URINARY 17-KETOSTEROIDS, RATE OF GAIN, FEED EFFICIENCY, AND CERTAIN BLOOD CONSTITUENTS OF BEEF CATTLE

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INTRODUCTION

Rate of gain and feed efficiency are influenced by hereditary factors as has been shown by numerous studies. There are two primary problems facing the researcher; namely, the selling of facts to the producer and the discovery of more effective and less expensive procedures which can be applied in early life of the animal for detecting the more desirable individuals. The former is a problem of extension; the latter requires additional basic research. Both rate of gain and feed efficiency can be determined to a fair degree of accuracy. However, the process involves a feed test that is expensive.

It is the opinion of many physiological geneticists that rate of gain and feed efficiency are the result of many complex biochemical reactions, and that these reactions are under genic control. If this concept be true, then it is conceivable that the study of some of the metabolites should be rewarding. If the same genes that affect growth in the feed lot are affecting growth earlier in life, an approach of this nature should yield a method by which the efficient animals could be detected

early in life (preweaning), and might well eliminate the necessity for a feed test. The application of such a concept will mean the use of new techniques (at least for geneticists) and experimental procedures as well as the adaptation of known procedures to the solving of problems of this nature. The geneticist will depend upon the biochemist for many of these techniques. He may need to modify some techniques to fit his particular purpose.

This has been the approach in beef cattle research at the Oregon Agricultural Experiment Station. MacDonald (77) and Williams (108) have obtained a large amount of data involving blood and urine chemistry. They have attempted to relate variations in chemical constituents of the blood and urine to variations in rate of gain and feed efficiency. Their work has shown great promise in uncovering facts regarding these economic traits. The experimentation at the Oregon Station has concerned itself largely with protein metabolism. Work of other researchers indicates the possibility of a relationship of the adrenal cortex to protein metabolism and its association with rate of gain and feed efficiency.

The present study was designed to continue the investigations on protein metabolism through blood chemistry; to investigate the relationship of adrenal cortex activity to protein metabolism; to adapt further

biochemical methodology to animal improvement research; and to increase our knowledge of metabolism and physiology in our larger meat animals.

REVIEW OF LITERATURE

Rate of Gain and Feed Efficiency

Performance testing is not new. Housman (1894) tells us of Robert Bakewell, who in the 18th century, made use of the tools which are now in general use by modern breeders (109, pp.27-31). Interest has been renewed only recently (98, pp.41-47; 110, p.90).

Black and Knapp (15, pp.72-77) concluded that a feed test from a body weight of 500 lbs. to a weight of 900 pounds would measure the performance of an individual beef animal. For a final evaluation they recommended using efficiency of gain during this period and carcass grade. They found that if animals were compared over a constant range in weight, rate of gain and feed efficiency were highly correlated. Efficiency of feed utilization could be predicted from rate of gain only when individuals were compared during the same weight range.

Koger and Knox (65, p.765) concluded that if animals, under similar environments, were compared at different periods, then there would be a positive relationship. This has been indicated by Nelms and Bogart (89, p.977). However, a negative relationship can be expected when a set of environmental conditions are experienced during one period and later removed or changed. Knapp and Clark

(61, pp.174-188) found no correlation between pre- and post-weaning periods, and concluded that gains prior to weaning were of no value in predicting feed lot gain. A negative correlation has been reported by several workers (15, p.75; 31, p.18; 16, p.34). This inconsistancy can be expected when one examines the heritability estimates for suckling gains, which are relatively low, (62, p.585). If the environmental variation could be reduced, possibly by providing adequate feed during the suckling period, the heritability estimates would undoubtedly be higher.

Heritability estimates for some economic traits have been reported by Knapp and Nordshog (63, p.69); and revised by Knapp and Clark (62, p.587); Kohli et al. (67, p.252); and others. All estimates indicate that the ability to gain rapidly and make efficient use of feed are highly inherited. Selection based on the performance of the individual, therefore, would be the most effective procedure in improving these traits.

Various attempts have been made to correlate some anatomical measurements with growth rates. One of the earliest studies of this nature was that of Hultz (52, pp.71-94). He found that very rangy calves made more rapid gains than very low-set calves and that cattle on a fattening ration changed type. Lush (76) concluded

that steers of many shapes gain well and steers which gain the same may be of many shapes. MacDonald and Bogart (78, p.460) more recently found that scores at the initiation or scores at the completion of the feed test were unrelated to rate or efficiency of gains.

Kohli, Cook and Dawson (66, pp.352-364) compared the relationship of various body measurements to rate and efficiency of gains, and they found that steers tended to vary independently of the body dimensions. It can be concluded that anatomical measurements are of little value in predicting rate of gain and feed efficiency.

MacDonald (77) determined the relation of certain blood and urine constituents to rate of gain and feed efficiency. He found indications of a relationship of blood urea nitrogen, blood amino acid nitrogen and urine urea nitrogen to rate of gain and feed efficiency. It is unfortunate that these data lacked statistical treatment. Williams (108) made a more extensive study of the blood constituents. He found blood creatinine to be negatively associated with rate of gain. However, he concluded that the blood constituents studied were not capable of ranking the animals as to rate of gain, feed efficiency or feed intake (108, p.74).

Attempts have been made to determine if an animal's ability to digest food is related to rate and efficiency

of gain. Baker, Colby and Lyman (6, p.731) found a positive relationship between the animal's ability to digest crude fiber and its feed efficiency. Nelms, Price and Bogart (90, p.217) found none of the digestive coefficients studied related to rate and efficiency of gains. This could possibly be due to lack of adequate techniques in determining digestibility.

Endocrine Control of Growth and Nitrogen Metabolism

Excellent reviews are available (9, pp.45-82; 72, pp.197-225) regarding endocrine control of nitrogen metabolism. Therefore, only the pertinent articles will be reviewed.

It has been demonstrated quite conclusively that injections of the growth hormone (somatatropin) will stimulate growth (37, p.71; 60, pp.747-760; 72, pp.210-211). The growth from growth hormone injections was continuous for long periods of time. Kleiber and Cole (60) measured the oxygen consumption as an index to metabolism. The oxygen consumption of the rats treated with growth hormone was 30 per cent less than that of the control animals. They feel that the response to the growth hormone is through a lowered metabolism rather than through changes in the protein and water content of new tissue. However, Marx et al. (80, pp.544-550) did not agree with

this conclusion. They found that the main effect of purified growth hormone was to decrease nitrogen excretion. Gordon et al. (45, p.318) have found evidence to support the latter contention. Bierring and Nielsen (13, pp.1015-1021) have observed that there was an increase in body water above that normally associated with protein tissue after the administration of the anterior pituitary extracts.

Further changes in body compostion after treatment with pituitary extracts, which caused accelerated growth, have been studied. Lee and Schaffer (69, pp.337-363) reported that treated rats maintained their initial composition and heat values, while the control animals showed decreases in the proportion of water, nitrogen, fat-free dry tissue, and ash and increases in fat and heat value of the tissue. The energy expended was 1.7 times the energy gained per gram weight increase for treated animals and 2.4 times the energy gained per gram weight increase for the controls. In studying the association of pituitary growth output with rate of growth in genetically different lines of swine, Baird et al. (5, pp.292-300), reported that rapidly growing animals showed a peak output of the hormone from 56 to 115 days of age while the slow growing animals plateaued in the output of the growth hormone during this period. Both lines showed a

reduction in growth hormone output as they approached maturity. This has been suggested as a possible reason for the growth stasis in mature animals.

It was found that growth hormone extracts were equally effective in adrenalectomized and intact animals (99, pp.234-240). In addition, those which were completely thyroidectomized responded to growth hormone extracts (97, pp.227-236). The gonads evidently are not required for the action of the growth hormone (104, pp. 468-471; 36, pp.511-546).

The relationship between the growth and sex hormones has been studied extensively. Bogart, Lasley and Mayer (19, pp.173-181) demonstrated that injections of estrone retarded growth in both normal and ovariectomized rats. Kim, Magee and Ivy (58, pp.525-528) felt that different levels of testosterone could explain the mechanism for the differences in growth rate between males and females. They reported female growth rate could be brought up to that of males by appropriate administration of testosterone proprionate.

Apparently testosterone acts by causing an increase in nitrogen retention. Perlman and Cassidy (93, pp.674-675) reported that dogs injected with testosterone proprionate retained an increased amount of nitrogen while on a high nitrogen diet. Dogs on low nitrogen diets

failed to show the increased nitrogen retention. Burris, Bogart and Oliver (26, pp.740-746) demonstrated that rate and efficiency of gains in steer and heifer calves could be increased by injections of testosterone. They concluded that since testosterone injections results in increased thyroxin output and reduced thyroxin storage that the growth associated with testosterone injections was probably mediated through the thyroid gland. Burris and Bogart (25, pp.1-6) reported that pituitaries, from calves having received testosterone, contained higher levels of thyrotropin than pituitaries from untreated animals. Also, pituitaries from steer and bull calves were higher in thyrotropin than those from heifers.

Growth and Associated Nitrogenous Metabolites

Protein is a basic constituent of all living tissue, and gain in body weight of young animals is largely an increase in muscle mass; therefore, it seems that protein metabolism should be considered if one is making a study of growth of young animals.

Amino acids may take any one of several pathways after being absorbed in the body. They may be stored, converted to tissue proteins, transaminated or deaminated, or even circulated in the original state. Various factors affect the blood amino acid level. Age and sex appear to

have little effect, though values for infants appear to be lower than for adults. This probably is due to the more rapid utilization of amino acids for tissue formation by the young. The ingestion of a protein meal in the postabsorptive state generally increases the level of whole blood amino acid nitrogen (106, p.1054). Certain diseases may affect the level of amino acid nitrogen in the blood. According to Steele, Reynolds and Bauman (101, pp.124-132), dietary changes will not alter the concentration of blood amino acids. MacDonald (77, p.112) reported a linear relationship between rate and efficiency of gains and blood amino acids in the young beef cattle, with faster and more efficient gainers exhibiting lower concentrations of amino acids. However, Williams (108, p.73) failed to show this relationship, but did find amino acid concentration affected by sex and feed intake.

The major portion of the nitrogen released by the catabolism of amino acids eventually appears in the urine as urea by means of the ornithine cycle or some similar system (68, pp.33-36). Austin, Stillman and Van Slyke (4, pp.91-104) pointed out that urea production was in direct proportion to the blood urea concentration, which indicated that either source (blood or urine) would serve for sampling for a measure of protein metabolism. Hersted et al. (49, pp.14-18) concluded from the protein

depleted rats that the protein catabolism proceeds in such a manner that the ratio of protein to body water remains fairly constant. This makes it possible for one to estimate the percentage protein involved in loss of body weight by converting the nitrogen from the increased urea production to protein by the factor 6.25, and multiplying by a factor of 5 to account for the associated body water (11, pp.198-226).

Blood uric acid is the product of purine-pyrimidine catabolism. In man it is the final end product but in most mammals the greater portion of it is oxidized to allantoin. Uric acid and allantoin are probably made up of the endogenous portion of nitrogen excretion. Blaxter and Wood (17, pp.11-25) pointed this out in their experiments with an Ayrshire calf on a nitrogen free diet. They found a very low excretion of urine, but uric acid and allantoin remained constant. Lennon (70, pp.521-572), reported that uric acid production in fasting humans decreased in the urine to the point at which the concentration in the urine was less than that in the blood.

The mechanism of kidney function relative to the excretion of uric acid has been investigated. Blaxter and Wood (18, pp.29-55) in their studies with the Ayrshire calf noted that there was an apparent inhibition of fat upon uric acid excretion rate. Earlier Alderberg

and Ellenberg (2, pp.379-385) had shown that this inhibition was not due to the effects of adding additional calories, because the addition of carbohydrates did not depress uric acid excretion. Freidman and Byers (42, pp. 684-687), using rats, found that the effect of ACTH on the production of urate was due to a direct action of the hormone on the kidney. The difference in uric acid excretion was due to tubular reabsorption of urate rather than due to an effect on glomerular filtration, since there was no change in the excretion of allantoin and creatinine. Christman (29, pp.303-348), in his review of purine-pyrimidine metabolism, concluded that most changes in the excretion of uric acid are due to impeded resorption rather than increased production.

The daily output of creatinine in the urine is constant for the individual (12, p.637). Unlike urea excretion, which is derived largely from exogenous sources, the creatinine output is practically independent of the protein level of the food. The creatinine excretion is therefore considered to be an index of the magnitude of the metabolism of the tissues and especially of muscle. Creatinine, unlike creatine, is purely a waste product and is generally considered an index to muscle metabolism.

The basis for modern knowledge of the adrenal cortex in protein metabolism rests in the now classical report

of Long, Katzin and Fry (75), in which it was shown that the administration of adrenal cortical extract increased the nitrogen excretion and liver glycogen of normal and adrenalectomized rats when fasted. Without treatment with adrenal cortical extracts the values for nitrogen excretion and liver glycogen were less than the normal for adrenalectomized rats. These observations have been repeatedly confirmed. Marx et al. (81, pp.102-105) demonstrated antagonism between the growth hormone and the adrenocorticotropic hormone as measured by body weight increase and by the degree of proliferation of the proximal epiphyseal cartilage of the tibia. The inhibition of the adrenocorticotropic hormone occurred in the absence of the pituitary gland and could not be attributed to the reduction in feed intake.

Hoberman (50, pp.431-367), using adrenalectomized rats, into which was injected glycine - N^{15}, obtained a reduced urinary nitrogen output and found that the amount of amino acid nitrogen formed from body protein was depressed. The amino acid nitrogen used in protein synthesis was increased. When the rats were fasted and chilled to stimulate over adrenal function the protein catabolism increased. When the adrenocorticotropic hormone was administered to hypophysectomized rats a depression in protein formation occurred. He felt this

indicated that insulin, not the growth hormone, was the target of the adrenal cortical hormones.

There has been a vast amount of investigation concerning the localization of the site and mode of action of the adrenal hormones in protein metabolism. By measuring the rate of urea formation in the nephrectomized rat treated with adrenal cortical extracts, Engel, Schiller and Pentz (35, pp.458-475) and Sayers et al. (96, pp.593-614) have demonstrated that an increase in nitrogen metabolism becomes apparent between 3 and 6 hours after the treatment. This time interval is comparable to that which has been found necessary to demonstrate other metabolic effects of the hormone. Ingle and his co-workers (56; 53) have shown that long continued administration of cortisone or adrenocorticotropic hormone results in considerable increase in nitrogen excretion. With the largest doses of the hormones, weight loss and increased nitrogen excretion persisted until death. With smaller doses nitrogen excretion often returned to normal despite continued administration of the hormone. They felt that this action was not due to depletion of mobilizable protein, but represented some type of endocrine and metabolic adaptation.

The effects of adrenal hormones on protein metabolism are widespread, but are not felt equally in all organs or

tissues. Somatic growth as a whole is suppressed by treatment with adrenal hormones. White and Dougherty (107) have shown that lymphoid tissue exhibits particularly rapid dissolution in response to these hormones. Other investigators have reported hair, skin, bone and connective tissue growth also are inhibited to a large degree. These effects appear to be exerted directly on the tissues in question, since Baker and Whitaker (7; 8) demonstrated atrophy of skin and inhibition of hair growth and wound healing on topical application of these hormones.

Under the proper experimental conditions, evidence can be found for an action of the adrenal cortex at just about all levels of nitrogen metabolism. A metabolic action of the adrenal cortex on the step between protein and amino acids can be demonstrated in studies on the rate of accumulation of amino acids in the plasma of eviscerate rats which have either been adrenalectomized or treated with cortisone. Bondy (20) and Ingle, et al. (55; 54) using this technique have shown that adrenalectomy depresses the release of amino acids from peripheral tissues, while cortisone increases it. In studies on urea production in nephrectomized rats that have received protein intravenously, there is likewise the suggestion that whole protein is more readily metabolized

in the presence of adrenal hormone excess and is not well utilized catabolically in the absence of the adrenal cortex (35; 21). Other evidence indicating a poor ability to mobilize and catabolize protein in the absence of the adrenal cortex is found in the failure of the fasted adrenalectomized animal to exhibit a negative nitrogen balance in response to injury (57).

Using the isotope technique, Hoberman (50), Barton (10) and Sinex (100) have obtained data which indicates that rates of both processes, anabolism and catabolism of protein are modified by adrenal hormone action or deficiency. At the present, there appears to be general agreement that the adrenal cortex does play an important role in the mobilization and eventual catabolism of protein and in the sparing of amino acids by decreasing their incorporation into protein, i.e., inhibiting protein anabolism. The question as to whether the adrenal cortex plays a significant role in the catabolism of amino acids is more controversial. There is evidence in both directions.

There is no satisfactory method for measuring the secretory activity of the adrenal cortex. With the exception of the method of Vogt, where young suprarenalectomized rats, submitted to stress from cold, are injected with cortical extracts (105, pp.431-456), which

unfortunately is limited in its application, the methods are indirect in their approach. Indices for the assessment of adrenocortical activity may be classified under four general headings, according to whether measurement is made of: 1) Alterations in the adrenal cortex, 2) alterations in the organism, 3) rate of excretion of steroids in the urine, or 4) titer of corticoids in the blood. It is obvious that the first two have limitations with regard to live animals. The latter is vague in that the corticoids have not been unequivocally identified as a cortical hormone or its metabolic product.

Clinical investigators (1; 14) have considered the 17-ketosteroids of the urine to be an index of the androgenic activity of the testes and the adrenal cortex. In normal subjects, under optimal conditions of the environment, adrenocortical activity is responsible for two thirds of the 17-ketosteroid excretion in the male and for the total 17-ketosteroid excretion in the female (95, p.258). However, it seems reasonable to expect less than one third of the 17-ketosteroids, of the male, to originate from the testes of the young animal (prepubertal). Recent data have indicated that the cortical hormone is degraded in the liver and other tissues of the body to 17-ketosteroids. The adrenocorticotropic hormone produces a parallel increase in urinary 17-ketosteroids,

chemocorticoids and biocorticoids (40; 83; 96; 103). The administration of cortisone is followed by an elavation in 17-ketosteroid excretion (95, p.258).

To summarize, performance can be determined by establishing rate of growth and rate of feed conversion. These economic traits have high heritability estimates. Therefore, the most effective procedure to improve these traits is to select on the basis of individual performance.

The determination of growth rate is fairly simple, but to determine feed efficiency an expensive feed test is involved. It would be desirable to predict performance of an individual at an early age, so that some selection could be practiced early in addition to eliminating need for the feed test.

Attempts have been made to correlate some anatomical measurements with performance, but this has been discouraging. Body type is not related to rate of growth and feed efficiency. Early growth will predict later performance only if the animals are under similar environments during both phases of growth, which is a rare situation.

More recently attempts have been made to relate nitrogen metabolism to growth rate. The latter method has shown some promise. It would seem likely that this

approach and/or the determination of hormonal activity would have a sound physiological basis.

The growth hormone stimulates growth rate by causing increased nitrogen and water retention. Testosterone stimulates growth by causing an increased nitrogen retention and by increasing the thyroxin output. Unfortunately, no satisfactory method of assay has been developed which can be applied to live animals.

The nitrogen metabolites have been studied extensively. Blood urea seems to have a closer relationship to rate of gain and feed efficiency than any of the other metabolites studied. Blood amino acid and blood creatinine have been reported to be related to rate of gain and feed efficiency, but to a lesser degree.

The adrenal cortex activity is related to protein metabolism. The cortical hormones affect protein metabolism by inhibiting the conversion of amino acids to protein and by increasing the catabolism of the already formed protein.

MATERIALS AND METHODS

The thirty-three purebred Hereford calves used in this study represent the offspring of three closed lines, namely: Lionheart, Prince and David. All three lines are maintained at the Oregon Agricultural Experiment Station and are essentially unrelated. The calves were unselected in that the entire offspring from each of the three lines were included in the study. However, all the calves were produced by animals which were selected on the basis of performance records.

Management

All calves were born during the spring of 1954 and were weaned at approximately 425 pounds body weight or November 19, 1954, whichever came first. Upon weaning, all calves were placed on an individual feeding regime. The procedure while on feed was essentially that as described by Nelms, Williams and Bogart (91, pp.1-2). They were fed a completely pelleted ration, composed of 2 parts chopped alfalfa and 1 part of concentrate. The calves were weighed weekly and the weekly feed intake was calculated after each weighing.

Urine Collection

Bull urine was collected as described by MacDonald

(77, p.33), using a Davol rubber funnel (22, p.479) strapped to the animal with a harness. A rubber hose connected the funnel to a 5 gallon carboy beneath the floor of a metabolism stall (22).

The heifer collecting unit was designed according to Hansard, Comar and Plumlee (47, pp.13-25). The attachment was a modification of that described by MacDonald (77, pp.35-44). Instead of attaching the straps by means of branding cement, a harness was designed (84) to hold the conduit in place.

In order to standardize environmental effects as much as possible, all animals were refused water and feed during the collection period. A 24-hour collection was obtained from all animals, which eliminated or minimized diurnal variations (94, p.81). All urine samples were collected under toluene. An aliquot sample was taken from the 24-hour collection and stored at 0°C under toluene until analyses could be completed.

Blood Collection

Ten milliliters of blood were drawn from the jugular vein through a stainless steel bleeding needle into a bleeding tube containing potassium oxalate at the rate of one milligram of potassium oxalate per milliliter of blood (48, p.491). All samples were drawn between the hours of 8:00 A.M. and 10:00 A.M. and the analysis was

initiated immediately.

Urinalysis

The 24-hour urine collection was measured at the end of the collection period by means of a graduated cylinder. The specific analyses included the hydrolysis, extraction and colorimetric determination of 17-ketosteroids.

The Hydrolysis and Extraction of 17-ketosteroids. The major portion of the 17-ketosteroids which occur in urine are in the form of their conjugates. The sulfates and glucuronosides of 17-ketosteroids have been isolated from urine (73, pp.113-114). The quantitative hydrolysis of the conjugated 17-ketosteroids has been the concern of many investigators (73, pp.113-134). Since there are three excellent reviews already published (73, pp.113-134; 82, pp.330-335; 44, pp.484-491), no attempt will be made to review all the original papers on the subject. There is no general agreement on the conditions of hydrolysis which leads to optimal results. Ideally, hydrolysis should quantitatively free the neutral steroids from their conjugated state and should do so without destruction or alteration of their molecular structure. The usual procedure is to boil the urine for 10 minutes after the addition of concentrated hydrochloric acid to the urine in the amount of one tenth the urine volume. It

is then cooled quickly and extracted.

Extraction of the free 17-ketosteroids after hydrolysis of the conjugates may be accomplished by shaking the urine in a separatory funnel with an organic solvent or by use of a continuous extractor. The latter procedure is very convenient when it becomes necessary to make many determinations.

Carbon tetrachloride, benzene and ether have been used widely for extraction. More urinary pigments are extracted by ethyl ether than by carbon tetrachloride or benzene (82, p.331). Although little of the urinary pigments are extracted by butyl ether (79, p.121; 30, p.883), it has the disadvantage of a relatively high boiling point (140°C.).

The urinary extract is washed with a solution of base in order to remove acids, phenols and some color. Talbot and associates (102, pp.365-377) introduced sodium hydrosulfite ($Na_2S_2O_4$) into the sodium hydroxide wash. This step results in bleaching the color of the extract and appears to be of value in that much undesirable coloring matter is lost.

The method of hydrolysis and extraction used in this study has been outlined by Engel (44, p.502). One hundred ml. of urine were heated to boiling under a reflux condenser, after which 10 ml. of hydrochloric acid

were added. Boiling was continued for exactly 10 minutes. The solution was cooled somewhat and 30 ml. of carbon tetrachloride (C.P. grade) was added, and the boiling continued for exactly 10 minutes. The carbon tetrachloride was removed by a separatory funnel and saved. The extraction was repeated by adding 30 ml. of fresh carbon tetrachloride and again, the solution was boiled for 10 minutes. The extract was removed and added to the first. The combined extracts were then washed with 20 ml. of distilled water, with 20 ml. 2 N sodium hydroxide, with 20 ml. distilled water, and with 20 ml. distilled water containing a small amount of sodium dithionite ($Na_2S_2O_4$). The extract was then evaporated to dryness and dissolved in 5 ml. of absolute alcohol. The colorimetric determinations were made from this extract as described below. The hydrolysis and extraction procedures were duplicated for all samples.

The Colorimetric Analytical Methods for Neutral 17-ketosteroids of Urine. Colorimetric analytical methods, with all their limitations, are the basis for the majority of quantitative investigations of the metabolism of the steroid hormones. Two excellent reviews cover the various methods available (87, pp.135-161; 82, pp.332-335) and have been summarized by Engel

(44, pp.492-494). The general method for the colorimetric determination of neutral 17-ketosteroids involves the Zimmerman reaction, which is based on the reaction of the group -CH_2CO- with m-dinitrobenzene in alkaline solution (112; 113). There have been innumerable modifications of the basic procedure proposed (82; 87). However, there are only two time tested variants in general use. One is probably as good as the other, both having limitations.

The basic feature of the Zimmerman reaction is in common with both procedures (Callow, Callow and Emmens (27) and Holtoroff and Koch (51)). The alcoholic solution is incubated with a concentrated, strongly alkaline solution of m-dinitrobenzene at a constant temperature of 25°C. in the dark. The reaction mixture is diluted with additional alcohol, and estimation of the intensity of color of the resulting product is accomplished in a photoelectric colorimeter or spectrophotometer. The essential difference between the two methods consists in the concentrations of potassium hydroxide and of alcohol in the reaction mixture. The principal advantage of the Holtoroff-Koch method is in the convenience afforded by the use of an aqueous solution of potassium hydroxide, which is stable for indefinite periods, in contrast to the alcoholic KOH of the Callow procedure, which is unstable.

The colorimetric determination used in this study was the Holtoroff-Koch method (51) as modified by Nathanson and Wilson (88). To 0.2 milliliters of alcoholic extract was added 0.2 ml of 2 per cent m-dinitrobenzene and 0.2 milliliters of 5N ± 0.25 potassium hydroxide. A standard (dehydroepiandrosterone acetate), a reagent blank and a color blank were run simultaneously with each set of determinations. The reaction mixtures were then incubated at 25°C. in the dark for 105 minutes. At the end of the incubation period the reaction mixtures were diluted to 10.6 milliliters with 70 per cent alcohol. The intensity of color was estimated using a Coleman Photoelectric Photometer employing a 1 cm. cuvette. Each alcoholic extract was determined in duplicate or triplicate.

Blood Analysis

The blood samples were analyzed for urea nitrogen, amino acid nitrogen, creatinine, uric acid and hemoglobin. The procedures used for the various constituents of the blood have been developed and employed for analyzing blood of human and other species. In the photometric determinations, optical density was determined using a Coleman Photoelectric Spectrophotometer employing a 1 cm. cuvette. The methods involve the obtaining of a protein

free filtrate (46, pp.469-471), as summarized by Hawk, Oser and Summerson (48, pp.493-494). The methods used are considered standard in this laboratory and have been described previously (77, pp.39-40; 108, pp.25-26).

Statistical Analysis

Since the data did not represent equal and proportionate numbers in all sub-classes, they were analyzed using a regression model as outlined by Anderson and Bancroft (3, pp.191-203). Additional interpretation of the results was made possible by methods provided by Calvin (28). A regression model was designed for each of the dependent variables. The analysis of variance was taken from the regression model. The 5 per cent significance level has been used throughout this study.

Rate of gain was determined by plotting the increasing live weight, while on the feed test, against time on arithmetic graph paper, and fitting a straight line to the points by the least squares method. Rate of gain is the average change in live weight per day.

Average feed efficiency was decided by calculating total feed consumed during the feed test and dividing by the amount of gain made. Feed efficiency throughout this study will be measured by amount of feed required per unit of gain. In other words, the most efficient animals required less feed per unit of gain.

Since weekly weights were taken, feed intake was calculated as the average feed consumed per week during the first 4 weeks of the feed test for the 500 pound measurement and during the last 4 weeks for the 800 pound measurement. Age-on-test was the number of days in age an animal was on the day in which the weight nearest 500 pounds was taken. Age at 800 pounds was the age of the calf in days at the weekly weight which most closely approximated 800 pounds.

RESULTS

A total of 33 purebred Hereford bulls and heifers were used in this study. The data on each animal include averate rate of gain on feed test (rate of gain), average feed efficiency (feed per 100 pounds gain), age (in days) at 500 pounds body weight and at 800 pounds body weight, feed intake (pounds feed per week), and some chemical data from blood and urine analyses. Blood was analyzed for urea nitrogen, amino acid nitrogen, creatinine, uric acid, and hemoglobin. Total urine volume per 24 hours, specific gravity and 17-ketosteroid concentration were obtained from urine samples. Blood and urine data were obtained both at 500 and at 800 pounds body weight. All data were determined on individual animals.

Rate of Gain and Feed Efficiency

Preliminary to analysis of data, the increase in body weight with time was plotted on arithmetic graph paper for each animal. Body weight was a linear function of time for each of the 33 animals for the period from 500 to 800 pounds body weight. It was decided, therefore, to use average daily rate of gain and average feed efficiency.

There are large differences between sexes in rate of gain and feed efficiency (Table 1), with male calves

Table 1

Average Rate of Gain, Average Feed Intake, and Feed Efficiency of the Test Calves from 500 Pounds Body Weight to 800 Pounds Body Weight

ITEM	LINE AND SEX							
	LIONHEART		PRINCE		DAVID		ALL	
	Males	Females	Males	Females	Males	Females	Males	Females
Number	5	6	7	5	6	4	18	15
Rate of Gain (lbs. per day)	2.85	2.12	3.08	2.09	2.85	2.42	2.92	2.19
Feed Efficiency (lbs. feed per lb. gain)	7.06	9.16	6.09	8.33	6.61	7.61	6.60	8.47
Average Feed Intake, 500-800 lbs. Body Weight (lbs. feed per wk.)	144	134	127	122	130	130	133	129

gaining at a faster rate than females. However, when analyzed statistically, these differences were significant only in the case of feed efficiency (Table 5). The various lines also exhibited marked differences. Here again, when treated statistically, significance occurred only in the case of feed efficiency. Both sex and line differences have been found to be significant in previous years (16, p.52; 31, p.22).

The age at which the animals reached 500 pounds body weight had a significant effect on rate of gain and feed efficiency (Table 6). This is in agreement with Dahmen and Bogart (31, p.16). Contrariwise, Nelms and Bogart (89, p.978) found that when feed efficiency was corrected for maintenance the younger calves were more efficient. The regression coefficients of .0044 and -.0071 (Table 6) for rate of gain and feed efficiency, respectively, indicate that each 10 days change in age at 500 pounds body weight is associated with a change of 0.044 pounds in gain per day and 7.1 pounds in feed required per 100 pounds gain.

Feed Intake

The average weekly feed intake for the experimental groups at 500 and 800 pounds body weight are shown in Tables 2 and 3, respectively. There was little or no

Table 2

Average Feed Intake, Age, Urinary 17-ketosteroid Excretion Rate, Blood Urea, Blood Amino Acids, Blood Creatinine, Blood Uric Acid and Hemoglobin of Test Calves at 500 Pounds

Line and Sex	Number of Animals	Feed Intake (lbs. per wk.)	Age on Test (days)	Urinary 17-ketosteroids (mg. per 24 hrs.)	Blood Urea Nitrogen (mg. per 100 ml.)	Blood Amino Acid Nitrogen (mg. per 100 ml.)	Blood Creatinine (mg. per 100 ml.)	Blood Uric Acid (mg. per 100 ml.)	Hemoglobin (gm. per 100 ml.)
Lionheart									
Males	4	110	215	121	13.8	6.8	1.18	2.24	11.5
Females	6	101	231	102	19.3	7.0	0.93	2.33	12.1
Prince									
Males	7	94	233	106	13.9	7.8	1.00	1.94	11.7
Females	5	96	228	98	12.9	6.1	1.14	2.31	12.4
David									
Males	6	98	244	123	10.6	7.0	0.92	1.52	11.9
Females	4	100	267	94	15.5	6.2	0.74	2.44	11.9
All									
Males	17	100	233	116	12.7	7.3	1.00	1.86	11.7
Females	15	99	242	98	16.2	6.5	0.95	2.35	12.1

Table 3
Average Feed Intake, Age, Urinary 17-ketosteroid Excretion Rate, Blood Urea, Blood Amino Acids, Blood Creatinine, Blood Uric Acid and Hemoglobin of Test Calves at 800 Pound Body Weight

Line and Sex	Number of Animals	Feed Intake (lbs. per wk.)	Age (days)	Urinary 17-ketosteroids (mg. per 24 hrs.)	Blood Urea Nitrogen (mg. per 100 ml.)	Blood Amino Acid Nitrogen (mg. per 100 ml.)	Blood Creatinine (mg. per 100 ml.)	Blood Uric Acid (mg. per 100 ml.)	Hemoglobin (gm. per 100 ml.)
Lionheart									
Males	5	159	326	99	11.6	8.4	1.32	1.96	13.0
Females	6	151	405	125	12.8	8.1	1.11	2.05	14.2
Prince									
Males	7	142	352	105	9.7	6.4	1.07	1.46	12.1
Females	5	136	383	134	11.4	6.6	1.02	1.26	12.7
David									
Males	6	148	371	136	10.2	6.5	0.85	1.55	13.2
Females	4	145	414	100	13.6	7.6	0.90	1.76	12.9
All									
Males	18	149	351	113	10.4	7.0	1.07	1.63	12.7
Females	15	144	400	121	12.5	7.5	1.02	1.71	13.4

effect of sex or line on feed intake (Tables 4 and 5), although the Lionheart line consumed slightly more than David or Prince lines, at both body weights.

Blood Constituents

Urea Nitrogen. All blood urea nitrogen averages fell well within the normal range (6-27 mg./100 ml.) presented by Dukes (32, p.54), with the exception of one animal (3.5 mg./100 ml.) at 500 pounds body weight. MacDonald (77, p.48) reported several females exceeding this range, but none were below. The means for blood urea nitrogen are presented in Table 2 for 500 pounds body weight and in Table 3 for 800 pounds body weight.

At 500 pounds body weight, both sex and line differences existed in blood urea nitrogen (Table 2). When analyzed statistically, these differences were significant. The females had a higher level of urea nitrogen in all cases, with the exception of the Prince line. The Prince line exhibited little sex difference in blood urea concentration at this body weight. The Lionheart line showed higher values of urea in both sexes than the other two lines.

At 800 pounds body weight the line difference had disappeared (Table 3). A sex difference existed, but was not as marked as at 500 pounds body weight. Neither

Table 4
Summary of Analysis of Variance of
Items Studied at 500 Pounds Body Weight

	Mean Squares				
Item	Lines	Sex	Line x Sex	Error	Standard Error
Degree of Freedom	2	1	2	25	
Feed Intake	32	2	77	98	10
Age	1816	1224	634	1124	34
Urinary 17-ketosteroids	84	2269	329	662	26
Blood Urea	57*	57*	27	13.5	3.7
Blood Amino Acids	1.32	1.59	2.23	2.56	1.60
Blood Creatinine	.18*	.07	.14*	.04	0.20
Blood Uric Acid	.02	2.03*	.43	.57	0.77
Hemoglobin	.30	.01	.31	1.18	1.1

* Indicates statistical significance at 5 per cent level of probability

Table 5

Summary of Analysis of Variance of Items Studied at 800 Pounds Body Weight

Item	Mean Squares				
	Lines	Sex	Line x Sex	Error	Standard Error
Degree of Freedom	2	1	2	27	
Rate of Gain	.14	.44	.18	.12	.35
Feed Efficiency	2.95**	1.68*	1.51	.35	.59
Feed Intake	316	23	15	102	10
Age	1322	4844**	1641	780	28
Urinary 17-ketosteroids	1351	3081	3389	1238	35
Blood Urea	5.69	27.21	3.34	12.28	3.5
Blood Amino Acids	3.04	3.12	1.42	1.93	1.39
Blood Creatinine	.05	.01	.05	.06	.25
Blood Uric Acid	.86**	.11	.12	.12	.35
Hemoglobin	3.63**	.24	1.6*	.46	0.7

* Indicates statistical significance at 5 per cent level of probability

** Indicates statistical significance at 1 per cent level of probability

lines nor sex differences were significant at this body weight. The values for all experimental groups were slightly less at 800 pounds body weight than at 500 pounds body weight.

Blood urea nitrogen was related to age at 500 pounds body weight. This relationship was significant when tested statistically (Table 6). The regression coefficient of -.070 (Table 6) indicates that for each difference of 10 days in age, on reaching 500 pounds there is associated a change in blood urea nitrogen of 0.7 mg./100 ml. The older animals had the lower values.

Blood urea nitrogen is not significantly related with feed intake (Table 7) on the ration used in these experiments.

Amino Acid Nitrogen. Like blood urea nitrogen, average amino acid nitrogen for all groups fell within the normal range (4-8.5 mg./100 ml.) presented by Dukes (32, p.54). One animal exceeded (14.9) this upper limit at 500 pounds body weight. Several animals of the Lionheart line either exceeded or approached the upper limit at 800 pounds body weight. There appeared to be little difference among the groups (Tables 2 and 3). A sex difference existed at 500 pounds in the Prince line, with males having the higher values, and at 800 pounds

in the David line, with the females having the higher values. The Lionheart line exhibited higher values at 800 pounds than the other lines. However, none of these differences were statistically significant. Williams (108, p.44) reported a sex difference in blood amino acid nitrogen concentration.

Blood amino acid nitrogen, unlike blood urea nitrogen, was not significantly related to age (Table 6).

The regression coefficient, 0.044 (Table 7), of feed intake on blood amino acid nitrogen approached significance. It is interesting to note that this is a positive relationship. Williams (108, p.44) found feed intake and blood amino acid nitrogen to be significantly related at 600 pounds body weight.

There was no apparent difference between blood amino acid at the two body weights.

Blood Creatinine. Several values fell below the normal range (1-2.07 mg./100 ml.) presented by Dukes (32, p.54). Eighteen of the animals at 500 pounds body weight fell below the lower limits as compared to 14 at 800 pounds.

Line and sex differences within the lines in blood creatinine exist at both 500 pounds and 800 pounds (Tables 2 and 3). However, the sex difference becomes less apparent at 800 pounds. When treated statistically only

the line difference and the line x sex interaction at 500 pounds body weight were significant. Even though the line difference is large, in favor of the Lionheart line, at 800 pounds, it lacks statistical significance.

Blood creatinine is apparently related to age. The regression coefficient (Table 6) of .0023 (statistically significant) indicates that for each 10 days difference in age on reaching 500 pounds there is an associated change of 0.023 mg. per 100 ml. in blood creatinine with the older animals having the higher values. This is in agreement with Williams (108, p.52). Blood creatinine is not related to feed intake (Table 7). There were no consistent changes in comparing 500 pounds measurements with the 800 pound observations.

Blood Uric Acid. Several animals exhibit values higher than those given by Dukes (32, p.54) as normal (0.05-2.08). Practically all the animals were in the upper limits. Blood uric acid also exhibits marked line and sex differences at both body weights (Tables 2 and 3), the differences becoming less at 800 pounds body weight. However, when analyzed statistically, the differences were significant only in the case of sex at 500 pounds and lines at 800 pounds body weight (Tables 4 and 5). The means are less at 800 pounds than 500 pounds body weight for all groups, except for the David males.

Neither age or feed intake had a significant effect on blood uric acid (Tables 6 and 7). However, age approached significance.

Hemoglobin. Hemoglobin values are expected to have a low degree of repeatability, from one time to another, due to variations in age, sex, season and health of the individual. They were not regressed on any of the other items in this study. The averages are presented in Tables 2 and 3. The analyses of variance are presented in Tables 4 and 5. At 800 pounds body weight, there is a significant effect of lines and line x sex interaction. The line effect, significant at 1% probability level, is due to the high values of the Lionheart line. The values obtained at 800 pounds are slightly higher than those at 500 pounds body weight.

Urinary 17-ketosteroids

The author is not aware of any values of urinary 17-ketosteroids for beef cattle in the literature. Whether the values obtained are in the normal range is not known. There seem to be definite line and sex differences at both weights (Tables 2 and 3). At 500 pounds body weight the males had consistently higher values than the females (Table 2). However, when these data were treated statistically the sex difference only approached

significance (Table 4). The line difference is smaller and also lacked significance.

At 800 pounds body weight the females had higher 17-ketosteroid excretion rates (Table 3) than the males. This was true in all groups except the David females. A possible explanation of this phenomenon will be presented in the Discussion. Here again the difference in ketosteroid excretion between the two sexes only approached significance (Table 5). Age was not related to urinary 17-ketosteroid levels in the urine.

Feed Intake in Relation to Rate of Gain and Feed Efficiency

Each individual animal was essentially on an ad lib feeding regime as no attempt was made to control feed intake. Therefore, considerable variation was noted. Klieber (59, p.257) suggested a possible relationship between feed utilization and appetite. MacDonald (77, p.74) reported the fastest and most efficient gainers eat less feed per day than slow gaining calves under same regime. In the present study, a positive and significant regression coefficient was found when feed intake was regressed on rate of gain. However, the correlation between feed intake and feed efficiency was not significant. The regression coefficient (Table 7) of 13.0 of feed

intake on rate of gain indicates that for each pound per day difference in rate of gain there is an associated change in feed intake of 13.0 pounds per week during the last 5 weeks of the feed test. The fastest gaining calves eat the most.

Blood Constituents in Relation to Rate of Gain and Feed Efficiency

The concentration of four blood constituents, namely: urea nitrogen, amino acid nitrogen, creatinine, and uric acid were regressed on rate of gain and feed efficiency. The regressions were calculated at 500 pounds and 800 pounds body weight.

Blood urea nitrogen was significantly related to both rate of gain and feed efficiency (Table 6) at 500 pounds body weight. As blood urea nitrogen decreased the animals gained faster and were more efficient converters of feed. The regression coefficients, -4.8 and 3.4, indicate that for each change in rate of gain of 1 pound per day and in feed efficiency of 1 pound feed per pound of gain there was an associated change in blood urea nitrogen of 4.8 and 3.4, respectively. This is in agreement with MacDonald, Evans and others reviewed in the literature (77, p.74; 41, p.211; 38, p.774).

The regression coefficients of rate of gain and feed efficiency on blood urea nitrogen at 800 pounds body

Table 6
Regression Coefficients and the 95 per cent Confidence Interval for the Items Studied at 500 Pounds Body Weight

INDEPENDENT VARIABLES	DEPENDENT VARIABLES: Urinary 17-ketosteroids (mg. per day)	Rate of Gain (lbs. per day)	Feed Efficiency (lbs. feed per lb. gain)	Blood Uric Acid (mg. per 100 ml.)	Blood Creatinine (mg. per 100 ml.)	Blood Amino Acid Nitrogen (mg. per 100 ml.)	Blood Urea Nitrogen (mg. per 100 ml.)
Age on test (days)	-4.0±32.0	.0044± .0019*	-.0071± .0033*	-.0068± .0043	.0023± .0011*	.0029± .0094	-.07± .04*
Urinary 17-ketosteroids (mg. per day)		-.0022± .0027	.0047± .0046	.0071± .0057	-.0012± .0015	-.0034± .0122	.019± .031
Rate of gain (lbs. per day)			-1.34± 0.20**	-0.55± 0.41	-0.11± 0.11	0.47± 0.88	-4.8± 2.0*
Feed efficiency (lbs. feed per lb. gain)				-0.14± 0.25	0.11± 0.06	-0.06± 0.53	3.4± 1.2*

* Indicates statistical significance at 5 per cent level

** Indicates statistical significance at 1 per cent level

Table 7
Regression Coefficients and the 95 per cent Confidence Interval for the Items Studied at 800 Pounds Body Weight

INDEPENDENT VARIABLES	DEPENDENT VARIABLES						
	Rate of Gain (lbs. per day)	Feed Efficiency (lbs. feed per lb. gain)	Feed Intake (lbs. per wk.)	Blood Uric Acid (mg. per 100 ml.)	Blood Creatinine (mg. per 100 ml.)	Blood Amino Acid Nitrogen (mg. per 100 ml.)	Blood Urea Nitrogen (mg. per 100 ml.)
Urinary 17-ketosteroids (mg. per day)	-.0028± .0019	.0016± .0032	0.010± 0.060	-.0003± .0020	.0004± .0014	.0195± .0067**	.0146± .0193
Rate of gain (lbs. per day)		-1.34± 0.20**	13.0± 5.1*	-0.04± 0.20	-0.02± 0.14	0.09± 0.78	-2.98± 1.88
Feed efficiency (lbs. feed per lb. gain)			-2.0± 3.3	-0.03± 0.12	-0.05± 0.08	0.35± 0.46	-0.85± 1.16
Feed intake (lbs. per wk.)				0.004± 0.007	0.002± 0.005	0.044± 0.026	0.045± 0.068

* Indicates statistical significance at 5 per cent level

** Indicates statistical significance at 1 per cent level

weight failed to show significance (Table 7). However, significance was approached in the case of rate of gain. As at 500 pounds the relationship was negative, i.e., faster gaining animals had lower urea nitrogen concentration. The relationship with feed efficiency was practically zero (Table 7).

Blood creatinine, unlike urea nitrogen, was not significant when regressed on rate of gain and feed efficiency. This again was the case at both body weights (Tables 6 and 7). However, at 500 pounds body weight the regression of creatinine on feed efficiency approached the significance level, the most efficient calves having lower values of creatinine.

Blood amino acid nitrogen did not approach significance when regressed on rate of gain and feed efficiency. This was the case at both weights (Tables 6 and 7).

Blood uric acid, like amino acid nitrogen, when regressed on rate of gain and feed efficiency was not significantly related (Tables 6 and 7).

In summary, significant regression coefficients were obtained when blood urea nitrogen at 500 pounds body weight was regressed on rate of gain and feed efficiency. The fastest and most efficient gainers had lower urea nitrogen values. This relationship approached significance at 800 pounds in the case of rate of gain. Blood

creatinine at 500 pounds when regressed on feed efficiency approached significance, with the most efficient animals having the lower values. No relationship was found when blood amino acid nitrogen and blood uric acid was regressed on rate of gain and feed efficiency.

Urinary 17-ketosteroid Excretion in Relation to Rate of Gain and Feed Efficiency

The regression coefficients obtained when urinary 17-ketosteroid concentrations were regressed on rate of gain and feed efficiency lacked significance in all cases (Tables 6 and 7). It is interesting that at both body weights the relationship is negative in case of rate of gain and in feed conversion. That is, the fastest and more efficient calves had lower values. This was the case at both weights. The significance of this relationship will be considered in more detail in the Discussion.

Urinary 17-ketosteroid Excretion in Relation to Certain Blood Constituents

In general, urinary 17-ketosteroids were not related to any of the blood constituents studied. This was true in all cases, except when blood amino acid at 800 pounds was regressed on 17-ketosteroids. This relationship was highly significant (P .01). The regression coefficient, .0195, of blood amino acids on 17-ketosteroids indicates

that for each change of 100 mg. per day in 17-ketosteroids excretion, blood amino acid nitrogen changes 1.95 mg. per 100 ml. The animal having higher excretion rates of 17-ketosteroids also has higher amino acid nitrogen levels in the blood.

DISCUSSION OF RESULTS

It is desirable to identify the outstanding individual when working with traits of high heritability. Progress will be more rapid when selections are based upon individual performance unless the population is highly inbred (71, p.210). For this reason, every animal in the present experiment was individually studied. Investigators have recognized that individual differences in metabolic patterns exist, but unfortunately, there have been only a few experiments designed to find the cause of these differences.

Since selection in beef cattle is based largely upon faster growth and more efficient conversion of roughage, and these traits are highly heritable, it is the goal of the breeder to identify the most outstanding individuals in respect to these traits. In view of these facts the discussion will be limited to a large degree to the relationship of the various metabolites to rate and efficiency of gains.

Feed Intake

Average feed intake was determined at the beginning and again at the end of the feed test. In addition the average feed intake for the entire feed test was obtained. It was felt that feed intake might be more closely related

to blood constituents if calculated in this manner, since the blood and urine analysis were determined at 500 pounds and 800 pounds. Feed intake varies considerably from one week to another, perhaps due in part to changes in temperature, humidity, etc. and, for this reason it would seem more appropriate to use an average of at least 3 or 4 weeks instead of one single day or week for establishing the initial and final values. Average feed intake at 800 pounds only was regressed on the other items studied.

There were no significant differences in feed intake due to sex or lines at the beginning or end of feed test (Tables 4 and 5). However, the small differences showed higher values for the males in most cases. The Lionheart line tended to have the greatest food intake. The females of this line generally consumed more feed than the males of the remaining lines. This is in accordance with past observations.

Even though rate and efficiency of gains are highly correlated, feed intake bears a closer relationship to rate of gain than it does to feed efficiency. This is born out when we look at the various lines. The Prince males have considerably lower feed requirements per unit of gain than the David or Lionheart lines, yet they have the lowest feed intake. The maintenance requirements

should be nearly the same as all groups were fed over same weight range. In view of this, it has been suggested by Nelms and Bogart (89, p.977) that animals in this line may have lower metabolic rates than the animals in the other lines studied.

When feed intake is regressed on rate of gain and feed efficiency within the lines and sexes, contradictory results may be obtained. According to Kleiber (59) the rate of production of body substances in growing animals depends on the stimulus for growth and on the level of energy. One might expect a positive relationship between rate of gain and feed intake in animals of similar breeding if this concept is true. Nelms and Bogart (89, p. 972) found this to be true for feed efficiency only when the maintenance factor had been removed.

In the present study, a positive and significant relationship was found when feed intake was regressed on rate of gain (Table 7). The regression coefficient of 13.0 indicates that for each change in rate of gain of one pound per day there is an associated change of 13.0 pounds in feed intake per week, with the faster gaining calves eating the most. The relationship between feed intake and feed efficiency was lower and was not significant. The fact that the females are eating almost an equal amount of feed and gaining at a considerably slower

rate than the bulls would indicate that differences in rate of gain could not be explained solely on the basis of a difference in feed intake. The possibility of a difference in the digestibility of feeds has been investigated. Palmer et al. (92, p.53) concluded that the difference between two strains of rats--one selected for high and one for low efficiency--could not be explained by differences in ability to digest and metabolize feed. Dunlop (33; 34), Baker, Colby and Lyman (6, pp.726-732) and Nelms, Price and Bogart (90) have reported evidence that tends to discredit the possibility of differences in digestibility.

Feed intake did not affect the concentration of the various blood constituents studied. Williams (108, p.73) reported that feed intake affects uric acid and amino acid nitrogen concentration in a similar study. However, the data had been corrected for rate of gain, feed efficiency and age. It seems unlikely that feed intake should affect the blood constituents, unless possibly the level of amino acid.

Blood Constituents

Blood Urea. Lines and sexes had significant effects upon blood urea concentrations at 500 pounds body weight.

The line difference is indicated by the higher values of about 3.0 mg. urea nitrogen per 100 ml. for the calves in the Lionheart line over the value for calves of the other lines at this body weight (Table 2). The females exhibited higher values, about 5.0 mg. urea nitrogen per 100 ml., than the males. This was true for all lines except the Prince line, where the males had slightly higher concentration of blood urea than the females.

The explanation for these differences may lie in a shift in metabolism. The animals exhibiting the higher urea values probably have a higher protein catabolism or they are deaminating more amino acids and thereby they have more circulating urea. One could expect the latter in the case of females, in that the diet was designed to provide protein for maximum growth and they are approaching maturity where less protein in proportion to energy is required. The lower values of the Prince females fit this concept. They tend to be large at maturity, and probably are still adding protein tissue to large degree at this body weight.

Age affected urea concentration. The significant regression coefficient of -.070 (Table 6), indicates that for each change of 10 days in age blood urea concentration changes .70 mg. per 100 ml., with older animals having lower values. This may be explained on the concepts

presented above. The calves that are older at 500 pounds body weight have had less desirable preweaning environmental conditions (presumably a lower milk supply). Since the test diet is designed to provide protein for maximum growth and animals are fed ad lib, they are, more than likely, making some compensation for being held back in earlier growth, thereby deaminating less of the amino acids and having a lower protein catabolism. The age at which an animal is tested should not affect the urea concentration had all animals been supplied an equal opportunity previous to starting the test and provided all animals were tested at the same body weight. This concept is supported by the fact that animals at 800 pounds have only slightly lower values than at 500 pounds body weight and yet they are 100 to 150 days older. Williams (108, p.64) reported that feed intake might be affecting urea concentration; however, the evidence in the present study does not support his findings.

It seems very likely that blood urea concentration at the initiation of a feed test might be the best predictive index for rate of growth that is available. This concept is supported when urea concentration is regressed on rate and efficiency of gains. The regression coefficient of -4.8 (Table 6) indicates that for each change in rate of gain of 1 pound per day there was an associated

change of 4.8 mg. per 100 ml. in blood urea, with the faster gaining calves exhibiting lower concentration of urea. In a similar manner, the more efficient calves had a lower blood urea concentration. There was associated with 1 pound of feed per pound gain a change in blood urea concentration of 3.4 (Table 6). Here again, the picture is complicated by age, in that age was significantly related to rate and efficiency of gains (Table 6). There is probably a compensatory effect due to the older calves being held back previous to reaching 500 pounds. Nelms and Bogart (89, p.977) report that when feed supply is adequate and calves are making maximum growth previous to test, the younger animals, not the older, will make the higher gains and be the more efficient.

The urea concentration at 800 pounds failed to show a significant relationship with rate or efficiency of gains (Table 7). However, significance was approached in the case of rate of gain. The association was practically negligible in the case of feed efficiency. This lack of association would indicate that the calves are probably plateauing in muscular growth and that changes in amino acid deamination and protein catabolism are not marked. This concept is supported when line and sex differences are considered, as both lacked statistical significance at 800 pounds body weight.

Blood Amino Acids. Blood amino acids failed to show any relationship to rate and efficiency of gains (Tables 6 and 7). This is in agreement with Williams (108, p.63), although MacDonald (77, p.71) reported a negative relationship between blood amino acids and rate and efficiency of gains. The regression of amino acids on feed intake approached significance. This would be in agreement with Williams (108, p.63) and contrary to other results (101, pp.124-132). This association of amino acids with feed intake would be the only logical relationship among the items studied, unless it is age. It seems reasonable to expect that a higher daily ingestion of protein would affect the amino acid level in the blood.

Amino acid concentration was apparently not affected by age (Table 6), although the females exhibited slightly higher values at 800 pounds than at 500 pounds. At 800 pounds body weight the calves in the Lionheart line had slightly higher values than calves in the other two lines, however, this difference lacked significance. It is interesting to note that the Lionheart line also had a higher feed intake at this body weight (Table 3).

Blood Uric Acid. Since uric acid is largely replaced by its oxidation product, allantoin, no conclusive results would be expected when one relates it to rate of

growth or efficiency of growth in beef cattle. However, the analysis for uric acid is run routinely in this laboratory, and the data were analyzed. Some interesting findings were uncovered on analysis. At 500 pounds body weight there was a significant sex difference with the females showing higher values (Table 4). This would suggest the possibility of the existence of genetic differences. This concept is supported when the data at 800 pounds are examined. At this body weight the significant sex difference has disappeared, but a significant line difference is apparent (Table 5). The difference is due to the higher values of the Lionheart line. Although line difference at 500 pounds was not significant, the Lionheart line had higher means (Table 2). Similarly, the females at 800 pounds exhibited higher concentrations of uric acid than the males.

Apparently neither age (Table 6) nor feed intake (Table 7) had any effect upon the concentration of blood uric acid. Williams (108, p.62) reported that feed intake had a significant effect on uric acid. He put forth the possible explanation of one blood constituent, such as amino acid concentration, which is affected by feed intake, interfering with the tublar resorption of uric acid. This idea has some support if one examines the

data in Table 3. The Lionheart line had significantly higher concentrations of uric acid and this line also had higher concentrations of blood amino acids.

Blood Creatinine. Creatinine concentration in blood is generally regarded as an index of muscular activity. At 500 pounds body weight a significant line and line x sex interaction was found (Table 4). The line difference was due to low creatinine concentration of the David line (Table 2). The interaction is probably due to Lionheart males having higher values than Lionheart females and vice versa in the Prince line. These determinations fit gross observations as to activity. The David line is generally considered quiet, as are the Prince males. No line or sex differences were apparent at 800 pounds body weight.

Age was significantly related to blood creatinine (Table 6). The regression coefficient, .0023, indicates that for each change in age of 10 days there is an associated change of 0.023 mg. creatinine per 100 ml. blood, with the older animals showing higher concentrations. This would indicate that the older animals at 500 pounds were more active than younger animals. However, the values at 500 pounds and 800 pounds body weight were similar. It would seem reasonable to expect less activity from the heavier animals. Feed intake apparently was

not affecting blood creatinine (Table 7). It might be expected that the more active animals would be eating more, but apparently this concept is not supported by these data.

Throughout the results mention has been made relative to some of the animals in which some of the blood constituents failed to fall within the normal range presented by Dukes (32, p.54). These values do not necessarily indicate abnormal individuals or faulty techniques. The majority of the animals making up the normal range were mature dairy cows and the values were determined by methods which have been succeeded by more precise methods.

Urinary 17-ketosteroids

There would appear to be definite sex differences in 17-ketosteroids excretion at both 500 pounds and at 800 pounds body weight. However, these differences lacked statistical significance (Tables 4 and 5). In general, there was a high degree of variation within each subclass. It is interesting to note that at 500 pounds body weight the males had higher excretion rates of 17-ketosteroids than the females. Since one third of the 17-ketosteroids are derived from the gonads in the case of males, one might expect them to excrete more than the females of this material. Also, the lower values of the Prince males is worthy of mentioning. Apparently, this

line is later maturing than the remaining lines (personal observations). If this is true the males in this line would be expected to have lower 17-ketosteroid excretion rates, since their gonads would not be as active as those of the other lines.

At 800 pounds body weight, the females exhibit higher values than the males (lacks significance), with the exception of the David line (Table 3), even though the origin of one third the urinary 17-ketosteroids are from the testes. This is to be expected since cortical hormones inhibit growth and the males gain at a faster rate than the females. The David line deviates from the general pattern in that the males have higher excretion rates of 17-ketosteroids than the females. The David females would be expected to have lower values than the female calves in the other two lines, since the average daily gain of the females in this line is about 0.30 lb. per day higher than that of females in the other lines (Table 1). The high values of the David males may be explained on the fact that 4 of the males in this line were well below the mean forrate of gain (Table 1), and were contributing to the higher mean for 17-ketosteroids noted in Table 3. The two remaining animals had very high rates of gain (3.35 and 3.67) and contributed less proportionally to the mean for 17-ketosteroids.

Apparently age had little effect on the 17-ketosteroid excretion rate (Table 6). However, the females showed approximately a 20 per cent increase from 500 to 800 pounds body weight. One would not expect feed intake to affect 17-ketosteroid excretion unless both were associated with another factor. The results show that feed intake was not affecting 17-ketosteroid excretion (Table 7) in this study.

When rate and efficiency of gains are regressed on 17-ketosteroid excretion rate, a negative relationship was found, i.e., those calves which gain more rapidly and convert feed to gains more efficiently had lower 17-ketosteroid excretion rates. This was true at both 500 and 800 pounds body weight (Tables 6 and 7). However, none of the regression coefficients were significant. These relationships are as would be expected, since the adrenal cortical hormones affect protein metabolism (75, pp.309-344) by interfering with protein anabolism and increasing protein catabolism.

Apparently, the rate of 17-ketosteroid excretion and the rate of gain are related. The regression coefficients are negative at both 500 and 800 pounds body weight. At both weights the regression coefficients approach significance. It would be expected that efficiency also would be associated with 17-ketosteroid excretion, since rate

of gain and feed efficiency are closely related. The regression of feed efficiency on urinary 17-ketosteroids at 800 pounds was considerably less than at 500 pounds body weight. This would indicate that feed efficiency and 17-ketosteroid excretion rate are not as closely related as rate of gain and 17-ketosteroid excretion rate. This might be expected since feed efficiency has a lower heritability than rate of gain. Apparently, the animal's environment affects feed efficiency to a larger degree than it does rate of gain.

It has been shown by Hoberman (50), Barton (10), Sinex (100) and others that the adrenal cortex does play an important role in the anabolism and catabolism of protein. In general, the adrenal hormones inhibit protein anabolism and increase protein catabolism. This being the case one would expect a piling up of amino acids in the blood. There is some indication of an increase in blood amino acids with increases in 17-ketosteroid excretion in this study. At 800 pounds body weight there was a significant relationship between blood amino acids and urinary 17-ketosteroids (Table 7). The regression coefficient of blood amino acids on urinary 17-ketosteroids of 0.0195 indicates that for each change in excretion of 10 mg. per 24 hours of 17-ketosteroids there is an associated change of 1.95 mg. in blood amino

acid nitrogen. Animals exhibiting high levels of blood amino acids had higher rates of urinary 17-ketosteroids. There seemed to be little relationship between the two at 500 pounds body weight. This might possibly be explained by the fact that the animals at 500 pounds body weight had been on the feeding regime for a short time, relative to the 800 pounds observations. Williams (108, p.34) working with suckling calves showed that the level of nitrogen metabolites in the blood became more stable after being placed under a relatively constant environment.

The predicative value of urinary 17-ketosteroid for rate of gain or feed efficiency in this study was low. However, certain possibilities seem apparent. Perhaps, with more research on the analytical procedures for 17-ketosteroids or application of a more precise index of cortical activity will reveal techniques which will predict performance of beef animals at a young age with a reasonable degree of accuracy.

A few comments seem pertinent. It might seem discouraging relative to the predictability of any one of the factors studied in predicting rate of gain or feed efficiency. It is unlikely that any one metabolite could predict these economic traits, since they are the result of many complicated biochemical processes. There are

several hormone systems acting upon these traits, each one affecting growth to some extent. The use of several criteria for evaluating an animal's potential for rate of gain and feed efficiency would seem to have merit. Probably each of these criteria used will require considerable detailed research before they can be used. In addition, research on the various interrelations will have to be completed. For many of the hormones new techniques for assaying will have to be established.

SUMMARY AND CONCLUSIONS

1. The association of certain blood constituents: urea nitrogen, amino acid nitrogen, creatinine and uric acid, and urinary 17-ketosteroid excretion rate with averate rate of gain and feed efficiency have been considered in 33 purebred Hereford males and females. The blood constituents and urinary 17-ketosteroids were determined at two body weights, 500 pounds and 800 pounds.

2. Average rate of gain and feed efficiency have been determined from 500 pounds body weight to 800 pounds. Body weight was a linear function of time during this period for each of the 33 animals.

3. There were no significant effects of sex or line on rate of gain, even though large apparent differences existed. The average rate of gain for the males was 2.92 and for the females 2.19 lbs. per day. However, age at 800 pounds body weight showed a significant sex effect with the males reaching 800 lbs. at a younger age than the females.

4. The males made more efficient gains than the females. The Prince and David lines were more efficient than the Lionheart line. A sex x line interaction existed, which was probably due to the lower feed

requirements of the females in the David line.

5. There were no differences in feed intake among any of the groups.

6. The females had higher blood urea concentrations at 500 pounds body weight than the males. The Lionheart line had higher urea concentrations than either of the two remaining lines at this body weight. Both sex and line effects lacked significance at 800 pounds body weight. The average urea concentrations were slightly higher at 500 pounds than at 800 pounds body weight.

7. There were no differences for blood amino acid among any of the groups at either 500 or 800 pounds body weight. The average amino acid concentration for the groups were similar at both body weights.

8. The David line had lower blood creatinine concentrations at 500 pounds body weight than the other two lines. A significant sex x line interaction also existed at this body weight. This was probably due to the high values exhibited by the Prince females. At 800 pounds body weight, there was apparently more variation within the groups and consequently no significant differences among groups were found. However, the David line had lower concentrations than the other two lines. The

average creatinine concentrations were similar at both body weights.

9. Blood uric acid showed a sex effect at 500 pounds and a line effect at 800 pounds body weight. The females had higher concentrations at 500 pounds than the males with little difference between the sexes at 800 pounds. The line difference at 800 pounds body weight is apparently due to the higher values of the Lionheart line. The average uric acid concentrations were slightly higher at 500 pounds than at 800 pounds body weight.

10. Blood hemoglobin showed a difference due to lines at 800 pounds body weight. The Lionheart line had higher values at this weight than either of the two other lines.

11. No significant difference between lines or sexes was found in 17-ketosteroid excretion rate at either 500 pounds or 800 pounds body weight. However, differences due to sex appeared to exist. At 500 pounds body weight the males had higher average excretion rates than the females, while at 800 pounds the females had higher average values. When comparing the means at the two body weights, the males were similar, while the females had higher average values at 800 pounds than at 500 pounds.

12. Age at 500 pounds body weight had a significant relationship with average rate of gain and feed efficiency. The older animals at 500 pounds made more rapid and efficient gains in body weight. It is felt that the older calves were compensating for the slower gains made prior to the feed test.

13. Age at 500 pounds was significantly related to blood creatinine and blood urea at 500 pounds body weight. The older animals had higher concentrations of creatinine and lower urea concentrations.

14. Rate of gain and feed efficiency were significantly related. For each increase of 1 lb. per day in rate of gain it required 134 lbs. less feed for each 100 lbs. gain made.

15. Feed intake at 800 pounds body weight was significantly related to rate of gain. The faster gaining calves had a larger weekly feed intake. Each increase in 1 lb. per day gain was associated with an increased feed intake of 13 lbs. per week. Feed intake was not significantly related to feed efficiency.

16. Blood urea at 500 pounds body weight was significantly related to rate of gain and feed efficiency. High concentrations of urea were associated with a low

rate of gain and a higher feed requirement per unit of gain. Each change in gain of 1 lb. per day is associated with a change in blood urea nitrogen of 4.8 mg. per 100 ml. blood. Each change in feed requirements of one lb. feed per lb. gain is associated with a change in urea nitrogen of 3.4 mg. per 100 ml. blood. The regression coefficients of blood urea at 800 pounds on rate of gain and feed efficiency were smaller than the corresponding coefficients at 500 pounds body weight and lacked significance.

17. The regression of urinary 17-ketosteroids at the two body weights on rate of gain and feed efficiency lacked significance. However, low excretion rates were associated with higher rates of gain and lower feed requirements per unit of gain. It is felt that some errors in techniques could be avoided with a more desirable urine collecting apparatus. It would seem that urinary 17-ketosteroids might provide an excellent tool for predicting rate of gain and feed efficiency since a wide range in excretion rates were found.

18. Urinary 17-ketosteroids at 800 pounds was related to blood amino acids at 800 pounds, with higher excretion rates being associated with higher concentrations of amino acids. This is in agreement with the

concept that the cortical hormones depress anabolism and increase protein catabolism.

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