning, age off test, suckling gain, average daily gain, feed per unit gain, weight per day of age, conformation score and condition score.

To determine the relationship of T_4 to beef cattle performance, simple correlation coefficients and least squares regression analyses were completed for T_4 with the performance data.

Results and Discussion

The data on circulating T_4 levels for all individuals combined were tested for effects due to age, sex, breed, line, sire and year by analysis of variance using the procedure for least squares analysis of data (Steel and Torrie, 1960). Highly significant differences were found for age and sex effects; no differences due to breed, line, sire or year were present.

As another means of testing for differences due to age, a paired t-test was made between the T_4 levels at 182 kg and 363 kg for the

individuals sampled in 1973. T_4 levels at 363 kg, with a mean of 8.69 ± 0.27 $\mu\text{g}/100$ ml, were found to be significantly higher ($P < 0.01$) than T_4 levels at 182 kg, at which time the mean was 7.62 ± 0.27 $\mu\text{g}/100$ ml. The relationship of T_4 levels measured at 182 kg body weight with age of the individual at the time the blood sample was taken is shown in Figure 1. T_4 levels, as this figure indicates, increased as the age of the animal when sampled increased. This is exactly opposite to the relationship of age with T_4 when sampling was done at 363 kg body weight (Figure 2). The relationship illustrated in Figure 2, where T_4 levels decrease with advancing age, agrees with the findings of Long et al. (1952). These authors report that blood PBI levels of dairy cows decrease with increasing age of the individual. The report of Reece and Man (1951) is supported by relationship shown by Figure 1, in that they found that the thyroid hormone levels of calves were lower than those of mature dairy cows.

It is possible that the change in the association of age with T_4 levels could be the result of differences in temperature at the time the samples were taken. Temperature is known to depress thyroxine levels in higher ranges and to elevate thyroxine levels in the lower ranges (Gashaw et al., 1974; Johnson and Yousef, 1966). As the date of taking the samples at 182 kg ranges from August to October, older calves, those that tended to wean later, would have been sampled at a time when temperatures are normally lower. This lowering of

AGE EFFECT ON T₄

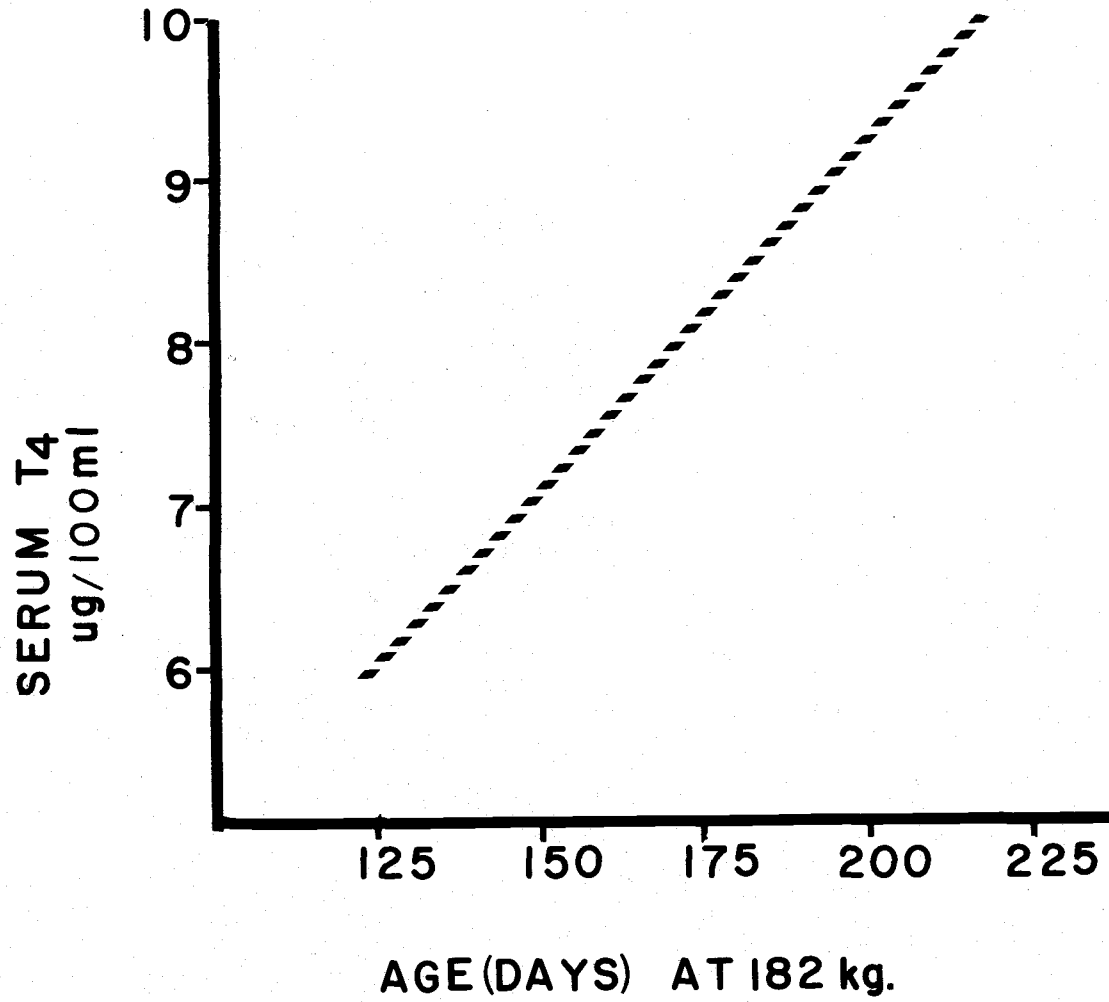


Figure 1. The relationship between age and T₄ levels at 182 kg.

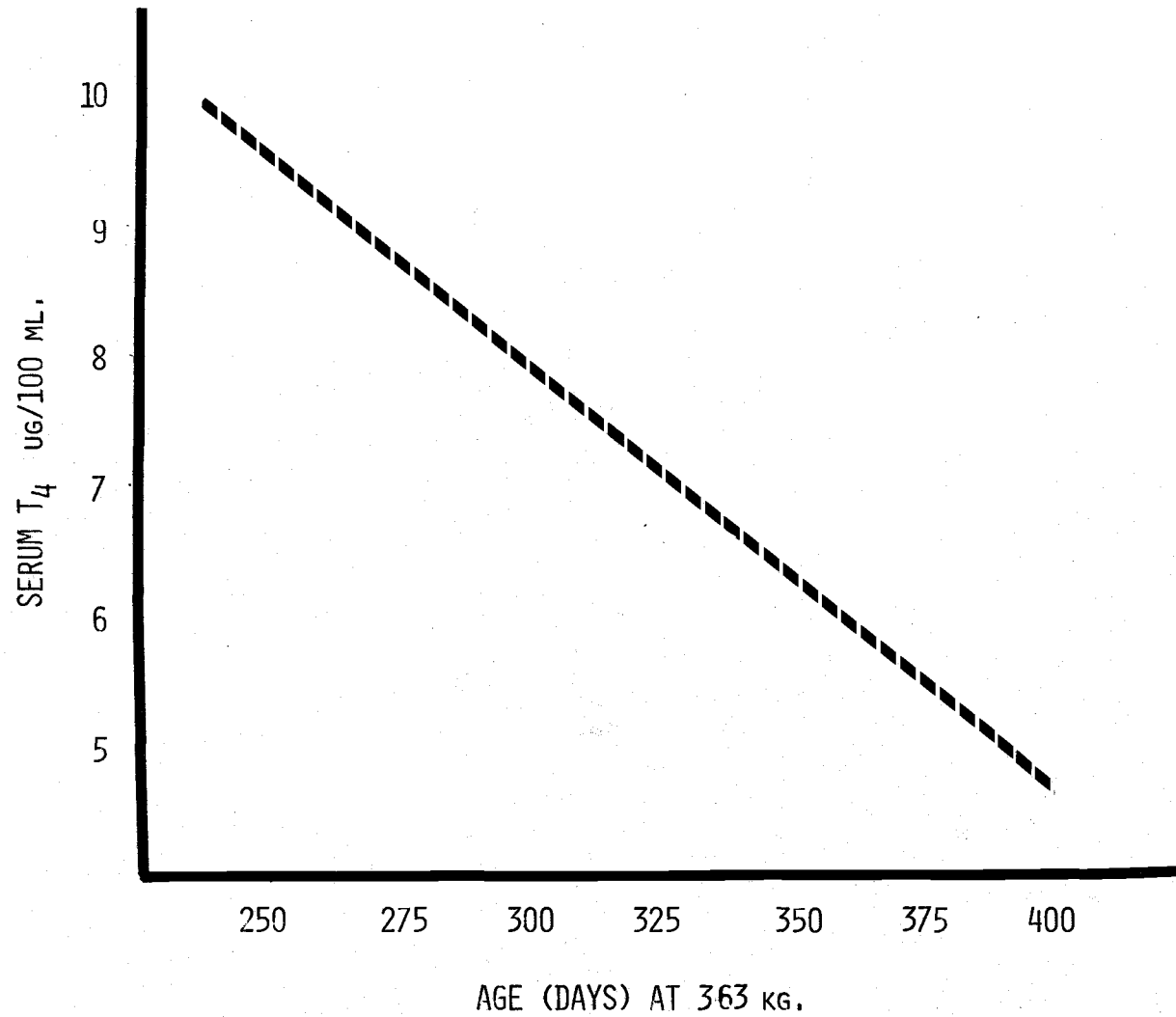


Figure 2. The relationship between age and T₄ levels at 363 kg.

temperature could have influenced T_4 levels to increase, thereby tending to increase the magnitude of the positive association of T_4 levels with age. This could also have had some influence upon the differences noted between T_4 levels taken at 182 kg and those taken at 363 kg. The temperatures are normally higher during the months (August through October) in which the 182 kg samples were taken than during the months (January through May) when the 363 kg samples were taken. As no data were collected concerning temperature fluctuations at the time of sampling, no definite effects due to temperature could be determined. Even if some temperature influence is involved, it is doubtful that effects due to temperature variations would have been sufficient to change the association between T_4 and age, because Pipes et al. (1963) found no significant seasonal effects on thyroxine levels in beef cattle, not even between summer and winter months. If this is true, then the differences in the relationships of T_4 levels with age depicted in Figures 1 and 2 would indicate the association of age with T_4 levels changes at some time between the taking of the samples at weaning (182 kg) and the samples following feed test (363 kg). A change during this time would be consistent with the findings of Blincoe (1958), who found the thyroxine levels to be somewhat stable in beef calves until seven months of age was reached at which time the thyroxine levels began to decline. This age would, in the case of most of the animals in this study, occur between the two

sampling times. This could explain some of the difference in association of age with T_4 levels at the two different sampling times. The association of age with T_4 levels could also be influenced by the relationship of T_4 with weight gains as will be discussed later.

Due to the highly significant differences observed between sexes, all further analyses of the data were completed for the two sexes separately. The means for T_4 levels at both sampling weights and for each sex are shown in Table 1.

Simple correlation coefficients were computed between T_4 levels and all production traits. Those correlation coefficients that were found to be significant are presented in Table 2. The lack of significant correlation coefficients between T_4 levels and production traits of heifers is most likely due to the relatively small number of heifers sampled.

The relationship of T_4 values determined at the end of the feed test (363 kg) with production traits for bulls is what would be expected based upon results reported in the literature. Average daily gain is influenced positively by higher T_4 levels, resulting in the animal's finishing test at a younger age (Gashaw et al., 1974). This is reflected in the negative relationship of T_4 levels with age at 363 kg body weight (Table 2). These results are consistent with those reported earlier by Anwar-Afghan (1967) and Bogart (1975). Using thyroid weight as an indicator of thyroid activity in beef cattle, these authors found that

Table 1. Mean values for serum T₄ levels (µg/100 ml) by sex and weight groups.

	182 kg		363 kg	
	Bulls (n=47)	Heifers (n=13)	Bulls (n=82)	Heifers (n=41)
T ₄	7.44 ± 0.32	8.30 ± 0.39	8.43 ± 0.20	8.86 ± 0.38

Table 2. Significant simple correlation coefficients between serum T₄ levels and performance trait values by sex and weight groups.

Performance Traits	T ₄ Levels			
	182 kg		363 kg	
	Bulls (n=47)	Heifers (n=13)	Bulls (n=82)	Heifers (n=41)
Age (182 kg)	0.560**	None		-0.331**
Age (363 kg)	0.320*		-0.339**	
Suckling Gain	-0.537**			
Average Daily Gain			0.346**	
Feed per Unit Gain			-0.319**	
Weight per Day of Age	-0.367*		0.331**	
Conformation Score	-0.356*			

* P < 0.05

** P < 0.01

thyroid weights were higher in lines and line crosses that gained more rapidly and efficiently than in the slower and less efficiently gaining lines. Also thyroid weights were found to decrease with increasing age at 455 kg. This would indicate a decrease in the level of circulating thyroid hormones with increasing age of the animal as was found to be true in the present study.

Since average daily gain is influenced positively by increasing T_4 levels, T_4 would be expected to have a negative influence upon feed per unit gain because of the highly significant negative relationship known to exist between average daily gain and feed per unit gain. This was found to be true as can be seen by the negative correlation between T_4 and feed per unit gain (Table 2). This also is in agreement with the results of Anwar-Afghan (1967) and Bogart (1975) as discussed above.

As higher T_4 levels result in more rapid and efficient gains, animals with higher T_4 levels finish test at younger ages and therefore have a higher weight per day of age than do slower gaining bulls with lower T_4 levels.

These relationships of T_4 with production traits of bulls were also tested by the least squares regression analysis method for the effect of T_4 on each trait. The significant F tests resulting from these analyses of variance are presented in Table 3. The results of these analyses indicate the same relationships already discussed.

Table 3. F values for significant effects of T_4 on average daily gain, weight per day of age and conformation score of bulls in each weight group.

Group	F Values			
	Average Daily Gain	Feed per Unit Gain	Weight per Day of Age	Conformation Score
Bulls (182 kg)			6.85**	6.38**
Bulls (363 kg)	9.82**	8.16**	8.85**	

** $P < 0.01$

For average daily gain, feed per unit gain and weight per day of age, T_4 was found to account for a highly significant portion of the variation in each trait. These results support earlier conclusions drawn from research utilizing thyroid weight as an indicator of thyroid function. Thyroid weights were found to be positively related to rate of gain and slaughter weight of beef cattle (Lucas et al., 1950). Mason et al. (1956) found that thyroid activity, as indicated by thyroid weights, was related to both efficiency and rate of gains in mice.

The relationship of T_4 levels determined at 182 kg with production traits of bulls differ somewhat from those relationships found at 363 kg. T_4 levels were negatively correlated (Table 2) with suckling gains. This influence of T_4 on suckling gains resulted in animals with higher levels reaching 182 kg at older ages; therefore,

a positive relationship exists between age at 182 kg and T_4 levels. The reason for this negative influence of T_4 on suckling gains is difficult to explain in light of the well documented positive influence of T_4 on gains at later stages of growth. It can only be hypothesized that possibly in some instances high T_4 levels resulted in elevated metabolic rates that required more energy resources than were available to the calf due to the lack of milking ability of the dam. This caused energy supplies available to be used for maintenance rather than growth.

Because of the high correlation between suckling gain and weight per day of age (Appendix Tables 2, 3, 4, and 5), increased T_4 levels at 182 kg had a negative effect on weight per day of age. This effect on weight per day of age is reflected by its negative correlation with T_4 levels at 182 kg (Table 2). As there is a highly significant positive relationship between weight per day of age and conformation score (Appendix Tables 2, 3, and 5), there is also a negative correlation between T_4 levels at 182 kg and conformation score. The effects of T_4 on weight per day of age and conformation score are highly significant as indicated by the F values in Table 3. No significant correlation was found to exist between T_4 levels at 182 kg and subsequent rate or efficiency of gains during performance testing.

Prediction equations (Appendix Table 6) were generated by backward stepwise regression analysis to determine the predictive

value of T_4 levels measured at 182 kg in relation to other production characteristics known at that time. Factors included in the model were birth weight, weaning age, suckling gain and T_4 levels (182 kg). Those regression coefficients found to be significantly different from zero ($P < 0.05$), thus indicating a possible predictive significance, are shown in Table 4. Significant regression coefficients were found only for bulls. The coefficient for the T_4 relationship with average daily gain was significant, while the coefficients for T_4 and suckling gain were found to have a significant relationship with feed per unit gain. By using the backward stepwise regression technique, it is possible to examine the changes that occur in the R^2 value as the different variables leave the model. This provides a second indicator of the biological value of any prediction coefficient. The amount of change in the R^2 value that takes place as the variables found to have significant coefficients are dropped from the model are also shown in Table 4.

Table 4. Significant regression coefficients and associated R^2 values for predicting average daily gain and feed per unit gain of bulls by T_4 levels and suckling gain (182 kg).

Performance Trait	T_4		Suckling Gain	
	Coefficient	R^2	Coefficient	R^2
Average Daily Gain	0.034*	0.066		
Feed per Unit Gain	-0.098**	0.166	-0.680*	0.103

* $P < 0.05$

** $P < 0.01$

The significant coefficient of 0.034 for T_4 in relation to average daily gain indicates that for each unit increase in T_4 concentration at 182 kg an increase of 0.034 kg in rate of gain per day will result (Figure 3). This is true, however, only if suckling gain, which is not a significant predictor in the model, is included. This indicates that for a given rate of suckling gain the above relationship of T_4 with average daily gain would exist, but this relationship would not be true if the correction for suckling gain were removed from the model. This fact reduces the value of T_4 as a predictor of average daily gain. Its value is further reduced by the fact that, as indicated by the R^2 value (Table 4), T_4 accounts for only a very small percentage of the variation in average daily gain. Therefore, although T_4 is statistically a significant indicator of future rates of gain, its actual biological value is questionable. In contrast, Gashaw *et al.* (1974) found that the level of circulating thyroid hormones have predictive significance in the case of heifers if used for similar weight cattle. However, considerably heavier animals were used in that study; the weights of the heifers at the start of the testing period in their study were similar to those of the cattle when finishing the testing period in the present study. Therefore, the correlations of T_4 levels at 363 kg with average daily gain probably are more comparable to the results of Gashaw *et al.* (1974), in which case the two experiments have similar findings. Table 2 shows that T_4 levels at 363 kg

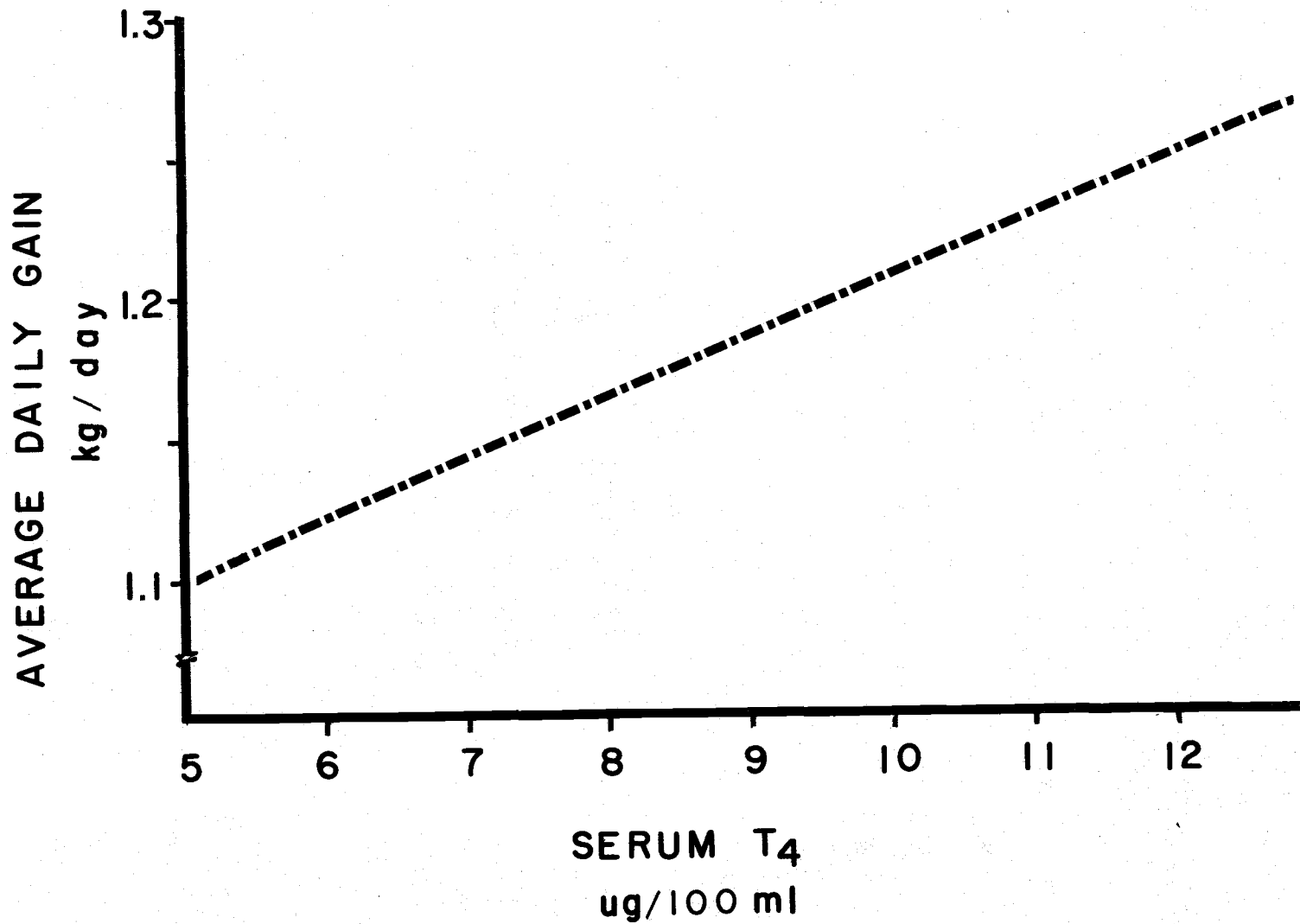


Figure 3. The predicted effect of T₄ levels at 182 kg on average daily gain.

do have a highly significant relationship with average daily gain.

Both T_4 and suckling gain have significant coefficients (Table 4) in the feed per unit gain model. The reliability of suckling gains as a predictor is not high (Clark et al., 1963). The predictive significance of suckling gain in the present data probably results from the interaction of environmental influences which in this case cause a positive association between suckling gains and postweaning efficiency of gains. This positive relationship between postweaning efficiency of gains and suckling gains is reflected in the negative correlation between suckling gain and feed per unit gain of bulls (Appendix Tables 3 and 5). The predictive value of T_4 is supported by the relatively large change in the R^2 value when that variable is removed from the model (Table 4). The regression coefficient indicates that for each increase of one unit in T_4 level at 182 kg, a decrease of 0.098 kg in the amount of feed required per kg of gain will occur (Figure 4). This relationship between T_4 levels at 182 kg and feed efficiency during performance testing further substantiates the work reported by Bogart (1975) in which it was found that calves with heavier thyroid glands gained more efficiently. This seems to be the result of the association of thyroxine with metabolic processes that stimulate the proliferation of lean rather than fat in the carcass. This is further indicated by the work of Anwar-Afghan (1967) and Bogart (1975) in which these authors found that higher thyroid weights were correlated with a higher

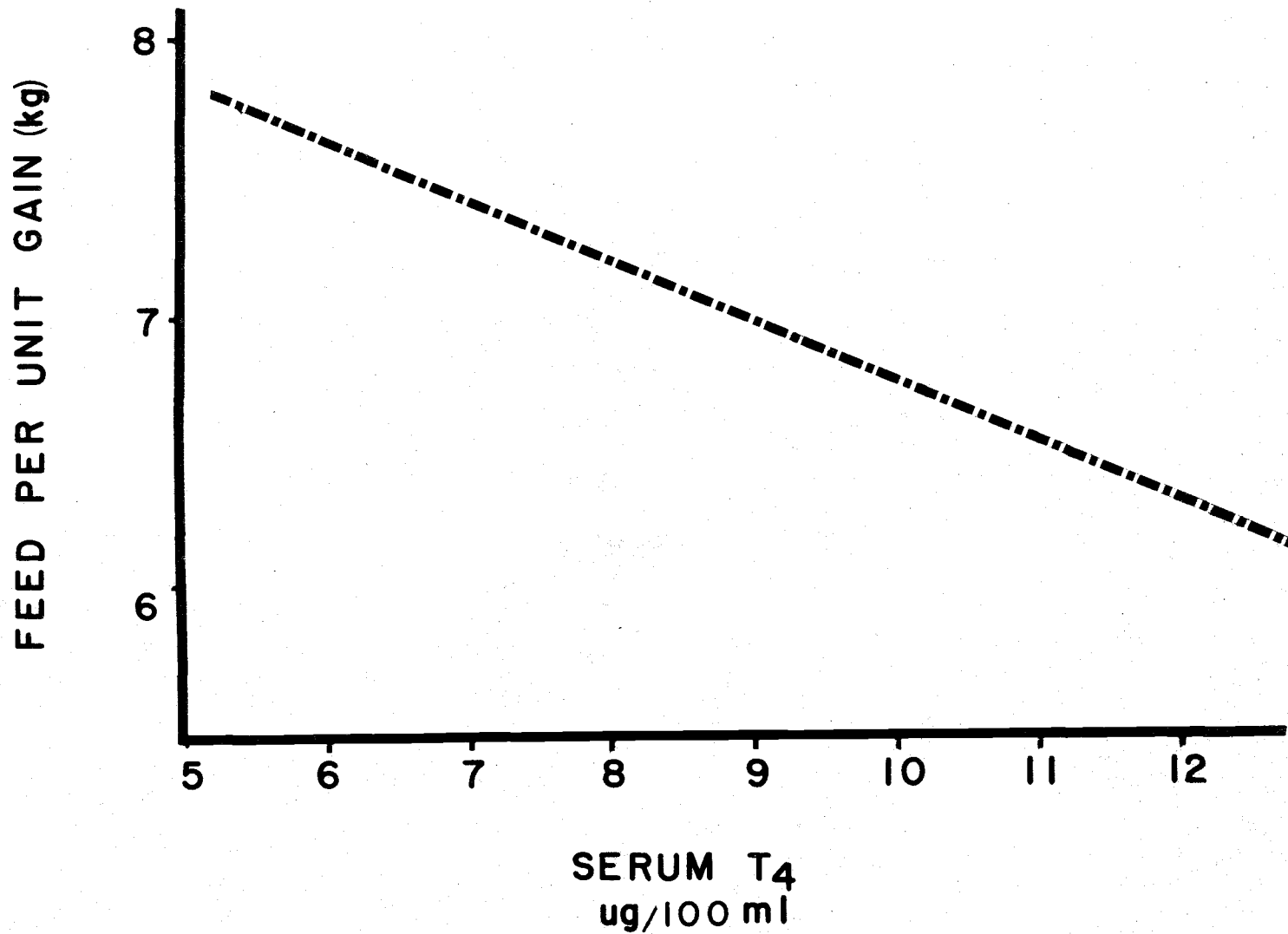


Figure 4. The predicted effect of T₄ levels at 182 kg on feed per unit gain.

percent lean in the carcass, while lower thyroid weights were associated with a higher percent fat in the carcass.

Summary

Serum T_4 levels were determined following weaning (182 kg) and performance testing (363 kg) for both bulls and heifers of the Hereford and Angus breeds. The relationship of T_4 levels with production traits was determined at both weights by computing simple correlation coefficients and by least square regression analyses. Significant ($P < 0.05$) relationships were found between T_4 levels at 363 kg and rate and efficiency of gain. Although T_4 levels at 182 kg were found to be statistically significant predictors of subsequent rates and efficiencies of gain only the predictive coefficient of T_4 for feed per unit gain was found to have appreciable biological value.

SECTION II: THE RELATIONSHIP OF NICOTINAMIDE
NUCLEOTIDE COENZYMES TO
PRODUCTION TRAITS

Materials and Methods

Liver samples were obtained by aspiration liver biopsy using the procedures of Erwin et al. (1956). This involved making a small incision, approximately 1.5 cm, parallel to the ribs in the twelfth intercostal space. This incision was made on the right side of the animal approximately 15 cm from the dorsal median plane. Following penetration of the body wall with a trocar and cannula (18 cm long and 8 mm in diameter), the liver was probed by pointing the cannula ventrally at an angle of 45° with the horizontal plane and anteriorly 45° with the saggital plane. The sample was removed by means of negative pressure created with a 40 ml syringe attached to the cannula, and immediately frozen in an ice bath consisting of dry ice in ethanol until they could be transported to the laboratory where they were stored at -15° C until assayed for coenzyme concentrations. These samples were collected at the same time and from the same sample population described in Section I.

The nicotinamide nucleotide coenzymes were extracted according to the procedures recommended by Ciotti and Kaplan (1957). Two extractions were needed, one for the oxidized forms utilizing cold 5%

trichloroacetic acid and another for the reduced forms utilizing hot 0.1 M sodium carbonate. The liver samples were homogenized in these solutions and the denatured protein was removed by centrifugation.

The fluorometric procedure for measuring nicotinamide nucleotide coenzymes was chosen for determination of liver concentrations. The specific methods followed for the determination of microgram quantities of the oxidized and reduced forms of NAD and NADP are those developed by Jacobson and Astrachan (1961). The principles involved in these procedures are: a) the fluorescence of oxidized nicotinamide nucleotides (NAD^+ and NADP^+) depends on the presence of the N-substituted nicotinamide ring in the oxidized form; b) the small fluorescence of the reduced nicotinamide nucleotides (NADH and NADPH) can be completely destroyed by acid; c) specific enzymes may be used to interconvert the oxidized and reduced forms or to rupture the nicotinamide ribose bond.

Two enzymic procedures were used to quantitatively assay microgram levels of the oxidized forms. First, total oxidized (NAD^+ + NADP^+) levels were determined by using Neurospora NADase to split NAD^+ and NADP^+ at the nicotinamide ribose linkage thereby completely destroying their fluorescent properties. NAD^+ levels of another aliquot were then assayed by reducing the NAD^+ with alcohol dehydrogenase and subsequently destroying the NADH formed by the

addition of acid. This results in total loss of fluorescence in the sample due to NAD^+ . These assays were run in duplicate for all samples. Two identical tubes were prepared for each sample; one underwent the enzymic reaction before addition of 50% TCA and the other in which the enzymic reaction was prevented by addition of the 50% TCA prior to the addition of the enzyme serves as a control. The difference in fluorescence between the reaction tube and the control tube is that due to the particular nucleotide(s) being assayed. The fluorescence values were converted to microgram quantities by comparison with a standard curve prepared using NAD^+ (98% purity) purchased from Sigma Chemical Company. Microgram quantity of NADP^+ was then determined by the difference between the total oxidized quantity of the quantity of NAD^+ .

Because the fluorescence of the reduced forms (NADH and NADPH) is small when compared to that of the oxidized forms, to measure small amounts they are first oxidized and the amount of the oxidized form is then measured. Again two assay procedures were employed. Total reduced forms ($\text{NADH} + \text{NADPH}$) were assayed by oxidizing both NADH and NADPH with an extract of *C. kluyveri*. NADH levels of a second aliquot were assayed by oxidizing NADH with alcohol dehydrogenase under conditions that would not oxidize NADPH . Duplicates and control procedures were followed as with the assays for oxidized forms. Again the fluorescence values were converted to

microgram quantities by comparison with a standard curve prepared with NADH (98% purity) purchased from Sigma Chemical Company. NADPH concentrations were obtained by difference between the total reduced concentrations and the concentrations of NADH.

Fluorescence was developed by the methyl ethyl ketone method (Ciotti and Kaplan, 1957) in which the oxidized forms of NAD and NADP react with the methyl ethyl ketone in alkaline solution followed by acidification to form a highly fluorescent product. The fluorescence of the samples was measured with an Aminco-Bowman spectrophotofluorometer according to the specifications of Chen and Hayes (1965).

In addition to the nicotinamide nucleotide concentration data obtained by the assays described, other coenzyme values and ratios were created by various transformation processes during statistical analysis. These were total coenzyme concentration ($\text{NAD}^+ + \text{NADP}^+$) + (NADH + NADPH), total NAD concentration ($\text{NAD}^+ + \text{NADH}$), total NADP concentration ($\text{NADP}^+ + \text{NADPH}$), and the ratios NAD^+/NADH , $\text{NADP}^+/\text{NADPH}$, and $(\text{NAD}^+ + \text{NADP}^+)/(\text{NADH} + \text{NADPH})$.

All of these coenzyme values for NAD and NADP were used to determine the relationship of liver nicotinamide nucleotide coenzyme concentrations with production traits of beef cattle. To determine these relationships, simple correlation coefficients and least squares regression analyses were completed for the coenzyme values with the performance data (see Section I).

Results and Discussion

No differences in coenzyme concentrations due to sex, breed, line or sire were found when the combined data were analyzed by least squares regression analysis for disproportionate subclass numbers (Steel and Torrie, 1960). However, since significant sex effects do exist in relation to the performance traits with which the data were to be compared (Clark et al., 1963), all subsequent analyses were made separately for bulls and heifers so that a better idea of the relationships of NAD and NADP to performance traits of both sexes could be obtained.

The mean concentrations of the nicotinamide nucleotide coenzymes for the parameters determined are presented in Table 5 for both sexes at each sampling weight. The mean concentration of NAD⁺ was greater than that of NADH in all cases, also the NADPH mean concentration was greater than that of NADP⁺. These patterns are in agreement with the concentration patterns previously found in sheep (Caiger et al., 1962) and beef cattle (Anwar-Afghan, 1967).

Total coenzyme concentrations were not found to have any significant relationship with age when tested within sampling groups. This was unexpected as most investigators have reported highly significant positive relationships between age and total coenzyme concentrations (Anwar-Afghan, 1967; Bogart, 1975; Caiger et al., 1962; Filsell et al., 1963). If tested between sampling weights, however,

Table 5. Mean values for nicotinamide nucleotide coenzyme concentrations ($\mu\text{g/g}$ of frozen liver tissue) by sex and weight groups.

Coenzyme	182 kg		363 kg	
	Bulls (n=47)	Heifers (n=13)	Bulls (n=82)	Heifers (n=41)
NAD	255.95 \pm 5.55	261.30 \pm 11.31	286.41 \pm 6.14	283.37 \pm 9.66
NAD ⁺	191.30 \pm 4.88	193.72 \pm 7.86	217.58 \pm 5.45	212.39 \pm 7.64
NADH	64.83 \pm 2.85	67.59 \pm 5.36	69.74 \pm 2.66	72.86 \pm 4.36
NADP	210.58 \pm 6.47	212.35 \pm 15.73	250.23 \pm 5.11	253.64 \pm 10.88
NADP ⁺	65.28 \pm 3.30	60.93 \pm 5.71	77.84 \pm 2.81	70.05 \pm 4.66
NADPH	147.21 \pm 5.72	151.42 \pm 15.55	172.20 \pm 4.91	183.50 \pm 9.02
NAD ⁺ + NADP ⁺	256.60 \pm 5.67	254.65 \pm 9.66	295.14 \pm 5.81	282.74 \pm 8.01
NADH + NADPH	216.29 \pm 6.31	219.00 \pm 15.81	243.32 \pm 4.91	253.87 \pm 8.72
Total coenzymes	473.05 \pm 8.45	473.65 \pm 17.81	537.77 \pm 8.02	537.32 \pm 10.53
NAD ⁺ /NADH	3.33 \pm 0.23	3.14 \pm 0.34	3.47 \pm 0.18	3.34 \pm 0.22
$\frac{\text{NAD}^+ + \text{NADP}^+}{\text{NADH} + \text{NADPH}}$	1.24 \pm 0.05	1.23 \pm 0.10	1.23 \pm 0.03	1.16 \pm 0.06

total coenzyme concentrations at 363 kg when the average age was 328 days, were found to be significantly ($P < 0.01$) greater than the total coenzyme concentrations at 182 kg when the average age of the individual was 185 days. A possible explanation for these findings is that the association of age with coenzyme concentrations at a given weight become significantly manifested only as the animals approach maturity. The report of Caiger et al. (1962) suggests this possibility as they were unable to discern differences in coenzyme concentrations between young animals at different ages. Significant differences did exist between the levels found in the young animals and those levels found in adult or mature sheep. They also determined that the coenzyme concentrations reached their maximum levels as the animals reached maturity. It would seem, therefore, that the degree of maturity and not age or weight per se is the main influence upon coenzyme concentrations. This could explain the discrepancy between the findings of Anwar-Afghan (1967) and the present study, for his measurements were taken at 454 kg when the animals would have been older and, therefore, closer to maturity.

In the present study, the relationship of coenzyme concentrations with age within sampling groups appears to depend upon the effect these coenzymes have on growth. For instance, the reduced forms, that have been shown to be involved in fatty acid synthesis (Longdon, 1957) and therefore associated with reduced growth rate

(Anwar-Afghan, 1967), are positively associated with age at both 182 and 363 kg body weight, while the values which indicate an increase in the oxidized forms that are associated with energy conversion (Dickens, 1961) result in increased rate and efficiency of growth. This is reflected in their negative correlation with age (Tables 6, 7, and 11). The net effect of these opposite relationships is to eliminate any significant correlation between total coenzymes and age.

The relationships of nicotinamide nucleotide coenzyme levels, measured at 363 kg, with production traits were determined by use of simple correlation coefficients and analyses of variance by the least squares regression method for disproportionate subclass numbers. The significant simple correlation coefficients for heifers and bulls are shown in Tables 6 and 7 respectively. In general, they indicate the same relationships with growth discussed in reference to age associations. For heifers (Table 6), NADP, NADPH, and NADH + NADPH influence weight per day of age negatively. These factors are all indicators of the relative concentrations of NADPH present in the liver cells. This coenzyme is known to be involved in lipogenesis (Flatt and Ball, 1969; Ingle et al., 1971; Longdon, 1957). Anwar-Afghan (1967) found that these coenzymes were associated with fat metabolism and had a negative influence upon rate and efficiency of gains. This would indicate why these coenzyme values have a negative effect on weight per day of age, while the coenzymes that are involved

Table 6. Significant simple correlation coefficients between nicotinamide nucleotide coenzymes and production traits of heifers (363 kg).

Coenzyme	Production Traits				
	Birth Weight	Age		Weight per Day of Age	Conformation Score
		182 kg	363 kg		
NAD			-0.335*		0.331*
NADH				0.319*	
NADP			0.368*	-0.360*	
NADP ⁺	0.315*				
NADPH		-0.344*	0.496**	-0.466**	-0.344*
NAD ⁺ + NADP ⁺			-0.315*		
NADH + NADPH			-0.384*	-0.333*	
NADP ⁺ /NADPH		-0.341*	-0.391*	0.371	
$\frac{\text{NAD}^+ + \text{NADP}^+}{\text{NADH} + \text{NADPH}}$			-0.427**	0.361*	

* P < 0.05

** P < 0.01

Table 7. Significant simple correlation coefficients between nicotinamide nucleotide coenzymes and production traits of bulls (363 kg).

Coenzyme	Production Traits					
	Age		Average Daily Gain	Feed per Unit Gain	Conformation Score	Condition Score
	182 kg	363 kg				
NADH					-0.329**	-0.366**
NADP				0.280*		
NADPH				0.330*		
NAD ⁺ + NADP ⁺						0.269*
NADH + NADPH		0.232*	-0.284*	0.354**		
NAD ⁺ /NADH					0.299*	0.355**
NADP ⁺ /NADPH	0.232*					
$\frac{\text{NAD}^+ + \text{NADP}^+}{\text{NADH} + \text{NADPH}}$				-0.270*		0.306*

* P < 0.05

** P < 0.01

in energy metabolism, such as NAD^+ and NADP^+ and, therefore, result in increased rate and efficiency of gains, influence weight per day of age positively. These relationships are more clearly shown in the case of bulls (Table 7) where the direct effects on rate and efficiency of gains are depicted. Again the reduced forms, $\text{NADH} + \text{NADPH}$, have the effect of reducing growth rate and feed efficiency (indicated by positive correlation with feed per unit gain). These coenzymes, which as shown by Longdon (1957) are associated with fatty acid synthesis, result in the animal's energy sources being tied up in fat deposits rather than being utilized for the proliferation of muscle and growth. This hypothesis is supported by the results of Anwar-Afghan (1967) and Bogart (1975), which indicate that increased levels of NADPH positively influence the percent fat in the carcass of beef bulls. The increase in percent fat was accompanied by decreased rate and efficiency of gains in the individuals with high concentrations of NADPH . The oxidized forms, indicated by the increased ratio of oxidized to reduced forms (Table 7), influence efficiency of gains positively. This would indirectly, due to the strong positive relationship between rate and efficiency of gains (Clark et al., 1963; also Appendix Tables 2 and 5), influence rate of gain positively.

These mechanisms appear to function in relation to conformation score in the case of bulls. NAD^+/NADH , which indicates an increase in NAD^+ relative to NADH and is associated with increased rate and

efficiency of gains, influences the deposition of muscle tissue as reflected in higher conformation scores. This supports the work of Anwar-Afghan (1967) in that he found the ratio of oxidized to reduced forms to be positively associated with percent lean in the carcass. NADH, a coenzyme which increases fat deposition, is negatively related with conformation score. Some problems arise, however, in interpreting the relationship of these coenzymes with condition score. The relationships tend to be and in fact are exactly opposite to what would be expected, with condition score being positively influenced by the oxidized forms and negatively influenced by the reduced forms. The reason for this appears to be the high positive correlation between conformation and condition scores (Appendix Table 3). From these data it would appear that condition score is a poor indicator of the effect of the nicotinamide coenzymes upon fat metabolism, at least at the stages of development being studied. This is probably due in part to the lack of objectivity in measuring condition score. These scores were assigned after visual appraisal, which allows the bias of the scorer(s) to influence their value. Because of these problems in assigning score to animals it has been found (Bogart, 1975; Clark et al., 1963) that there is no positive relationship between type scores and production traits in beef cattle.

These relationships of nicotinamide nucleotide coenzymes, when measured at 363 kg, with production traits were also indicated to be

the result of influences of NAD and NADP by the least square regression analyses. The significant F values resulting from these analyses are shown in Tables 8 and 9 for heifers and bulls respectively.

Simple correlation coefficients computed between the levels of nicotinamide nucleotide coenzymes measured at 182 kg and production traits indicate the same types of relationships that are true at 363 kg. The heifers have less significant relationships primarily because of the very small sample size involved. The only relationships that do exist between coenzyme levels and production traits for heifers (Table 10) involve condition scores. The problems associated with trying to determine the effect of coenzyme levels on condition score were discussed previously. Again, the effects appear to be the opposite of the expected and are probably due to the positive correlation with conformation scores and not the result of the actions of the coenzymes themselves.

The involvement of the oxidized forms in metabolic mechanisms associated with growth is indicated by the simple correlation coefficients for bulls (Table 11). $NAD^+ + NADP^+$, $NAD^+/NADH$ and $(NAD^+ + NADP^+)/ (NADH + NADPH)$ have a positive influence upon suckling gain. The influence of the $NAD^+/NADH$ ratio on rate of gain may be the result of its relationship with other enzyme systems. Bogart (1975) states that more rapidly gaining calves have significantly higher levels of phosphatase activity, a higher ratio of alkaline to acid

Table 8. F values for significant effects of nicotinamide nucleotide coenzymes (363 kg) on weight per day of age and conformation score of heifers.

Source	F Values	
	Weight per Day of Age	Conformation Score
NADH	4.20*	4.54*
NADPH	10.24**	4.97*
NADH + NADPH	4.60*	
NADP ⁺ + NADPH	5.49*	
NAD ⁺ /NADH		4.56*
NADP ⁺ /NADPH	5.89*	
$\frac{\text{NAD}^+ + \text{NADP}^+}{\text{NADH} + \text{NADPH}}$	5.56*	

* P < 0.05

** P < 0.01

Table 9. F values for significant effects of nicotinamide nucleotide coenzymes (363 kg) on average daily gain, feed per unit gain, conformation score and condition score of bulls.

Source	F Values			
	Average Daily Gain	Feed per Unit Gain	Conformation Score	Condition Score
NADH			8.76**	9.19**
NADPH		8.82**		
NADH + NADPH	6.30*	10.31**		
NAD ⁺ + NADP ⁺				
NADP ⁺ + NADPH		6.12*		5.61*
NAD ⁺ /NADH			7.06*	10.36**
$\frac{\text{NAD}^+ + \text{NADP}^+}{\text{NADH} + \text{NADPH}}$		5.68*		7.44**

* P < 0.05

** P < 0.01

Table 10. Significant simple correlation coefficients between nicotinamide nucleotide coenzymes and condition score of heifers (182 kg).

Trait	Coenzymes				
	NADP	NADPH	NADH + NADPH	$\frac{\text{NADP}^+}{\text{NADPH}}$	$\frac{\text{NAD}^+ + \text{NADP}^+}{\text{NADH} + \text{NADPH}}$
Condition Score	-0.645*	-0.830**	-0.819**	0.769**	0.830**

* P < 0.05

** P < 0.01

Table 11. Significant simple correlation coefficients between nicotinamide nucleotide coenzymes and production traits of bulls (182 kg).

Coenzyme	Production Traits					
	Age		Suckling Gain	Weight per Day of Age	Conformation Score	Condition Score
	182 kg	363 kg				
NADH	0.295*		-0.304*	-0.325*		
NADPH	0.422**		-0.339*			
NAD ⁺ + NADP ⁺	-0.298*		0.324*			0.337
NADH + NADPH	0.333*					
NAD ⁺ /NADH	-0.330*	-0.345*	0.343*	0.386**	0.306**	
$\frac{\text{NAD}^+ + \text{NADP}^+}{\text{NADH} + \text{NADPH}}$	-0.421**	-0.349*	0.417**	0.375*		0.294*

* P < 0.05

** P < 0.01

phosphatase activity, and less variation in activity and ratio of the phosphatase activities from one weight to the next than slowly growing calves. The influence of the NAD^+/NADH ratio on the activity of alkaline phosphatase has been shown by Romasamy and Butterworth (1973). These investigators found that the activity of alkaline phosphatase in pig kidney can be changed four-fold by the alteration of the NAD^+/NADH ratio from zero to infinity. If this type of influence is true in beef cattle, then an increase in the NAD^+/NADH ratio could stimulate higher levels of phosphatase activity resulting in the increased rate and efficiency of gains.

These values indicating increased activity of the oxidized forms influence weight per day of age positively as the increased growth rates result in younger ages at the end of the feed test. The NAD^+/NADH ratio also influences conformation scores positively, indicating a positive influence on muscular development. The paradox of these coenzymes also positively influencing condition score is true in this case also, but again, probably is due to the interrelationships already discussed.

The reduced coenzymes influence growth rates negatively as indicated by the negative correlation of NADH and NADPH with suckling gain. Because of the reduced growth rates associated with these coenzymes, they are associated with a decrease in weight per day of age.

When tests for the effects of the coenzymes on production traits were made by the least squares regression analyses, the same relationships discussed above were found to be significant. The F values resulting from these tests are shown in Tables 12 and 13.

In an attempt to determine the value of nicotinamide nucleotide coenzyme levels in predicting future performance of beef cattle, backward stepwise regression analyses were completed. Factors included in the model were those that would normally be available at weaning time; birth weight, age at weaning, suckling gain, and the coenzyme value being tested. These coenzyme values were those determined at the time of weaning or 182 kg. Those regression coefficients found to be significantly ($P < 0.05$) different from zero were considered as having possible value in prediction of performance. The changes in R^2 values were also noted as each variable left the model. These changes indicate the amount of variation in the dependent variable which is accounted for by the inclusion of the particular independent variable being deleted. In this case, the variable of interest would be the value for one of the nicotinamide coenzymes. A small change in the R^2 value would indicate that the particular coenzyme being tested accounted for little of the variation in the production trait when in the model. Therefore, even though the coefficient of that variable might be statistically significant it would

Table 12. F values for significant effects of nicotinamide nucleotide coenzymes (182 kg) on condition score of heifers.

Source	F Values	
	Condition Score	
NADPH	24.27**	
NADH + NADPH	22.40**	
NADP ⁺ /NADPH	15.88**	
NADP ⁺ + NADPH	7.82*	
$\frac{\text{NAD}^+ + \text{NADP}^+}{\text{NADH} + \text{NADPH}}$	24.27**	

* P < 0.05

** P < 0.01

Table 13. F values for significant effects of nicotinamide nucleotide coenzymes on weight per day of age, conformation score and condition score of bulls.

Source	F Values		
	Weight per Day of Age	Conformation Score	Condition Score
NAD ⁺ + NADP ⁺			5.65*
NADH	5.19*		
NAD ⁺ /NADH	7.69*	4.55*	
$\frac{\text{NAD}^+ + \text{NADP}^+}{\text{NADH} + \text{NADPH}}$	7.18*		4.17*

* P < 0.05

** P < 0.01

not be expected to have much biological value as a selection criterion for improving beef cattle performance.

The significant regression coefficients and their associated R^2 values are shown in Table 14 for heifers. At first inspection, these figures look very impressive due to the high R^2 values. However, the fact that these coefficients are all associated with condition score, which has been shown to bear little relationship to fat metabolism, places their value in question. Also due to the small sample size ($n = 14$), any predictive properties of these relationships would be highly speculative.

Table 14. Significant regression coefficients and associated R^2 values for predicting condition scores of heifers by nicotinamide nucleotide coenzyme levels (182 kg).

Coenzyme	Coefficient	R^2
NADP	-0.005**	0.607
NADPH	-0.007**	0.689
NADH + NADPH	-0.007**	0.671
$\text{NADP}^+/\text{NADPH}$	1.362**	0.591
$\frac{\text{NAD}^+ + \text{NADP}^+}{\text{NADH} + \text{NADPH}}$	1.110**	0.688

**
P < 0.01

Several regression coefficients were found to have some statistically significant predictive value in the case of bulls (Table 15). However, as can be seen by the small R^2 values associated with them, these coefficients would have little biological value in predicting future performance of beef cattle.

Summary

The relationship between nicotinamide nucleotide coenzymes and production traits was determined at 363 kg and 182 kg for both bulls and heifers. The reduced coenzymes were found to negatively influence rate and efficiency of gains, while the oxidized coenzymes were found to be positively associated with rate and efficiency of gains. These associations between coenzyme levels and growth rate were also reflected in the relationships between coenzymes and age within sampling group. When tests between the 363 kg and 182 kg samples were made, coenzyme levels were found to be positively associated with age. Discussion of the actions of the coenzymes that result in these relationships is presented. Despite the relationships that were found, none of the coenzyme parameters determined were found to have value as biological indicators of future performance in beef cattle.

Table 15. Significant regression coefficients and associated R^2 values for predicting weight per day of age, conformation score and condition score of bulls by nicotinamide nucleotide coenzyme levels (182 kg).

Coenzyme	Weight per Day of Age		Conformation Score		Condition Score	
	Coefficient	R^2	Coefficient	R^2	Coefficient	R^2
NADH	-0.005*	0.004				
NADPH	0.002*	0.015				
NADP ⁺ /NADPH	-0.116*	0.028				
NAD ⁺ /NADH	0.068**	0.009	0.142*	0.017		
$\frac{NAD^+ + NADP^+}{NADH + NADPH}$	0.327*	0.000			0.598*	0.011
NAD ⁺ + NADP ⁺					0.006*	0.017

* P < 0.05

** P < 0.01

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APPENDIX

Appendix Table 1. Mean values of production traits by sex and weight groups.

Production Traits	182 kg		363 kg	
	Bulls (n=47)	Heifers (n=13)	Bulls (n=82)	Heifers (n=41)
Birth Weight (kg)	34.79 ± 0.64	32.59 ± 0.85	34.21 ± 0.50	30.73 ± 0.64
Age in Days (182 kg)	181.38 ± 3.56	157.62 ± 5.87	182.74 ± 2.61	165.46 ± 3.60
Age in Days (363 kg)	320.66 ± 5.69	335.69 ± 9.22	324.89 ± 4.65	334.66 ± 3.99
Suckling Gain (kg/day)	0.86 ± 0.03	0.93 ± 0.03	0.85 ± 0.02	0.87 ± 0.02
Average Daily Gain (kg/day)	1.36 ± 0.03	0.97 ± 0.03	1.36 ± 0.03	1.04 ± 0.01
Feed per Unit Gain	6.24 ± 0.14	8.09 ± 0.25	6.15 ± 0.10	7.59 ± 0.13
Weight per Day of Age (kg)	1.16 ± 0.02	1.03 ± 0.03	1.15 ± 0.01	1.03 ± 0.01
Conformation Score	12.25 ± 0.11	12.55 ± 0.14	12.33 ± 0.09	12.73 ± 0.07
Condition Score	11.86 ± 0.10	12.55 ± 0.13	11.88 ± 0.08	12.60 ± 0.07

Appendix Table 2. Significant simple correlation coefficients between production traits of heifers (363 kg).

	Age		Suckling Gain	Average Daily Gain	Feed per Unit Gain	Weight per Day of Age	Conformation Score
	182 kg	363 kg					
Birth Weight	-0.487**			-0.354*			
Age at 182 kg		0.748**	-0.895**	0.356*		-0.753**	
Age at 363 kg			-0.763**			-0.982**	-0.542**
Suckling Gain						0.805**	
Average Daily Gain					-0.703**		0.421**
Feed per Unit Gain							-0.436**
Weight per Day of Age							0.520**

* P < 0.05

** P < 0.01

Appendix Table 3. Significant simple correlation coefficients between production traits of bulls (363 kg).

	Age (363 kg)	Suckling Gain	Average Daily Gain	Feed per Unit Gain	Weight per Day of Age	Conformation Score	Condition Score
Age at 182 kg	0.692**	-0.881**			-0.752**	-0.366**	-0.279*
Age at 363 kg		-0.806**	-0.640**	-0.619**	-0.978**	-0.731**	-0.546**
Suckling Gain				-0.235*	0.846**	0.435**	0.403**
Average Daily Gain				-0.827**	0.599**	0.630**	
Feed per Unit Gain					-0.552**	-0.575**	-0.362**
Weight per Day of Age						0.701**	0.487**
Conformation Score							0.721**

* P < 0.05

** P < 0.01

Appendix Table 4. Significant simple correlation coefficients between production traits of heifers (182 kg).

	Age (363 kg)	Suckling Gain	Average Daily Gain	Feed per Unit Gain	Weight per Day of Age	Conformation Score
Age at 182 kg	0.849**	-0.950**			-0.875**	
Age at 363 kg		-0.811**	-0.682*	0.801**	-0.984**	-0.598*
Suckling Gain					0.869**	
Average Daily Gain				-0.700**	0.673**	
Feed per Unit Gain					-0.773**	

* P < 0.05

** P < 0.01

Appendix Table 5. Significant simple correlation coefficients between production traits of bulls (182 kg).

	Age (363 kg)	Suckling Gain	Average Daily Gain	Feed per Unit Gain	Weight per Day of Age	Conformation Score	Condition Score
Age at 182 kg	0.740**	-0.867**			-0.787**	-0.438**	-0.382**
Age at 363 kg		-0.817**	-0.538**	0.641**	-0.977**	-0.709**	-0.522**
Suckling Gain				-0.317*	0.861**	0.537**	0.477**
Average Daily Gain				-0.808**	0.509**	0.492**	
Feed per Unit Gain					-0.607**	-0.499**	
Weight per Day of Age						0.707**	0.483**
Conformation Score							0.680**

* P < 0.05

** P < 0.01

Appendix Table 6. T_4 prediction equations for average daily gain and feed per unit gain of bulls (182 kg) when suckling is held constant.

$$Y (\text{ADG}) = 1.20 + 0.034 (T_4)$$

$$Y (\text{F/G}) = 3.10 + 0.098 (T_4)$$
