# THE INFLUENCE OF DIFFERENT LEVELS OF DIETARY POTASSIUM ON WHOLE-BODY $^{40}K$ COUNT, BLOOD SERUM AND MUSCLE POTASSIUM CONCENTRATION IN STEERS

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# CHAPTER I

## INTRODUCTION

There are presently several $^{40}K$ whole-body counters of various kinds being used to evaluate live animals for pounds of fat-free lean. The ultimate objective of the use of these instruments appears to be in their usefulness as an aid in the selection of breeding animals. This method has the principle advantage of providing an estimate of a carcass trait (total pounds of fat-free lean) to be made on live animals.

Several prediction equations using net $^{40}K$ count have been developed to estimate fat-free lean in live animals. The precision of these equations is dependent on a number of factors. Several of these factors and their importance are discussed in the next chapter of this thesis.

The value of $^{40}K$ counters depends on how precisely they estimate fat-free lean in live animals. One of the factors which may influence the precision of these estimates is the influence of dietary potassium. Very little information exists in this area. Therefore, this study was conducted to evaluate the influence of three levels of dietary potassium on net $^{40}K$ count per minute, blood serum potassium concentrations and muscle tissue potassium levels of steers. A further objective was to characterize the relationship between animal weight and $^{40}K$ count.

To eliminate the difficulties associated with holding animals off feed and water, it would be desirable to evaluate animals unshrunk. This would cause less strain to animals and would reduce the economic

loss a producer may suffer due to reduction in animal weight. Thus, the possibility of $^{40}K$ evaluation on unshrunk animals was also investigated.

# CHAPTER II

# REVIEW OF LITERATURE

## Introduction

The search for a non-destructive method of evaluating live animals has led to the development of instruments which detect potassium-40 ($^{40}K$) to predict lean in meat animals. One such instrument now in use at the Oklahoma Live Animal Evaluation Center is the Nuclear Electronic $^{40}K$ Counter. One of the major principles on which this machine is based is that potassium contains a fixed, measurable proportion of naturally occurring radioactive atoms ($^{40}K$) which give off very small amounts of gamma radiation. Anderson (1959) reported that 0.01% of all naturally occurring potassium was in the form of the $^{40}K$ isotope. Kulwich, Feinstein and Golumbic (1960) indicated that $^{40}K$ has an isotope abundance of 0.0119% and that potassium from different sources is quite constant in $^{40}K$ content.

This principle is further supported by Forbes (1963) and Ward, Johnson and Tyler (1967) who reported that $^{40}K$ comprised 0.012% of all naturally occurring potassium. This appears to give this principle a sound basis.

The second major principle, that nearly all of the potassium in the body of the live animal is found in the muscle, has resulted in some conflicting reports.

Lawrie and Pomeroy (1963) indicated that most potassium is mainly

associated with the intracellular, nonfat phase in the body and that the quantity of potassium in muscle tissue is constant. Kirton and Pearson (1963), however, reported a potassium concentration in separable fat of lambs of 0.82 grams of potassium per kilogram of fat as determined by flame photometry. The authors state that this confirms the presence of potassium in fat in amounts that cannot be ignored. Bone potassium was also found to comprise 11% of the potassium content of dressed lamb carcasses.

Bennink et al. (1968) reported that the average potassium content of 14 samples of steer adipose tissue was 12.6 grams potassium per kilogram fat-free dry solids as compared to a mean for all meat samples of 14.0 grams potassium per kilogram fat-free dry solids. Ward, Johnson and Tyler (1967) reported a high negative correlation ($r = -.94$) between fat percent and potassium concentration (expressed as grams potassium per kilogram ground meat) for 42 samples of ground beef from three different animals. When potassium was expressed as grams potassium per kilogram dry matter, a negative correlation of -.997 was obtained. These high negative correlations suggest that the potassium concentration of these ground beef samples was closely related to the fat percentage.

In a more detailed study involving 90 steers, Lohman and Norton (1968) reported that potassium was found in all steer tissue. Standard trimmed lean comprised 37.8% of the steers body mass and contained 53.4% of the total body potassium. Carcass bone contained 12.4% of total body potassium and the gastrointestinal tract 16.4%. The gastrointestinal tract was found to be the most variable source of potassium and carcass adipose potassium contributed very little to the variation in total body

potassium. Kirton et al., (1961) reported similar potassium concentrations in the lean of sheep (50% of the total potassium in the lean), however, Pfau (1965) found 69% of the total body potassium in the muscle of swine. These reports point out that there is a considerable amount of the total body potassium located in other than lean tissue. The majority of the research done in this area does not support the statement of Anderson (1959), made with no experimental evidence, that there is no potassium in fat and very little in bone. The importance of these other sources of body potassium to the precision of predicting lean tissue in live animals has not yet been determined.

## $^{40}K$ Estimates of Lean in Carcass and Live Animal

Various workers have reported the reliability of the use of naturally occurring $^{40}K$ to predict the lean in both live animals and carcasses. More success has been obtained in predicting the composition of carcass components than either whole carcasses or live animals.

A very high association ($r = .96$ to $.98$) has been demonstrated between $^{40}K$ count and lean content of hams (Kulwich, Feinstein and Anderson, 1958; Kulwich, Feinstein and Golumbic, 1960; Kulwich et al., 1961a). Pringle and Kulwich (1961) suggest a nearly linear relationship between lean content of hams and $^{40}K$ count. Highly significant ($P < .01$) correlations between percent separable fat, -.865, and percent separable lean, 0.798, and $^{40}K$ counts per minute per pound of intact beef rounds have also been reported (Kulwich et al., 1961b).

When $^{40}K$ count was used to predict carcass composition the results were not so encouraging. Early work with sheep and swine indicated significant but low correlations between carcass potassium and lean (Kirton

et al., 1961; Kirton, Gnaedinger and Pearson, 1963). Large standard errors of estimates associated with resulting regression equations suggested potassium was of questionable value for predicting carcass composition.

Since that time evidence suggests the association between carcass $^{40}K$ count and carcass lean is somewhat higher than reported by these workers. Carcass $^{40}K$ count has been reported to account for from 53 to 90.3% of the variation in carcass lean muscle mass in sheep (Judge et al., 1963; Lohman et al., 1965; Breidenstein et al., 1965a).

Breidenstein et al. (1965b) also indicated twelve minute $^{40}K$ count on one side of the carcass, when added to the basic model accounted for 91.3% of the variation in the weight of carcass lean muscle mass of pigs. The basic model included constants for breed, sex, age, live weight and carcass weight. This is somewhat higher than the percent of the total variation in carcass lean muscle mass of steers accounted for by carcass $^{40}K$ count (81 and 88% in two phases) reported by Lohman et al. (1966).

Mullins et al. (1968) reported a correlation of 0.70 between percent four lean cuts and percent potassium in the carcass as measured by $^{40}K$ in 32 pigs slaughtered at 90.0 kg. Moser (1970) studied 53 Yorkshire barrows slaughtered over five weight groups (100, 150, 200, 250 and 300 lbs.). Correlation coefficients between first and second carcass $^{40}K$ counts per minute ranged from 0.88 to 0.96 over the five weight groups. Correlation coefficients between $^{40}K$ carcass counts per minute and standard trimmed lean cuts, averaged over duplicate counts on the same carcass, ranged from 0.22 to 0.78. Average correlation coefficients between $^{40}K$ carcass counts per minute and fat-free lean ranged from 0.16 to 0.83.

Mullins et al. (1969) reported consistently higher relationships between the percent potassium in the carcass, as determined by $^{40}K$ count, and yield of lean cuts and fat than similar comparisons between the percent potassium in the live animals and yields of lean cuts and fat. The standard errors associated with $^{40}K$ determined in the live animal were also greater than for $^{40}K$ determined in the carcass. McLellan (1970) reported a correlation coefficient, pooled over the average of two carcass $^{40}K$ counts and four weight groups, between carcass $^{40}K$ count and fat free lean of 0.82 in 31 steers, and heifers. Garrett and Pennoyer (1970) found a correlation of 0.90 between nitrogen in the carcass and carcass potassium as measured by flame photometry on samples of 48 steers.

The literature also contains considerable evidence to suggest the relationship between whole body $^{40}K$ count and carcass composition is high enough to indicate $^{40}K$ counters may be of some benefit in estimating lean in live animals. Also evident, is that many sources of variation exist that may affect the accuracy of prediction of lean by $^{40}K$ counting.

Cheek and West (1955) determined the total body potassium and the lean body mass of 30 rats and concluded that a close relationship existed between these two variables. Muldowney, Crooks and Bluhm (1957) arrived at a similar conclusion when studying the relationship between total exchangeable potassium and lean body mass in normal humans. A correlation of 0.90 was reported but this is a very questionable value because accurate estimates of carcass composition of normal humans is impossible. Early work with hogs (Zobrisky et al., 1959) also indicated $^{40}K$ content may be of use as a non-destructive index for determining

protein to fat ratios in livestock.

One of the early studies using $^{40}K$ to determine carcass composition of live animals was conducted with sheep (Kirton et al., 1961). Potassium - 40 activity was determined on 10 shorn lambs before and after washing. Washing reduced the potassium content by 0.94 grams potassium per kilogram live weight. Correlations between percent protein in the carcass and live $^{40}K$ count before and after washing were 0.80 and 0.83 respectively. These results led the authors to conclude that although significant correlations were found between the gamma activity of the live animals and their carcass composition, in general these relationships did not appear high enough for practical importance. Due to the small number of animals used in a correlation study, the validity of these data is questionable.

Kirton, Gnaedinger and Pearson (1963) studied the relationship between potassium content and composition of 24 pigs. The empty body contained an average of 176.8 grams of potassium (S.D. = 14.4) while the carcasses, which comprised 77% of the empty bodies, contained 143.7 grams (S.D. = 12.7) or 81% of the potassium in the empty body. This compared with 81% of the carcass potassium in the muscle of pigs reported by Stant, Martin and Kessler (1969) and 84% in swine reported by Pfau and Kallistratos (1963). It was found that one standard error of a regression equation comprised 13% of the range in ether extract and 17% of the range in protein when body potassium was used for predicting composition. These authors concluded that percent potassium does not appear to be sufficiently precise for determining differences between individual live pigs, but may be useful for selection of groups of pigs. Judge et al. (1963) reported correlations between $^{40}K$ counts per minute per

pound of live weight and percent excess fat of live weight ranging from - .72 to - .89 in lambs. The correlations between $^{40}K$ activity and percent edible portion (live weight) were significant in only one of four cases (r = .75).

Fitting constants for breed, sex, live weight, and carcass weight to 24 pigs (Breidenstein et al., 1965b), accounted for 44.7% (S.E. = 2.68 kg.) of the variation in weight of carcass lean muscle mass. Eight minute $^{40}K$ count on live animals added to the basic model accounted for 87.7% of the variation and the standard error of estimate was reduced to 1.29 kilograms. A similar study with sheep (2 wethers, 16 ewes and 9 rams) reported by Breidenstein et al. (1965a) indicates the basic model, consisting of constants for sex, age, live weight and carcass weight, accounted for 59.5% (S.E. = 0.47 kg.) of the total variation in carcass lean muscle mass. However, when potassium content of the live body was added to the basic model, 72.6% of the variance was accounted for and the standard error of the estimate was reduced to 0.40 kg. Lohman et al. (1963), also working with sheep (16 ewes and 11 rams) found 76.6% of the variation in carcass lean muscle mass accounted for by whole body potassium but a much higher standard error of estimate of 2.9 kilograms.

In a study involving 42 steers (21 in each of two consecutive years) Lohman et al. (1966) demonstrated the importance of gastrointestinal contents to $^{40}K$ counting. When steers were held off water for 12 - 18 hours prior to $^{40}K$ counting, whole body potassium accounted for 51% of the variation in carcass lean muscle mass with a standard error of estimate of 10.2 kilograms. However, when steers were fed a diet low in radioactivity (oats) for seven days prior to counting, whole body potassium estimates of carcass lean muscle were markedly improved.

Eighty-eight percent of the total variation was accounted for with a standard error of estimate of 5.3 kilograms. The combination of weight and $^{40}K$ data on this group of steers increased ($P < .01$) the variation accounted for and decreased ($P < .01$) the standard error of estimate for carcass lean muscle mass to 4.4 kilograms. The standard error of estimate based on weight alone was 7.1 kilograms and 6.9 kilograms for the first and second year respectively.

Mullins et al. (1968) reported the correlation between percent four lean cuts of 32 pigs and percent potassium in the live animal ($^{40}K$ measurement) was 0.60. Moser (1970), however, reported correlations ranging from - .37 to 0.96 between live animal $^{40}K$ counts per minute and lean cuts of 53 pigs. Higher correlations were generally associated with heavier pigs. A similar situation was reported by Mullins et al. (1969). Significant ($P < .01$) positive relationships were observed between potassium content of 32 live pigs and trimmed hams ($r = 0.66$), percent ham and loin ($r = 0.57$) and percent four lean cuts ($r = 0.64$). Although most correlations of this study were significant, they accounted for only a small portion of the variation in chemical constituents and yield of cuts.

McLellan (1970) studied 31 steers and heifers of four slaughter weight groups. His data indicated no obvious trend in which the association between count and fat-free lean increased or decreased as live weight changed. There was also no detectable difference in $^{40}K$ count-fat-free lean association from the 24-hour shrinkage period to the 72-hour period. Pooled correlation coefficients in the case of both the 24-hour and 72-hour shrinkage periods were 0.80. The standard error of estimate based on weight alone was 15 pounds. When $^{40}K$ count was used

in a prediction equation, the standard error of estimate was 10.8 pounds. In equations involving both count and weight, the standard error of estimate ranged from 8.8 to 10.5 pounds.

## Sources of Variation in $^{40}K$ Counting

Thus far this review indicates that there is a real relationship between $^{40}K$ count and fat-free lean, although there are a number of factors which affect the precision with which an animal's composition can be estimated by $^{40}K$ count. The detection efficiency of a radiation detector for a gamma emitter distributed within a large sample is influenced by self absorption and by sample-to-detector geometry (Twardock et al., 1966). Background depression and efficiency of counting $^{40}K$ radiation from the animal are directly related to the weight of the animal and instrument calibration factors can markedly affect the predictability of carcass lean muscle mass (Lohman et al., 1966).

In addition to the electronics of the various detectors and sample size and shape, which influence prediction precision, the literature contains numerous conflicting reports on the constancy of potassium concentrations within the various body components. The importance of these factors to the precision of predicting fat-free lean is not well established.

Cheek and West (1955) found young rats have a lower potassium content per unit of lean body mass than adults. This was based only on the intercept of the regression line. However, Weiner and Fields (1969) reported that potassium concentration of blood plasma of 324 female sheep to be very constant. In agreement with this work, Long et al. (1965) made 3827 determinations of potassium blood serum on 316 sheep and in-

dicated a very slight but inconsistent decrease in serum potassium levels as age increased. Allen, Anderson and Langham (1960) studied 1590 human subjects of both sexes ranging in age from less than one year to 79 years. The average concentration of potassium per kilogram of gross body weight showed a steady decline with increasing age. It was suggested this age effect is due to a slight loss of body potassium rather than to an increase in protoplasmic mass. Estimates of potassium concentration were made with a $4\pi$ liquid scintillation counter. Significant ($P < .01$) decreases in potassium concentration as age increased were reported for muscle tissue of pigs (Lawrie and Pomeroy, 1963).

Stant, Martin and Kessler (1969) slaughtered 24 barrows at five different weights (23, 46, 68 and 91 kg). This study also revealed a tendency for older pigs to have a lower muscle tissue potassium concentration than young pigs but it is difficult to determine whether this was influenced by weight or age. Muscle tissue showed a linear decrease in potassium concentration with increase in weight. There was also a significant ($P < .05$) quadratic increase in chemically determined grams potassium in the carcass and a significant ($P < .05$) cubic decrease in grams potassium per kilogram carcass associated with increasing live weight. The presence of significant interactions between Yorkshire-Durocs and Yorkshire-Chester White crossbred barrows and weight groups indicated that potassium followed different developmental patterns in the two breed types.

## Biological and Technical Sources of Variation Associated With Estimates of Muscle Potassium

The use of whole body potassium as a quantitative index of the

fat-free body assumes potassium is maintained in a relatively constant amount within the lean muscle mass. Workers do not agree in this area. It also appears the technique used to estimate potassium concentration is very important.

Green, McNeill and Robinson (1961) found quite constant potassium concentrations, in the whole body of calves and pigs. However, Lawrie and Pomeroy (1963) slaughtered litter mate pigs at three weights (150, 200 and 250 lbs.) and studied potassium concentrations of five different muscles. A mean potassium concentration of 0.35% (wet tissue basis) was reported in the longissimus dorsi. Potassium concentrations of the psoas major and rectus femoris were significantly higher ($P < .01$) than those of longissimus dorsi, triceps and extensor carpi radialis (0.37% vs 0.31%). These authors suggest that since concentrations of potassium may differ by at least 30% between muscles the assessment of total muscle may be inaccurate if based on the $^{40}K$ gamma ray emissions. Pfau and Kallistratos (1963) however, determined the potassium concentrations of all muscles from a single pig and concluded that potassium content was relatively constant. Pfau et al. (1963) also compared the potassium content of the semimembranosus and longissimus dorsi muscles from two different breeds and found nonsignificant differences between muscles and between breeds.

Gillett, Pearson and Kirton (1965) slaughtered six Hampshire and six Yorkshire barrows between 186 and 220 lbs. Six muscles were excised from each carcass. The percent decrease in potassium concentration determined by flame photometry between rectus femoris (4.11 gms K per kg wet tissue) and psoas major (3.64 gms K per kg wet tissue) was 11.9% ($P < .05$). Maximum percent decrease between extreme values was 21%. It

is interesting to note that in these data ranking of the muscles for potassium concentrations was the same in each breed with very small differences reported in potassium concentrations of the same muscles between breeds.

In a comparable study with steers (seven Angus, seven Herefords and two Shorthorns) Gillett et al. (1967) excised all or parts of eight muscles for study of potassium concentration as determined by flame photometry. An average potassium concentration of 3.73 grams per kilogram wet tissue was reported for all muscles with the highest concentration of 3.95 grams per kilogram in the semitendinosus and the lowest of 3.44 grams per kilogram in the supraspinatus. Regardless of the basis for expression of potassium content of the muscles (wet, fat-free-moisture-free or protein) significant ($P < .05$) differences between muscles existed. These data indicated no breed differences in potassium concentration on a wet basis but when expressed on a fat-free-moisture-free basis significant differences ($P < .05$) occurred. Duggleby and Seebeck (1967) measured 152 samples of muscle, fat and bone from seven steers for potassium concentration. Potassium estimates were made with a sodium iodide crystal. Highly significant differences were reported between muscle groups and between animals. Average concentrations in the longissimus dorsi from forequarter and hindquarter were 3.26 and 3.85 grams per kilogram respectively ($P < .001$). It was indicated that animal differences were not related to carcass weight. In a limited study involving eight wholesale cuts of beef from five animals, Ward, Johnson and Tyler (1967) indicated the differences in potassium concentration, expressed on a fat-free basis, were not significant. When samples of each of these cuts were ground and analyzed for potassium and fat, potassium

concentration showed a variability that was obviously related to the fat percent of the sample. The coefficient of variation when potassium was expressed on a wet basis was 17.2%. This was reduced to 9.0% when expressed on a fat-free basis.

Muscle to muscle variability in potassium concentration of sheep is also reported (Gillett et al., 1968). Potassium content of muscles ranged from 0.31 to 0.45%. On a wet basis (gms K per kg muscle) the potassium content of all muscles differed significantly ($P < .05$). The percent decrease between highest and lowest muscles was 8% and, contrary to above reports, this increased to a 12.77% decrease when potassium was expressed on a fat-free moisture-free basis. Four of the 25 lambs studied had high blood potassium levels (1.44 gms per kg as compared to 0.42). Blood potassium level did not appear to affect muscle potassium concentrations.

The potassium content differences for ground beef from one side of each of 12 steers (fraternal or identical twins) and for eight wholesale cuts of three dairy cows was made by Bennink et al. (1968). No significant differences were found among wholesale cuts within cows or among cows, or among ground beef samples for different steers. Potassium concentrations were estimated by use of atomic absorption spectrometry and by $^{40}K$ activity. Means from the two methods were similar, however, the correlation coefficient between the two determinations was relatively low ($r = 0.676$). Standard deviations and ranges were much greater when potassium was estimated by $^{40}K$ ($^{40}K$ S.D. = 0.5 gms per kg fresh meat, atomic absorbtion spectrometry = 0.3). Mullins et al. (1969) also found significant differences ($P < .05$) in the potassium concentration (wet tissue basis) between untrimmed and trimmed ham, loin, shoulder, and

belly and jowl of 32 pigs. The level of potassium in the composite right side (0.202%) determined by flame photometry was higher than the levels of the live pigs (0.131%) and their carcasses (0.176%) as determined by $^{40}K$ counting. When potassium was expressed on a fat-free moisture-free basis, the soft tissue of the ham, loin, and shoulder contained practically the same percent potassium.

In a detailed study of 98 steers Lohman, Ball and Norton (1970) evaluated the biological sources of variability of lean muscle potassium concentration of beef cuts. The steers were Holstein, Angus-Holstein, Charolais-Angus or Angus fed a diet either high or low in roughage. Half of the steers were implanted with diethylstilbestrol and the steers were slaughtered in one of four predetermined weight groups (306, 385, 465 and 544 kg). The lowest average potassium concentration (fat-free dry matter basis) was in the rib, 13.72 grams per kilogram, the highest in the round, 15.27 grams per kilogram. There was no consistent trend in potassium deviations from one breed to another or from one weight to another or for hormone treatment or energy level. There was a significant association of weight group with potassium when determined by atomic absorption spectrophotometry, but not when determined by gamma ray spectrometry. Both methods of analysis indicated that Angus-Holsteins had about 5% less potassium than Angus ($P < .05$). The technical error associated with the average potassium estimate for each steer was 3.6%. Accounting for technical error and differences among breed types and treatments, the remaining variation, 3.0% is an estimate of biological variation of potassium per kilogram fat-free dry matter of muscle in steers.

Using the same steers Lohman, Dieter and Norton (1970) examined the

technical variation in the measurement of potassium in the lean muscle of steers. About 1800 samples (including 400 reruns for duplicates that differed from each other by more than 5%) were analyzed by use of atomic absorbtion spectrophotometry and averaged 60 samples per run in 33 runs over a years time. A significant difference of about 10% in the average potassium content of the dry matter was found between blocks (runs). The error variance component, of which 67.6% was associated with the block effect, 0.4% with the cuts X blocks interaction, 19% with the steers X cuts X blocks interaction, and 13% with the mean of duplicates, totaled .00826 $gm^2$, a standard deviation of .091 gm and CVe of 9.6%. Rerunning extracts from each of five cuts six times over a month resulted in failure to observe a stable relation among cuts in potassium concentration as measured by atomic absorption spectrophotometry as indicated by significant interactions of cuts with runs. These data point to serious limitations in the procedure used to estimate potassium by atomic absorption spectrophotometry.

## Influence of Dietary Potassium to $^{40}K$ Counting

It has been demonstrated in the pig (Kirton, Gnaedinger and Pearson, 1963) and in cattle (Lohman and Norton, 1968) that the gastrointestinal tract contents are the most variable sources of potassium in the body. Little information is available on the effect of varying levels of dietary potassium on potassium concentrations of body components or on how various diets affect the prediction of carcass composition by $^{40}K$ techniques.

Lohman et al. (1966) reported that steers fed a high roughage diet averaged 3% higher whole body potassium than those fed a low roughage

diet and also had 5% less carcass lean muscle mass. Oats fed seven days prior to slaughter reduced the standard error of estimate of predicting carcass lean muscle mass from 10.2 to 5.3 kgs. Lohman and Norton (1968), separating steers into various body components based on anatomical function, found an 18% (nonsignificant difference) higher potassium concentration in the blood and mesenteric fat in steers fed a high roughage diet than those on a low roughage diet. No other dietary effects were noted. Johnson et al. (1968) also illustrated that two levels of dietary potassium had no influence on the potassium concentration in the milk of eight high producing Holstein cows. The high potassium diet contained 2.6 times as much potassium as the low level diet. Clark et al. (1970) analyzed the potassium concentration of 164 beef carcasses. The energy intake of these cattle was varied by varying the corn to corn silage ratio of the diet. In trials I and II (124 carcasses) there was a higher ($P < .05$) level of potassium in the fat-free dry matter when cattle received only corn silage for the entire feeding period compared to cattle fed a full feed of corn at least the last half of the feeding trial, 1.07% to 1.04%. In trial III (40 steers) no differences were found.

In order to effectively study dietary potassium levels and how they affect the precision of $^{40}K$ estimates of fat-free lean, it is necessary to review the factors involved in potassium absorption and the mechanisms which regulate potassium levels in the body.

Ruminants normally consume potassium in excess of dietary requirements and rarely, if ever, would a deficiency occur in a natural diet. Potassium apparently enters the blood by a diffusion process, and most is absorbed from the rumen (Parthasurathy and Phillipson, 1953; Perry, Cragle and Miller, 1967) and from the omasum (Oyaert and Bouckaert,

1961). These studies also indicated that no absorption occurred until the concentration in the rumen exceeded that of carotid blood. Phillips and Code (1966) also found potassium absorption in dogs to be concentration dependent.

Lambi (1965) states that since the vast majority of body potassium is within the cells, the disparity between the serum potassium concentration and the body stores of this ion tend to make the diagnosis of potassium depletion of this ion more difficult. This review also points out that the renal tubules of the kidney are the mechanism for eliminating excess potassium and that in man the daily urinary potassium excretion is very similar in amount to the daily potassium dietary intake. This was further illustrated by Keynes and Harrison (1967). Before the animals were fed, KCl solutions were administered by fistula to three ewes with catheterized bladders. After a control injection of water only, there was a delay of one to three hours before potassium excretion rose, and maximum potassium excretion was reached after six hours. After KCl injection there was a delay of about one hour before potassium concentration rose. Peak values were appreciably greater than for controls and were reached after two to three hours. When KCl was infused into the jugular vein in concentrations of 0.25 M KCl at rates of 2.7 milliliters per minute for as long as three hours, similar results were obtained, except that the time lag for urine potassium concentration to increase was virtually eliminated. Beal and Budtz (1968) also found that 89 percent of dietary potassium was excreted in the urine of sheep and 11 percent in the feces. There was also a correlation of 0.99 between total potassium intake and excretion.

This literature review indicated that there are several sources of

variation associated with $^{40}K$ estimates of fat-free lean in live animals. However, studies designed specifically to evaluate the influence of dietary potassium to $^{40}K$ estimates of lean in live animals are limited. These studies also do not determine the influence of dietary potassium on blood serum or muscle tissue potassium levels.

# CHAPTER III

## MATERIALS AND METHODS

### Introduction

Three levels of dietary potassium were evaluated in a Latin Square experimental design with 36 crossbred steers. Potassium concentrations of the diets were 1.31%, 1.03% and 0.29%. The experiment was designed to study the influence of potassium in the diet on net $^{40}K$ count of steers and on potassium concentration of blood serum and muscle tissue.

Upon purchase, all steers were placed on the same diet for a two-week adjustment period. This standardized the management of all steers prior to being placed on treatment. Following this adjustment period the steers were weighed, and $^{40}K$ counted and blood samples were taken from each steer. Weight and $^{40}K$ count were taken without any shrinkage and after being held off feed and water for 24 hours.

Following this adjustment period the steers were placed on treatment with one-third of the steers receiving each treatment. The steers received this treatment for two weeks and appropriate samples and measurements were again obtained. The steers were then again placed on treatment for a two-week period, however, each steer received a different treatment than previously. Data were again collected and the steers were placed back on treatment and received that diet which they had not previously received.

The experimental plan utilized also allowed the importance of the

carry-over effect of each ration to be evaluated. With the adjustment period, the experimental period was 60 days long and appropriate samples and measurements were obtained at 14 day intervals. Each steer then received the same diet during the adjustment period, and at the completion of the study every steer had received each of the three diets for a two-week period. No steer, however, received the same diet twice. Carry-over effect is then defined as the influence of the diet in the preceeding period. If carry-over effect exists, a measurement in any period is influenced by the diet received in that period and the diet received in the preceeding period so that if no adjustment for carry-over effects was made this would be confounded with the effect of the ration in the present period.

The $^{40}K$ counter used was the Nuclear Electronic $^{40}K$ Counter located at the Oklahoma Live Animal Evaluation Center (hereafter referred to as evaluation center). This counter is described by McLellan (1970) and Moser (1970).

## Animals

The animals used in this study were 36 Angus-Hereford crossbred steers purchased from the Ark-Valley feedlot, Arkansas City, Kansas. The feedlot contained approximately 120 steers of this breeding in one pen, all from the same ranch in northeastern Oklahoma. An attempt was made to select a random sample of steers weighing between 800 and 950 lbs. The steers were trucked to the Oklahoma State University beef center on May 7, 1970, where, with the exception of days on which the steers were $^{40}K$ counted, they were housed throughout the study. The

steers were penned three to a pen on slatted floors in a shed open to the south.

## Experimental Design

The design of the experiment involved 12 3 x 3 Latin Squares. Columns of the squares were two week feeding periods, rows were steers, and treatments were three different diets as shown in Table I. The diets were a high potassium diet with natural feeds as the source of potassium (Diet A), a high potassium diet with added KCl as the largest potassium source (Diet B), and a low potassium diet of natural feeds (Diet C). The feeds were analyzed for potassium content by methods described below. Average potassium concentrations for diets A, B and C were 1.31%, 1.03% and 0.29% respectively. These values were somewhat lower than the expected concentrations of 1.46% for rations A and B and 0.571% for ration C, as calculated by average values given for these feeds by Morrison (1959).

To estimate carry-over effect of rations from one period to the next, the selection of Latin Squares was balanced so that each treatment was administered an equal number of times following each other treatment. Table II illustrates the six orders that the rations could be administered to each steer.

The facilities at the evaluation center limited the number of steers which could be handled at one time so the 36 steers were assigned to three groups of 12 steers per group. Steers within a group were handled together for purposes of data collection. The method used to assign steers to squares and groups was to first complete the treatment orders in each Latin Square to give the balanced design discussed above.

TABLE I

COMPOSITION OF THE DIETS (PERCENT)

| Ingredient | Diets | | |
|---|---|---|---|
| | A | B | C |
| Alfalfa | 40.00 | --- | --- |
| Wheat straw | --- | 38.60 | 39.25 |
| Shelled corn | 39.25 | 48.60 | 49.50 |
| Soybean oil meal | 10.00 | 9.10 | 9.25 |
| Molasses | 10.00 | --- | --- |
| Salt | 0.50 | 0.50 | 0.50 |
| Aerofac | 0.25 | 0.25 | 0.25 |
| Urea | --- | 0.75 | 0.75 |
| Limestone | --- | 0.50 | 0.50 |
| KCl | --- | 1.70 | --- |

A = High potassium diet (natural feedstuffs) K = 1.31%.

B = High potassium diet (KCl added) K = 1.03%.

C = Low potassium diet (natural feedstuffs) K = 0.29%.

TABLE II

DESIGN OF EXPERIMENT

| | Order of Treatments | | | | | |
|---|---|---|---|---|---|---|
| Period | 1 | 2 | 3 | 4 | 5 | 6 |
| I | A | B | C | A | B | C |
| II | B | C | A | C | A | B |
| III | C | A | B | B | C | A |

Six steers received each of the six treatment orders.

Squares were then randomly allotted to the three groups and the steers were randomly assigned a row of a square. With the exception of the time the steers were at the evaluation center, all steers were managed as one large group.

In any one period, the steers of one square were on different ration so that the steers of a square were never penned together. The steers were penned, three to a pen, with one steer from each group in each pen. These three steers were always penned together while on treatment. Four pens were designated to each ration and these remained the same throughout the experiment. Steers were moved to designated pens for each period.

Prior to being placed on the respective ration according to the Latin Square plan, all the steers received the same diet for a two week adjustment period. Hereafter, the adjustment period will be designated Period I, and the three periods of the design as Periods II, III, and IV.

## General Procedures

Upon arrival at the Oklahoma State University beef center, the steers were all placed on a mixture of approximately one-half ration A and one-half ration C. The steers were fed this ration for two weeks prior to going on separate rations according to the experimental plan. During Period I the steers were cleaned of all excess manure and mud balls and numbered tags were placed in their ears.

At the end of each two week feeding period the steers were trucked to the evaluation center, where they remained approximately two days and then trucked back to the pens. While at the evaluation center the

data were collected.

The animals were hand fed twice daily to maximum feed consumption (the amount varied according to individual pen consumption patterns). Ration B was prepared at each feeding by hand mixing 1.7 lbs. of pre-weighed KCl to each 100 lbs. of ration C. It is very likely that the KCl did not get uniformly mixed throughout each 100 lbs. of feed. However, because all of this 100 lbs. was fed before mixing a new batch and between 60 and 70 lbs. of the ration was placed in each pen each day, it is felt there was a fairly uniform concentration of the KCl in ration B among pens.

Rations A and C were ground, mixed and sacked at the Fort Reno Experiment Station and trucked to Stillwater. Throughout the experiment four different batches of feed were prepared. At each feeding grab samples of each diet were taken and stored in a plastic bag for potassium analysis. All the feed samples from any one batch were mixed together before analysis but no mixing was done between batches.

## Collection of Data

Since all the steers within each group remained together for purposes of data collection and each group was handled alike during each period, only the day by day routine procedure for one period will be given. In describing this procedure, day one refers to the fourteenth day of any feeding period. All data obtained are shown in Table III.

### Day 1

At approximately 5:00 p.m. the steers were sorted and Group I was trucked to the evaluation center, about one mile. Upon arrival at the

TABLE III

WEIGHTS AND MEASUREMENTS TAKEN[a)]

| |
|---|
| 1. Unshrunk weight |
| 2. Unshrunk $^{40}K$ count |
| 3. 24 hr. shrunk blood serum potassium concentration |
| 4. 24 hr. shrunk weight |
| 5. 24 hr. shrunk $^{40}K$ count |
| 6. 24 hr. shrunk muscle tissue potassium concentration[b)] |

a) All potassium concentrations were determined in duplicate by atomic absorption spectrophotometry.

b) In period 3 only.

evaluation center, the steers were separated into three groups of four each and placed in concrete, slatted-floor holding pens. The steers were then washed with soap and water. The steers remained in the holding pens overnight with feed, the same diet the steers had been receiving throughout the period, and water available.

Day 2

At about 6:00 a.m. the $^{40}K$ counting began with a potassium chloride standard source counted first. The unshrunk animal counting continued without stop until all 12 animals in this group had been counted. This was completed around noon. The KCl standard source was also counted at the beginning and the end of each counting day.

A 10 minute background count was taken before and after each animal count to estimate the amount of natural radiation in the air at the time of the sample count. The background count was obtained with the animal crate in the counter, so that, as nearly as possible, the steer was the only additional $^{40}K$ source introduced into the counter. The net count of each animal was obtained by averaging the before and after background counts and subtracting from the steers gross 10 minute count.

The counting order was established by randomizing the three rations and the four steers within each ration. If the ration order came up A, B and C, the first steer in A was counted, then the first in B followed by the first in C. This procedure was repeated until all 12 steers in that group had been counted. This randomization was redone each time a count was begun on a group of steers. Immediately prior to being counted the weight was taken on each steer.

At 12:00 noon of Day 2 the feed and water were removed from all

steers in Group I and all were penned together for twenty-four hours.

At approximately 4:00 p.m. of Day 2, Group II began the same procedure as Group I on Day 1.

Day 3

At 6:00 a.m., Group II was counted in the same manner as Group I had been counted on Day 2. Immediately after counting the KCl source after Group II, the 24 hour shrunk $^{40}K$ counting of Group I began. This was generally between 12:00 noon and 12:30 p.m. Counting procedure was again identical to that described above with one exception. Just prior to being weighed, blood samples were collected from each steer. Blood samples were taken by jugular puncture. Approximately 10 mls of blood were collected in polyethylene centrifuge tubes. Samples were allowed to stand at room temperature until all samples had been taken from the steers in a group. Serum was separated by centrifuging[1] whole, clotted blood at 8,000 rpm for 10 minutes. The serum was removed by decantation, placed in sterile, stoppered plastic tubes and stored at $0^{o}F$ until chemical potassium analysis was determined (approximately eight weeks from the last counting period).

Upon completion of the shrunk $^{40}K$ counting of Group I, the steers were trucked back to the beef unit pens (between 5 and 6 p.m.) and placed on their respective treatment for the next period. At this time Group III was trucked to the evaluation center and the first days procedure of Group I was performed on Group III.

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[1]Sorvall Superspeed Automatic Refrigerated Centrifuge Model RC2-B, Ivan Sorvall, Inc., Norwalk, Conn.

Day 4

The procedure on day four was identical to Day 3 only it involved Groups II and III.

Day 5

Since there was no unshrunk group to count on this day, the counting began with the KCl standard source at 12:00 noon. The same procedure as described above for Group I, Day 2 was then repeated on Group III.

This same procedure was repeated at the end of each two week feeding period with the exception of the sampling of muscle tissue from each steer described below.

Muscle Biopsy

Following the counting of each steer in Period 3, a small sample of muscle tissue was surgically removed from the longissimus dorsi of the right side of each steer. Surgery was performed by members of the Oklahoma State University veterinary staff.

The steers were restrained in a metal holding chute. The area of the tenth rib was washed with soap and water and the hair clipped. This area was then washed with alcohol. A 4 sq. in. area was anesthetized and 4 in. vertical and longitudinal incisions were made. The fat was peeled away from the muscle and discarded. The fascia (connective tissue over the muscle) was also removed. Two samples, approximately five grams, were removed, one dorsal and one ventral, with surgical clippers. Samples were blotted free of excess blood with absorbent towels, wrapped in saran and wrapped again in aluminum foil. Samples were then stored

in four ounce sample jars at 0°F until analyzed for potassium. Incisions were disinfected and sutured.

There was excess swelling in this area in about half of the steers after a few days. Because of this, about one week after surgery each steer was run through a chute and the incision of those steers with excess swelling was opened and allowed to drain. The surgery did not appear to affect performance to a marked degree.

## Chemical Analyses

Serum, feed and muscle potassium was determined by atomic absorption spectrophotometry using a Perkin Elmer Model 303[2] spectrophotometer equipped with a digital concentration readout.[3] The methods used were as described by the manufacturer. Samples were prepared according to techniques outlined by Stuedemann (1970). In order to measure variation due to technique and instrumentation, duplicate analysis were conducted on each sample. Potassium analysis was completed approximately three months after the fourth period experimental data were collected.

Duplicate 2 gm. samples of air dry feed from each batch were ashed in a muffle furnace[4] for twelve hours at 575 to 600°C (temperature was attained gradually). The ash was dissolved in a 1:3 HCl (one part concentrated HCl to three parts of glass distilled water), transferred to a 25 ml volumetric flask and diluted to volume with 1:3 HCl. With an

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[2]Perkin-Elmer Corp., Norwalk, Conn.

[3]Model DCR 1. Perkin Elmer Corp., Norwalk, Conn.

[4]Hevi-Duty Electric Multiple Unit Furnace, Hevi-Duty Heating Equipment Co., Watertown, Wis.

automatic diluter this preparation was diluted 100 times by adding 0.1 ml to 9.9 mls of glass distilled water. Potassium concentrations were read from these dilutions.

Blood serum samples were allowed to thaw at room temperature. Serum was diluted 50 times by adding 0.1 ml of serum to 4.9 mls of glass distilled water. Potassium concentrations were read from these dilutions.

Muscle tissue samples were also thawed at room temperature and dried for twenty-four hours at 100°C. Dried samples were ashed and dissolved in acid as described for feed. This preparation was diluted 75 times by adding 0.1 ml to 7.4 mls water and potassium concentrations were read from this dilution.

Dilution techniques for all samples allowed potassium concentrations to be read in the range of 1-10 ppm.

## Statistical Procedures

Means, analyses of variance and estimates of carry-over effects were determined for all traits except muscle tissue potassium concentration by methods described by Cochran, Autrey and Cannon (1941) and by Lucas (unpublished notes on experimental designs in animal science).

To estimate carry-over effects, the observed performance of any steer in any period is expressed as a linear function of the effects of the steer, the period, the ration being received in that period, the ration received the previous period and experimental error. If carry-over effects of treatments from one period to the next are present, then the measurement of the direct effects of a treatment is confounded with carry-over effects. Thus, these data were first analyzed for the presence of carry-over effects. If carry-over effect did not exist, the

analysis for all traits was simplified to analyze only the direct treatment effects. Because the design provides less replication for estimates of carry-over effects than for the direct effects, estimates of carry-over effects are considerably less precise than those for direct effects.

To determine the importance of carry-over effects of treatments, steer weights, net $^{40}K$ counts and blood serum potassium were first analyzed for carry-over effects utilizing the mathematical model for carry-over effects presented below. If this analysis showed carry-over effects to be nonsignificant, each trait was analyzed using the non-carry-over effects model.

One carry-over effect and one non-carry over effect model is presented. It should be noted that each model expresses the three groups of steers as a source of variation, however, the group term applies only to the analysis of blood serum potassium. This was done because the blood serum samples taken from a group of steers on any day were handled together during chemical potassium analysis. Thus, the effect of handling the samples as a group is a measure of the technical variation associated with the potassium analysis of blood serum. The grouping of steers into three groups was done completely at random, so the effect of the groups should be a random variable and is combined with the effect of square in the analyses of weight and net $^{40}K$ count. Also, in the analyses of these two traits, unshrunk and shrunk data were analyzed separately.

The mathematical model for the presence of carry-over effect was:

$$Y_{ijkl} = u + G_i + S_{ij} + R_{ijk} + P_l + (GP)_{il} + (SP)_{jl} + (ST)_{jm}$$
$$+ C_m + D_m + (GC)_{im} + (GD)_{im} + e_{ijklm}.$$

When carry-over effects were negligible the model was:

$$Y_{ijklm} = u + G_i + S_{ij} + R_{ijk} + P_l + (GP)_{il} + T_m + (GT)_{im} + e_{ijklm},$$

where:

$Y_{ijklm}$ is an individual observation of one of the traits.

$u$ is an effect common to all observations, the overall mean.

$G_i$ is the effect of the ith group of steers (applies only to blood serum potassium). i = 1, 2, 3.

$S_{ij}$ is the effect of the jth square of the ith group of steers. For blood serum potassium, j = 1, 2, 3, 4. For other traits all steers are considered as one group so i = 1 and j = 1, 2 : ·· 12.

$R_{ijk}$ is the effect of the kth steer in the jth square in the ith group. k = 1, 2, 3.

$P_l$ is the effect of the lth period. l = 1, 2, 3.

$(GP)_{il}$ is the effect of the interaction of the lth period and the ith group (applies only to blood serum potassium).

$(SP)_{jl}$ is the effect of the interaction of the lth period and the jth square.

$(ST)_{jm}$ is the effect of the interaction of the mth treatment, unadjusted for carry-over effects, with the jth square. m = 1, 2, 3.

$T_m$ is the direct effect of the mth treatment. If carry-over effects are present, this term is confounded with carry-over effects. It represents the direct effect of the mth treatment in any period, plus any carry-over effect of another treatment that may exist.

$C_m$ is the carry-over effect of the mth treatment (preceeding treatment). It represents the treatment effects that exist from a treatment administered in the period immediately preceeding the period in which measurements were made.

$D_m$ is the direct effect of the mth treatment when carry-over effects are present. It represents the direct effect of a treatment after the true carry-over effect of the preceeding treatment.

$(GC)_{im}$ is the interaction of the carry-over effect of the mth treatment with the ith group.

$(GD)_{im}$ is the interaction of the direct effect of the mth treatment when carry-over effects are present with the ith group.

$e_{ijklm}$ is the random error unique for each observation.

In the construction of these models, the assumption was made that errors were normally and independently distributed about a mean zero and a common variance, $\sigma^2$.

Sources of variation and degrees of freedom for steer weight, net $^{40}K$ count and blood serum potassium utilizing the carry-over effects present model are shown in Table IV. It should be noted that in this analysis the sum of squares for treatments unadjusted for carry-over effects is calculated and subtracted from total sum of squares, although it is not included in the carry-over effects model. Unadjusted treatment sum of squares and not adjusted treatment sum of squares are subtracted from total sum of squares, along with all other terms, to calculate error sum of squares. This is done to eliminate any bias the effect of treatment adjustments may have on error.

Sums of squares for adjusted direct effects, carry-over effects and

TABLE IV

SOURCES OF VARIATION AND DEGREES OF FREEDOM FOR NET $^{40}K$ COUNT, WEIGHT AND BLOOD SERUM POTASSIUM UTILIZING THE CARRY-OVER-EFFECTS-PRESENT MODEL

| Source of Variation | Degrees of Freedom | |
|---|---|---|
| | a | b |
| Total | 107 | 107 |
| Group | * | 2 |
| Square[c] | 11 | 9 |
| Row (steers) | 24 | 24 |
| Period | 2 | 2 |
| Group x Period | * | 4 |
| Square x Period | 22 | 18 |
| Treatment (unadjusted)[d] | (2) | (2) |
| Square x Treatment | 22 | 18 |
| Treatment Carry-Over Effect | 2 | 2 |
| Adjusted Treatment Direct Effect | 2 | 2 |
| Adjusted Direct Effect x Group | * | 4 |
| Carry-Over Effect x Group | * | 4 |
| Error | 22 | 18 |

[a]Degrees of freedom for weight and net $^{40}K$ count.

[b]Degrees of freedom for blood serum potassium.

[c]Squares within group for analysis b.

[d]See text for explanation.

*Does not apply to these traits.

their interactions were calculated (as described by Lucas) as follows:

$$D_m = (t^2 - t - 1)\, T_m + tR_m + \Sigma_m + P_1 - tM,$$

where:

$D_m$ = adjusted total for the mth treatment,

$t$ = number of treatments ($t = 3$),

$T_m$ = the total of the mth treatment,

$R_m$ = the overall sum of the observations immediately following the mth treatment.

$\Sigma_m$ = the sum of the animal totals for those animals receiving the mth treatment in the last period.

$P_1$ = the sum of all observations in period 1.

$M$ = the grand sum.

The adjusted direct effect sum of squares (SSD) is given by:

$$SSD = \left[\frac{1}{qt(t + 1)(t - 2)(t^2 - t - 1)}\right] \Sigma D_m^2.$$

where:

$q$ = total number of Latin Squares.

The total carry-over effect of the mth treatment is given by:

$$Q_m = tD_m - t(t + 1)(t - 2)\, T_m + (t + 1)(t - 2)M$$

The carry-over effect sum of squares (SSC) is given by:

$$SSC = \left[\frac{1}{qt^3(t + 1)(t - 2)}\right] \Sigma Q_m^2.$$

To calculate the interaction of these sources of variation with groups (done only for blood serum potassium), the same values were calculated within each group using the same formulas as above. Within group ad-

justed direct effect, and carry-over effect totals are then represented by the following symbols:

$G_iD_m$ = adjusted direct effect total of the mth treatment in the ith group of steers.

$G_iQ_m$ = total carry-over effect of the mth treatment in the ith group of steers.

The adjusted direct effect by group sum of squares is given by:

$$\left[\frac{1}{nt(t+1)(t-2)(t^2-t-1)}\right] \Sigma G_i D_m^2,$$

where:

n = the number of Latin Squares per group.

The sum of squares for the carry-over effect by group interaction is:

$$\left[\frac{1}{nt^3(t+1)(t-2)}\right] \Sigma G_i Q_m^2.$$

When carry-over by group interaction was non-significant, the sum of squares and degrees of freedom for this term and for error were pooled to test for the presence of carry-over effects.

Treatment means when carry-over effects were suspected were calculated as follows:

Overall adjusted direct effect mean, $\bar{D}_m = \frac{D_m}{qt(t+1)(t-2)} + \frac{M}{qt^2}$,

and Carry-over effect mean, $\bar{Q}_m = \frac{Q_m}{qt(t+1)(t-2)}$.

The variance of treatment means were calculated as follows:

$$V\bar{D}_m \text{ (adjusted direct)} = \frac{S^2(t^2-t-1)}{qt(t+1)(t-2)},$$

$$V\bar{Q}_m = \frac{tS^2}{q(t+1)(t-2)},$$

where:

$S^2$ = error mean square.

When the test for carry-over effects was nonsignificant, traits were analyzed by the model for no carry-over effects. The sources of variation and degrees of freedom for $^{40}K$ net count, weight and blood serum potassium under this model is shown in Table V. The residual term represents all interactions of period with squares and groups and treatments with squares and groups. When residual mean square and error mean square were of similar magnitude they were pooled to test for significance of those terms above them in the analysis.

With this analysis, the variance of treatment means were calculated as follows:

$$V\bar{Y}_m \text{ (unadjusted for carry-over effects)} = \frac{tS^2}{qt^2} .$$

To study the relationship between net $^{40}K$ count and weight, the simple regression coefficient, regressing count on weight, was calculated for each treatment. Separate coefficients were calculated for shrunk and unshrunk data.

The performance of animals on each treatment also varied considerably so these same coefficients were used to correct net $^{40}K$ count treatment means to a constant weight. Mean net $^{40}K$ count for each treatment was adjusted to the overall mean weight as follows:

$$\hat{Y}_m = \bar{Y}_m + b\ (\bar{X}_m - \bar{X}..)$$

where

$\hat{Y}_m$ = adjusted mean net $^{40}K$ count for the steers on the mth treatment.

TABLE V

SOURCES OF VARIATION AND DEGREES OF FREEDOM FOR NET COUNT, WEIGHT AND BLOOD SERUM POTASSIUM WHEN CARRY-OVER EFFECTS WERE ABSENT

| | df | |
|---|---|---|
| Source of Variation | a | b |
| Total | 107 | 107 |
| Group | * | 2 |
| Square[c] | 11 | 9 |
| Row (steers) | 24 | 24 |
| Period | 2 | 2 |
| Group x Period | * | 4 |
| Treatment | 2 | 2 |
| Group x Treatment | * | 4 |
| Error in Square | 24 | 24 |
| Residual | 44 | 36 |

a) Degrees of freedom for net $^{40}K$ count and weight.

b) Degrees of freedom for blood serum potassium.

c) Squares within group for analysis b.

* Does not apply to these traits.

$\bar{Y}_m$ = unadjusted mean of the mth treatment.

b = the simple regression coefficient of net $^{40}K$ count on weight.

$\bar{X}_m$ = mean weight of the mth treatment.

$\bar{X}..$ = overall mean weight.

Separate adjustments were made for unshrunk and shrunk data. The regression coefficients used to make the correction in each case was the coefficient calculated by pooling over treatments.

Muscle tissue potassium was analyzed as a split-plot design by methods of Snedecor and Cochran (1967). Missing data were estimated with a formula by Cochran and Cox (1968). Rations were the main-plot treatments and animals the main-plots. Sub-plots were the position of the longissimus dorsi (dorsal or ventral) from which muscle samples were taken. The mathematical model utilized was:

$$Y_{ijk} = u + M_i + B_j + (BM)_{ij} + e_{ij} + T_k + (BT)_{jk} + (MT)_{ik} + (MBT)_{ijk} + \delta_{ijk}.$$

where,

$M_i$ is the effect of the ith main plot treatment (rations applied to animals). i = 1, 2, 3.

$B_j$ is the effect of the jth group of steers (12 steers per group). j = 1, 2, 3.

$(BM)_{ij}$ is the interaction of the ith treatment with the jth group.

$e_{ij}$ is the random error unique to the observation on each animal.

$T_k$ is the effect of the kth sub-plot (position of the muscle

extraction). k = 1, 2.

$(BT)_{jk}$ is the interaction of kth sub-plot treatment with the jth group.

$(MT)_{ik}$ is the interaction of the kth sub-plot treatment with the ith main-plot treatment.

$(MBT)_{ijk}$ is the interaction of the ith main-plot treatment with the jth group and the kth sub-plot treatment.

$\delta_{ijk}$ is the random error unique to the observation on each individual muscle sample.

In the construction of this model, the assumption is made that $e_{ij}$ is a normally distributed random variable with a mean of zero and a variance, $\sigma^2_m$, and that $\delta_{ijk}$ is a random variable with mean zero and a variance, $\sigma^2_I$.

The variance for a main-plot treatment mean is

$$\frac{\text{main-plot error mean square}}{n}$$

where

n = number of animals per treatment

In the analysis of variance, the main-plot error mean square estimates $(\sigma^2 I + t\sigma^2_m)$, where t equals three, the number of treatments. For comparisons between two sub-plots that are within main-plots, and for any comparison among treatments that is entirely within main-plots, the basic error variance is $\sigma^2 I$, estimated by the sub-plot error mean square.

Comparisons between treatment means for all traits were made by making all possible comparisons between means using the LSD method as described by Snedecor and Cochran (1968).

# CHAPTER IV

# RESULTS AND DISCUSSION

## Background Analysis

All $^{40}K$ counts, both background and steer counts, were taken as five consecutive two-minute counts. In order to study the distribution of these counts, the average was obtained for each individual 10-minute count (the average of five consecutive two-minute counts) and expressed as $^{40}K$ counts per two minutes. Each individual two-minute count was then deviated from this mean and expressed as a deviation in counts per two-minutes.

The range in deviations when only background was being counted was - 740.2 to 1751.6 counts per two-minutes and the standard deviation of deviations was 209.8 counts per two-minutes. This is based on 1,730 individual two-minute counts deviated from the mean two minute count of 346 ten-minute counts. The distribution of these deviations is illustrated with a histogram in Figure 1. The width of each bar in the histogram represents one-half of a standard deviation. Although no test for normality was made on these data, there is not substantial evidence to suggest these deviations are not normally distributed. The mean deviation, zero, ± one standard deviation contained 72.3% of the observations, slightly greater than the 68.26% expected in a normally distributed population. The mean ± two standard deviations contained 95.5% of the observations and 99.1% fell within the range of three standard de-

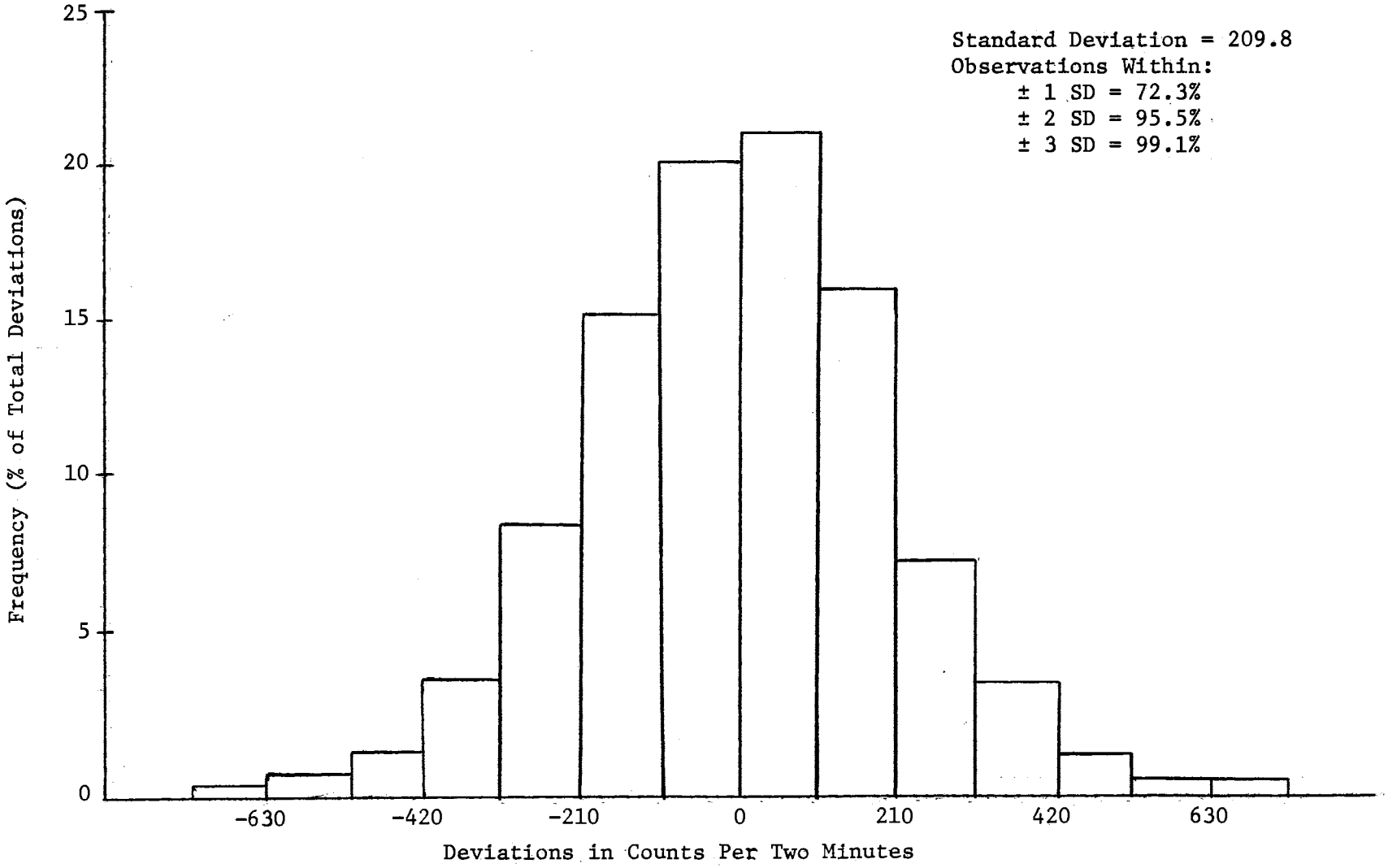


Figure 1. Histogram of Deviations of Two Minute Background Counts Deviated From the Mean of Five Consecutive Two Minute Counts

deviations of zero. Although the extremely large deviations were all positive, only four exceeded 800.

The deviations of two-minute counts from the mean of five consecutive two-minute counts when a steer was in the counting chamber are illustrated with the histogram in Figure 2. The range in these deviations was from -2,449.6 to 2042.2 counts per two minutes and the standard deviation of the deviations was 389.8 counts per two minutes. This is based on 1435 individual two-minute counts deviated from 287 ten-minute steer counts. This increase in the size of deviations is expected since the radiation being measured at this time includes that naturally present plus that introduced by the steer. The average two-minute count when a steer is in the chamber is almost twice the average two-minute background count.

The shape of this distribution is almost identical to that of background deviations. Zero ± one, two and three standard deviations included 73.6, 95.9 and 99.2% of the observations respectively. Large deviations were equally distributed on the negative and positive side.

Both of these distributions of deviations had somewhat more values within the range of ± one standard deviation than expected. However, these distributions suggest that, within a short time period, the disintegration of the radioactive atoms measured (primarily $^{40}K$ and a small amount of $^{137}Cs$) is fairly random and approaches normality, if it is not normal. The variance within five consecutive two-minute background counts, when plotted against the mean of these five counts, showed no consistent trend in the relationship between the size of the mean of five consecutive two-minute counts and the variance within these five counts. Large variances appeared to be associated as frequently with small means

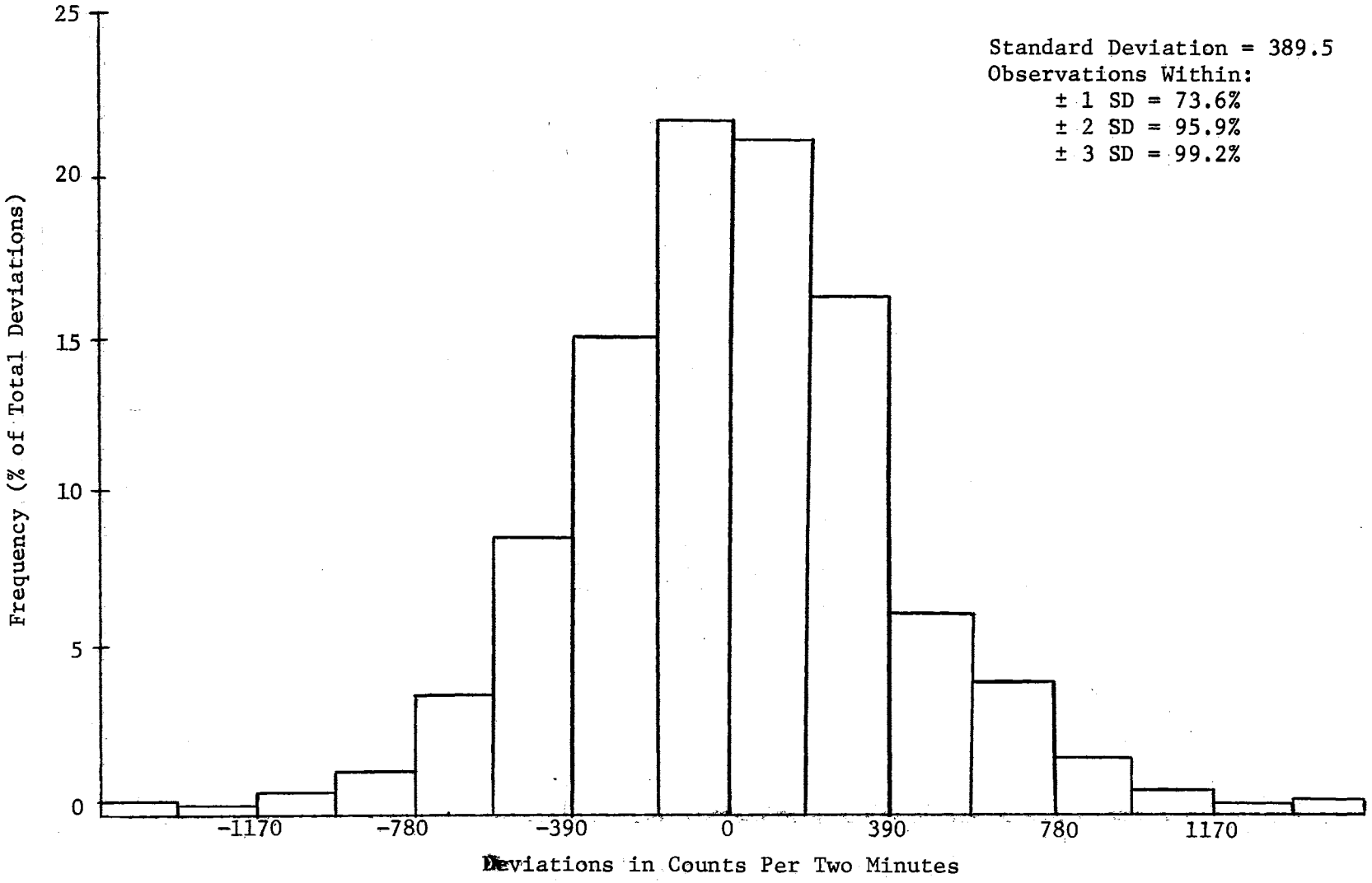


Figure 2. Histogram of Deviations of Two Minute Background Plus Steer Counts Deviated From the Mean of Five Consecutive Two Minute Counts

as with large means. Assuming these deviations are normally distributed and that the size of the variance does not depend on the size of the mean, $^{40}K$ data from this $^{40}K$ counter can be analyzed without making transformations to stabilize the variance.

## Animal Performance

The mean weight for the steers on each treatment at the end of each period is shown in Table VI. The average unshrunk and shrunk weight and respective standard deviations at the end of Period I (period in which all steers were on the same ration) was 868 lbs. (SD = 89.8) and 818 lbs. (SD = 67.3) respectively.

The average weight gain per steer on rations A, B and C measured from unshrunk weights was 9.3, 33.0 and 27.5 lbs. respectively. Weight gains per steer measured from shrunk weights were 6.9, 26.6 and 28.5 lbs. for rations A, B and C respectively. There is no apparent reason for the marked difference in performance of the three rations. Ration A was the alfalfa based diet which was expected to cause the highest gain per period.

## Carry-Over Effects

The analyses of variance for carry-over effects for each trait, shrunk and unshrunk, are given in Appendix Tables XII, XIII and XIV. Carry-over effects were nonsignificant in each case as all tests of significance for the presence of carry-over effects resulted in F values less than 1.0.

Carry-over effect means and standard errors of these means are shown in Table VII. The means in all cases except two (unshrunk net $^{40}K$

TABLE VI

MEAN WEIGHTS AT THE END OF EACH PERIOD FOR STEERS FED THE VARIOUS DIETS

| | Unshrunk Steer Weights, lbs | | | | Shrunk Steer Weights, lbs | | | |
|---|---|---|---|---|---|---|---|---|
| | | Diets | | | | Diets | | |
| Period | Pretrial[a] | A | B | C | Pretrial[a] | A | B | C |
| 1 | 876 | | | | 818 | | | |
| 2 | | 876 | 934 | 872 | | 833 | 886 | 831 |
| 3 | | 935 | 896 | 909 | | 880 | 836 | 855 |
| 4 | | 901 | 924 | 988 | | 846 | 876 | 928 |
| Overall mean | | 904 | 918 | 923 | | 853 | 866 | 872 |
| SE[b] | | 2.91 | 2.91 | 2.91 | | 2.09 | 2.09 | 2.09 |

[a]All steers recieved a mixture of Diets A and C in Period 1.

[b]Standard errors apply only to the overall means.

TABLE VII

CARRY-OVER EFFECT MEANS AND STANDARD ERRORS FOR EACH TRAIT AND EACH RATION

| Item | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | A | | B | | C | |
| | Mean | SE | Mean | SE | Mean | SE |
| Unshrunk Weight, lb | 3.03 | 4.13 | 0.78 | 4.13 | - 3.82 | 4.13 |
| Shrunk Weight, lb | 0.51 | 3.27 | 2.26 | 3.27 | - 2.77 | 3.27 |
| Unshrunk Net $^{40}K$ Count[a] | -5.38 | 75.79 | 104.33 | 75.79 | -98.96 | 75.79 |
| Shrunk Net $^{40}K$ Count[a] | 0.85 | 82.90 | 71.55 | 82.90 | -72.40 | 82.90 |
| Blood Serum Potassium, ppm | -1.48 | 6.81 | - 0.90 | 6.81 | 2.33 | 6.81 |

[a] Net $^{40}K$ count per minute per steer.

count for ration B and C) are smaller than the standard errors of these means. All but one of the carry-over effect means for ration C (the diet with the lowest potassium concentration) are negative. Ration B (ration with added KCl) had all positive carry-over means, except one, and carry-over means for ration B were larger than carry-over means of ration A, although ration A had a higher potassium concentration than ration B (1.31% vs 1.03%).

It appears that the ration fed in any two-week period did not affect the weight, net $^{40}K$ count or blood serum potassium levels in the following period. This would indicate that feeding a standard diet for a two-week period prior to $^{40}K$ counting would allow $^{40}K$ comparisons to be made among the steers within a group free from the effects of previous rations these animals may have been receiving.

## Non-Carry-Over Treatment Effects

The relationship between weight loss in 24 hours shrink and net $^{40}K$ count loss in 24 hours shrink is presented in Table VIII. The mean loss in weight and respective standard deviation was quite similar for all treatments. Average weight loss was somewhat lower during Period I (pretrial), but this may be a reflection of the lighter weight of all steers during this period.

The average net $^{40}K$ count loss in 24 hours shrink was not as uniform among treatments. As would be expected, those steers receiving the two highest potassium diets (A and B) lost considerably more count than those on diet C. The steers receiving the mixture of diets A and C, pretrial, however, lost the least net $^{40}K$ count in 24 hours shrink.

The variation that exists between weight loss and net $^{40}K$ count

TABLE VIII

MEAN 24 HOUR WEIGHT LOSS AND NET $^{40}K$ COUNT LOSS AND REGRESSION COEFFICIENTS REGRESSING NET $^{40}K$ COUNT LOSS ON WEIGHT LOSS IN 24 HOURS

| Treat. | No | Mean Weight Loss, lb | SD | Mean $^{40}K$ Count Loss[a] | SD | Ratio[b] | b | $S_b$ |
|---|---|---|---|---|---|---|---|---|
| Pretrial | 35 | 44.8 | 15.12 | 534.3 | 454.96 | 11.9 | 11.46* | 4.77 |
| A | 36 | 52.5 | 11.70 | 845.3 | 434.65 | 16.1 | 12.44* | 5.99 |
| B | 36 | 52.0 | 12.98 | 850.2 | 474.94 | 16.3 | 4.48 | 6.23 |
| C | 36 | 51.3 | 17.46 | 598.4 | 347.02 | 11.7 | 9.86** | 2.96 |
| Ave. | 143 | 50.2 | 14.71 | 708.3 | 449.21 | 14.1 | 10.19** | 2.43 |

*$P < .05$

**$P < .01$

[a]Counts per minute (cpm)

[b]Mean cpm divided by mean weight

loss is evident from observation of the regression coefficients (regressing count loss on weight loss) and the respective standard errors of these coefficients. The regression coefficients for three of the four treatments were significantly greater than zero, pretrial and diet A ($P < .05$) and diet C ($P < .01$). Although the coefficient for diet B (4.48) was lower than all others and nonsignificant, the large standard error associated with this coefficient does not suggest it is estimating a value different than the other three coefficients. Consequently, all coefficients were pooled. The pooled regression coefficient of net $^{40}K$ count loss on weight loss in 24 hours shrink was 10.19 counts per minute per pound ($P < .01$). The standard error of estimate of a prediction equation to predict count loss from weight loss was 424.9 counts per minute. This compares to the overall standard deviation of net $^{40}K$ count loss in 24 hours shrink of 708.3 counts per minute. With the exception of diet B, the regression coefficients were not greatly different than the average net $^{40}K$ count loss per pound of weight loss. This does not give very good evidence for a linear relationship between these two variables but does suggest a linear relationship may exist.

This indicates that as weight loss during shrink increases, net $^{40}K$ count loss also increases, but this relationship does not appear close enough to very accurately predict $^{40}K$ count loss from weight loss. Some of the variation in weight loss and $^{40}K$ count loss during shrink is undoubtedly due to different feed consumption patterns by steers while at the evaluation center. Some steers appeared to eat very little while others ate quite well. As a result, when unshrunk $^{40}K$ counting was done, some steers may have already been partially shrunk (12 hours off feed and water) and others may really have been measured unshrunk. The

time lag of six hours from beginning to end of the counting of any one group would also result in steers being counted not at 24 hours shrink, but between 24 and 30 hours shrink.

The relationship between net $^{40}K$ count per minute and steer weight is presented in Table IX. The regression of net $^{40}K$ count per minute on weight in pounds, measured from shrunk and unshrunk observations, and their respective standard errors are shown for each treatment. All regression coefficients are significantly greater than zero ($P < .01$). There is a tendency for coefficients to be higher for high potassium diets and on unshrunk measurements, although the largest coefficients, both shrunk and unshrunk, were obtained during Period I when steers received the mixed diet (mixture of A and C). This may reflect the influence of lighter weight cattle. A higher percentage of each pound gained by lighter steers should be muscle than each pound gained by heavier steers and consequently should theoretically cause a greater increase in net $^{40}K$ count.

The size of the coefficients and their standard errors are very similar for all treatments both shrunk and unshrunk. As a result all coefficients were pooled to obtain one estimate of the increase in net $^{40}K$ count per minute for each increase of one pound in weight. Unshrunk and shrunk coefficients were $6.69 \pm 0.87$ and $5.63 \pm 0.80$ net $^{40}K$ counts per minute per pound of gain respectively. These coefficients were then used to correct the average $^{40}K$ count of steers on each treatment to the overall mean weight of steers. This adjusted for the differences in gain by steers on each ration discussed above.

Adjusted net $^{40}K$ count treatment means and their respective standard errors are presented in Table X. The respective analyses of variance

TABLE IX

REGRESSION COEFFICIENTS, REGRESSING NET $^{40}K$ COUNT PER MINUTE PER STEER ON WEIGHT IN POUNDS, FOR EACH TREATMENT MEASURED FROM SHRUNK AND UNSHRUNK DATA

| Item | Treatment | No | $b^b$ | $S_b$ |
|---|---|---|---|---|
| Unshrunk | pretrial[a] | 36 | 8.61 | 1.83 |
| | A | 36 | 8.51 | 1.74 |
| | B | 36 | 7.40 | 1.54 |
| | C | 36 | 5.63 | 1.87 |
| | pooled | 144 | 6.69 | 0.87 |
| Shrunk | pretrial[a] | 35 | 7.29 | 1.93 |
| | A | 36 | 7.08 | 1.56 |
| | B | 36 | 6.15 | 1.52 |
| | C | 36 | 5.93 | 1.42 |
| | pooled | 143 | 5.63 | 0.80 |

[a]Period I when all steers received the same treatment.

[b]All regression coefficients are significantly greater than zero ($P < .01$).

TABLE X

ADJUSTED NET $^{40}K$ COUNT MEANS FOR EACH TREATMENT MEASURED UNSHRUNK AND SHRUNK

| Item | Treatment | Mean[c] | SE |
|---|---|---|---|
| Unshrunk[a] | A | 14105.4 | 48.50 |
| | B | 13958.9 | 48.50 |
| | C | 13657.9 | 48.50 |
| Shrunk[b] | A | 13254.7 | 43.08 |
| | B | 13121.2 | 43.08 |
| | C | 13036.7 | 43.08 |

[a]All means are corrected to a common weight of 914.94 pounds.

[b]All means are corrected to a common weight of 863.43 pounds.

[c]$^{40}K$ counts per minute.

are shown in Appendix Table XVI. The diets steers received significantly affected net $^{40}K$ count both unshrunk and shrunk ($P < .01$).

The mean unshrunk net $^{40}K$ count for steers on ration A was 146.6 counts per minute (cpm) higher ($P < .05$) than the mean for steers on diet B and 447.5 cpm higher ($P < .01$) than the mean for steers on diet C. The mean $^{40}K$ cpm for steers on diet B was also 301 cpm higher ($P < .01$) than the mean of steers on diet C.

Differences between treatments were not as large after 24 hours shrink. The mean for steers receiving diet A was 133.5 cpm higher ($P < .05$) than the mean of steers receiving diet B and 218.0 cpm greater ($P < .01$) than the mean of steers receiving diet C. There was a nonsignificant difference of 84.5 cpm between rations B and C.

Measurements of $^{40}K$ for each diet were made on the same steers. Weight adjusted treatment means should then be making comparisons of the same steers at a constant weight and the average pounds of fat-free lean for steers on each diet should be similar. Since diet significantly affected net $^{40}K$ count it is interesting to compare estimates of fat-free lean from the adjusted mean net $^{40}K$ count of each diet assuming average fat-free lean for steers on each diet is the same. This was done using the prediction equation presented by Frahm, Walters and Odell (1970) developed with similar weight (average of 848 lbs) yearling Angus bulls. The equation $\hat{Y} = 75.5 + 0.0145$ (cpm), results in estimates of average fat-free lean of 267.7, 265.8 and 264.5 pounds for the steers on diets A, B and C respectively. These differences are not large considering the effects of diet were highly significant. Each of these estimates fall well within the range of one standard error of estimate for this equation (8.4 pounds) of each other. Since this is a prediction

equation, the standard error of estimate is estimating the variation in the population of pounds of fat-free lean at any given $^{40}K$ cpm. The estimates used here are means estimating mean pounds of fat-free lean of a group. It would be interesting to know the standard error of the mean pounds of fat-free lean at any given $^{40}K$ cpm from this prediction equation. This would make a more valid comparison among estimates, however this value is not known.

Even though differences in estimates of fat-free lean are not large between diets, these data give some indications that a fat-free lean prediction equation should be developed while cattle are receiving a standard diet of known potassium content. It would then be desirable to feed this diet, or one of similar potassium content, to cattle for about two weeks prior to $^{40}K$ evaluation. This may or may not reduce the standard error of a prediction equation, but would give one more confidence that the predictions and associated standard errors would apply to those cattle being evaluated.

Differences in fat-free lean estimates would be greater based on unshrunk $^{40}K$ counts, however no prediction equations are available to make this comparison.

Keeping animals off feed and water for a period of time is not only hard on animals but may also be expensive in terms of weight loss to producers. However, these data do not suggest animals should be evaluated without being shrunk. The possibility of removing only feed and not water should perhaps be explored.

Period (time) and row (animals) also significantly ($P < .01$) affected net $^{40}K$ count both unshrunk and shrunk. Since an equal number of animals received each treatment in each period, this source of variation

represents primarily the growth of animals over time. The significant effect of animals is more difficult to interpret. It compares the average $^{40}K$ count of steers in rows of the squares. Some of this variation is undoubtedly due to differences in weight and some to differences in lean content of animals of similar weight, however animal to animal variation in potassium concentration would also influence this value. Based on the muscle and blood analysis discussed below where animals were one of the largest sources of variation, the significant affect of animals on net $^{40}K$ count can be interpreted to mean animal to animal variation in potassium content may be an important source of variation to $^{40}K$ evaluation.

Muscle tissue and blood serum potassium concentration means and respective standard errors are shown in Table XI.

Analyses of variance for blood serum potassium (carry-over effects negligible) and muscle tissue potassium are given in Appendix Tables XVII and XVIII. Although the rank of blood serum potassium means was the same as the potassium concentration of diets, the diets did not significantly ($.25 > P > .05$) affect blood serum potassium concentration. The average serum potassium level of 191.13 ppm (equivalent to 19.113 mg/100 ml) is slightly lower than the average of 23.2 mg/100 ml determined by Field, Weiner and Wood (1969) and the average of 23.0 mg/100 ml reported by Stuedemann (1970) for sheep.

The highly significant ($P < .01$) group mean square indicates that technical variation was a significant source of variation in potassium analysis. The standard error of duplicate analysis was 18.147 ppm. Because all the samples from any group of steers in any period were handled together for potassium analysis, the significant animal mean square

TABLE XI

MEAN MUSCLE TISSUE AND BLOOD SERUM POTASSIUM CONCENTRATIONS FOR EACH TREATMENT

| | Blood Serum[a] | | Muscle Tissue[b] | |
|---|---|---|---|---|
| Treatment | Mean | SE | Mean | SE |
| Pretrial | 200.5 | 21.63[c] | | |
| A | 196.1 | 4.32 | 2.93 | 0.24 |
| B | 189.3 | 4.32 | 2.64 | 0.24 |
| C | 188.0 | 4.32 | 2.90 | 0.24 |
| Ave. | 191.1 | 2.49 | 2.82 | 0.14 |

[a]Parts per million.

[b]Gms K per kg wet tissue.

[c]Standard deviation of blood serum potassium concentration during Period I when all steers received the same diet.

($P < .01$) indicates blood serum potassium concentrations are not constant between animals. Potassium concentrations ranged from 133 to 306 ppm. An estimate of the variation of blood serum potassium concentration is the overall standard deviation of 18.57 ppm (calculated from the mean of duplicate analysis). This compares to a mean of 200.5 ppm (SD = 21.63) in Period I based on thirty-five observations when all steers were receiving the same treatment. The $CV_e$ of 9.7% indicates the amount of variation that exists between animals in blood serum potassium concentrations.

The analysis of variance for muscle tissue potassium is shown in Appendix Table XVIII. There was no significant effect of diet on muscle tissue potassium. The mean potassium concentration for diets A and C (high and low potassium diets) were very similar while the mean of diet B was somewhat lower (Table XI). The position of muscle extraction also did not affect potassium concentration. The mean of all samples extracted ventrally was 2.86 gms K/kg (SE = 0.87) as compared to a mean of 2.79 gm K/kg (SE = 0.87) for samples extracted from the dorsal position. It would not be expected that these means differ since these samples were extracted only about four inches from each other.

The overall mean potassium concentration of muscle tissue, 2.82 gms K/kg ± 0.139, is somewhat higher than that reported by Lohman, Ball and Norton (1970) of 2.53 gms per kg in rib but is lower than the 3.1 gms per kg reported by Bennink *et al.* (1968) and 3.68 gms per kg reported by Gillett *et al.* (1967) in rib.

Contrary to blood serum potassium analysis, the group mean square was nonsignificant. This may in part be explained by the fact that potassium concentrations of all muscle samples were read from the atomic

absorbtion spectrophotometer in two days time (although several days were used for sample preparation) while blood serum samples were analyzed over a period of about two weeks. Because of the many mechanical adjustments made on the instrument during analysis, it was apparent this machine is not consistent over time. The inconsistency of this method of potassium analysis is also apparent from the relatively large standard error of duplicate analysis of 0.322 gms/kg. The coefficient of variation of 11.07% based on the mean of duplicates is in agreement with the 9.6% reported by Lohman, Dieter and Norton (1970). These authors discuss the limitations of atomic absorbtion spectrophotometry to estimate potassium in lean.

An estimate of the variation between animals in muscle tissue potassium concentration is the standard deviation calculated from the mean of the two samples extracted from each animal, each analyzed in duplicate. This is 0.436 gm K/kg. The resulting coefficient of variation of 15.46% is somewhat higher than that for blood serum. Since muscle potassium was calculated on a wet tissue basis, some of this variation is quite likely caused by differences in fat content of the tissue samples. The standard deviation is somewhat higher than that reported by Lohman, Ball and Norton (1970) of 0.14 gms/kg but the coefficient of variation is in agreement with that of 17.2% reported by Ward, Johnson and Tyler (1967).

The simple correlation between muscle tissue potassium concentration and blood serum potassium concentration, calculated across treatments, was 0.846 ($P < .01$). This is calculated from the average concentrations of the thirty-six steers in Period III. This rather high relationship and the variation between animals in each of these variables

discussed above could in part explain the standard errors of estimates of $^{40}K$ prediction equations. Based on these data it seems reasonable that two steers could be of the same weight and have very similar pounds of total fat-free lean and yet have quite different $^{40}K$ counts simply because of the animal to animal variation in potassium concentration.

Because muscle tissue samples were collected only in Period III, no estimate of treatment carry-over effects could be made on muscle tissue in the manner described for other traits. However, no steers received the same diet in Period III as in Period II and an equal number of steers receiving any diet in Period III had received each of the other two diets in Period II. A comparison of means (mean of six observations) did not indicate any influence of preceding diet on muscle tissue potassium.

It appears from these data that the primary influence of dietary potassium to $^{40}K$ counting is the effect on potassium content of gastrointestinal tract and contents, even after 24 hours shrink. Dietary potassium did not significantly affect blood serum potassium or muscle tissue potassium, although blood serum potassium levels were higher in steers on high potassium diets. The steers on the highest potassium diet also had the highest muscle tissue potassium levels. The animal to animal variation in blood serum and muscle tissue potassium levels could also be important sources of error in estimating fat-free lean by $^{40}K$ counting.

# CHAPTER V

## SUMMARY

Three levels of dietary potassium were evaluated using a Latin Square experimental design to determine the importance of potassium in the diet on the $^{40}K$ count of 36 steers. The influence of potassium in the diet on blood serum and muscle tissue potassium concentration was also evaluated. Levels of potassium studied were 1.31% (diet A), 1.03% (diet B) and 0.29% (diet C). Estimates of carry-over effects of rations from one period to the next were also evaluated.

Potassium-40 measurements were made on each steer unshrunk and after 24 hours off feed and water. The weight of each steer was taken at the time of counting. From these data, the relationship between net $^{40}K$ count and weight as affected by different levels of potassium in the diet was studied. Blood and muscle samples were collected only after 24 hours shrink. Blood samples were collected after each feeding period, however muscle samples were taken only in one period. Muscle samples were taken by biopsy techniques.

### Carry-Over Effects

The ration fed in any two week period did not significantly affect the weight, net $^{40}K$ count or blood serum potassium level of steers in the following period. The mean carry-over effect of each treatment was small. Carry-over effect means were centered about zero with eight

means being positive and seven negative. Thirteen of the fifteen carry-over effect means were smaller than their associated standard errors and none were significantly different from zero.

## Non-Carry-Over Treatment Effects

The average weight loss during the shrink was 50.2 lbs. This weight loss was similar for all treatments, but the average loss in $^{40}K$ count during shrink varied considerably among treatments. The average net $^{40}K$ count loss per steer for the two high potassium diets was 847.7 counts per minute compared to a loss of 534.3 counts per minute when all the steers received the same diet during the pretrial period. The regression of $^{40}K$ count loss on weight loss ranged from 4.48 count loss per minute per pound weight loss for diet B, to 12.44 for diet A. These were the two high potassium diets, however, the differences between regression coefficients for the different treatments were not significant. The variation within treatments in $^{40}K$ count loss in 24 hours was also evident by the large standard deviations for this trait for each treatment (347.02 to 474.94 counts per minute).

Dietary potassium did not significantly affect the relationship between weight and $^{40}K$ count in these steers. The regression of $^{40}K$ count on weight resulted in significant ($P < .01$) coefficients for all treatments measured from both shrunk and unshrunk data, however, no significant differences in regression coefficients between treatments existed. There was a tendency for these coefficients to be higher for high potassium diets and for unshrunk measurements. The pooled regression coefficient of $^{40}K$ count on weight was $6.69 \pm 0.87$ and $5.63 \pm 0.80$ counts per minute per pound for unshrunk and shrunk measurements respectively.

Net $^{40}K$ count was adjusted to the overall mean weight of steers with the pooled regression coefficients of $^{40}K$ count on weight. Ration significantly ($P < .01$) affected unshrunk and shrunk net $^{40}K$ count. Unshrunk differences between adjusted means of treatments A and B, A and C, and B and C were 146.6 ± 68.6, 447.5 ± 68.6 and 301.0 ± 68.6 counts per minute respectively. Shrunk differences between adjusted treatment means of A and B, A and C, and B and C were 133.5 ± 60.9, 218.0 ± 60.9 and 84.5 ± 60.9 counts per minute, respectively. Although these differences do not cause large differences in estimates of fat-free lean of these steers using present prediction equations, it appears prediction equations should be developed utilizing net $^{40}K$ count while cattle are receiving a standard diet of known potassium content. These data provide evidence that feeding of this standard diet for two weeks prior to $^{40}K$ evaluation will allow comparisons to be made among a group of cattle free from the effects of any previous ration these cattle may have been receiving. It appears that present prediction equations and their associated standard errors of estimate do not apply to cattle that have received different rations prior to $^{40}K$ evaluation, but only to cattle being evaluated which have received a diet similar in potassium content to that received by cattle with which the equations were developed.

Potassium in the diet did not significantly affect blood serum, or muscle tissue potassium levels. The overall mean potassium blood level was 191.1 ± 2.49 parts per million. The average potassium concentration of the muscle tissue extracted from the longissimus dorsi was 2.82 ± 0.14 gm/kg.

The highly significant mean square for animals in the analysis of net $^{40}K$ count and blood serum potassium indicate animal to animal varia-

tion in potassium concentration may be a large source of variation to $^{40}K$ estimates of fat-free lean in live animals. A highly significant correlation ($P < .01$) of 0.85 was also found between blood serum potassium and muscle potassium.

These data suggest that the primary influence of dietary potassium to $^{40}K$ counting is the effect on potassium content of the gastrointestinal tract and content and not on the potassium concentration of the intracellular fluids. This influence coupled with natural animal to animal variation in potassium content could be major sources of variation that affect the precision of $^{40}K$ estimates of fat-free-lean in cattle.

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# A P P E N D I X

TABLE XII

ANALYSES OF VARIANCE FOR WEIGHT, UNSHRUNK AND SHRUNK,

UTILIZING THE CARRY-OVER EFFECTS PRESENT MODEL

| Source of Variation | df | Unshrunk MS | Shrunk MS |
|---|---|---|---|
| Total | 107 | | |
| Square | 11 | 16439.9 | 18831.8 |
| Animals (row) | 24 | 25251.7 | 22070.6 |
| Period | 2 | 17423.2 | 11307.3 |
| Square x Period | 22 | 333.7 | 154.7 |
| Treatment[a] (unadjusted) | (2) | 3401.0 | 3390.0 |
| Square x Treatment | 22 | 319.8 | 151.3 |
| Adj. Direct Effect | 2 | 2173.3 | 2519.9 |
| Carry-Over Effect | 2 | 195.3 | 103.9 |
| Error | 22 | 273.2 | 170.9 |

[a]Treatment sum of squares included only to calculate proper error sum of squares.

TABLE XIII

ANALYSES OF VARIANCE[a] FOR NET $^{40}K$ COUNT, UNSHRUNK AND SHRUNK, UTILIZING THE CARRY-OVER EFFECTS PRESENT MODEL

| Source of Variation | df | Unshrunk MS | Shrunk MS |
|---|---|---|---|
| Total | 107 | | |
| Square | 11 | 44837.2 | 35300.6 |
| Animals (row) | 24 | 21369.2 | 17881.2 |
| Period | 2 | 64784.9 | 13080.5 |
| Square x Period | 22 | 696.7 | 447.8 |
| Treatment[b] (unadjusted) | (2) | 10654.0 | 1124.4 |
| Square x Treatment | 22 | 991.1 | 512.2 |
| Adj. Direct Effect | 2 | 11359.0 | 1438.7 |
| Carry-Over Effect | 2 | 113.4 | 57.3 |
| Error | 22 | 919.0 | 1099.6 |

[a] All variances times $10^{-2}$.

[b] Treatment sum of squares included only to calculate proper error sum of squares.

TABLE XIV

ANALYSIS OF VARIANCE FOR BLOOD SERUM POTASSIUM

UTILIZING THE CARRY-OVER EFFECTS PRESENT MODEL

| Source of Variation | df | MS |
|---|---|---|
| Total | 107 | |
| Group | 2 | 5410.8 |
| Square in Group | 9 | 2108.0 |
| Animals (row) | 24 | 2144.8 |
| Period | 2 | 7814.6 |
| Group x Period | 4 | 2072.1 |
| Square in Group x Period | 18 | 476.1 |
| Treatment[a] (unadjusted) | (2) | 1334.8 |
| Square in Group x Treatment | 18 | 540.6 |
| Adj. Direct Effect | 2 | 851.3 |
| Carry-Over Effect | 2 | 133.0 |
| Adj. Direct x Group | 4 | 873.5 |
| Carry-Over x Group | 4 | 1094.8 |
| Error[b] | 18 | 662.9 |

[a] Treatment sum of squares included only to calculate proper error sum of squares.

[b] Error and Carry-Over Effect x Group were pooled to test for the presence of carry-over effects.

TABLE XV

ANALYSIS OF VARIANCE FOR WEIGHT, UNSHRUNK AND SHRUNK, UTILIZING THE CARRY-OVER NEGLIGIBLE MODEL

| Source | df | Unshrunk MS | Shrunk MS |
|---|---|---|---|
| Total | 107 | | |
| Square | 11 | 16439.87 | 18831.81 |
| Animals (row) | 24 | 25251.67** | 22070.63** |
| Period | 2 | 17423.23** | 11307.26** |
| Treatment | 2 | 3401.01** | 3390.01** |
| Error in Square | 24 | 266.69 | 165.30 |
| Residual[a] | 44 | 326.79[a] | 153.04 |

[a]Error in Square and Residual were pooled for making tests of significance.

**($P < .01$).

TABLE XVI

ANALYSES OF VARIANCE[a] FOR NET $^{40}K$ COUNT, UNSHRUNK AND SHRUNK, UTILIZING THE CARRY-OVER EFFECTS NEGLIGIBLE MODEL

| Source of Variation | df | Unshrunk MS | Shrunk MS |
|---|---|---|---|
| Total | 107 | | |
| Square | 11 | 44837.2 | 35300.6 |
| Animals (row) | 24 | 21369.2** | 17881.2** |
| Period | 2 | 64784.9** | 13080.5** |
| Treatment | 2 | 18595.6** | 4339.4** |
| Error in Square[b] | 24 | 851.8 | 1012.8 |
| Residual | 44 | 843.9 | 479.9 |

[a] All variances times $10^{-2}$.

[b] Error in square and residual were pooled for all tests of significance.

** $P < .01$.

TABLE XVII

ANALYSIS OF VARIANCE FOR BLOOD SERUM POTASSIUM UTILIZING THE CARRY-OVER EFFECTS NEGLIGIBLE MODEL

| Source of Variation | df | MS |
|---|---|---|
| Total | 215 | |
| Group | 2 | 5410.85** |
| Square in Group | 9 | 2107.99** |
| Animals (row) | 24 | 2144.84** |
| Period | 2 | 7814.60** |
| Group x Period | 4 | 2072.19* |
| Square in Group x Period | 18 | 476.05 |
| Treatment | 2 | 1334.76 |
| Group x Treatment | 4 | 1135.75 |
| Square in Group x Treatment | 18 | 540.58 |
| Error in Square[b] | 24 | 690.73 |
| Sampling Error[a] | 108 | 340.99 |

[a]Calculated from duplicate analyses.

[b]Error in square, Square in Group x Treatment and Group x Treatment were pooled for test of significance of all terms above them.

*$P < .05$.

**$P < .01$.

TABLE XVIII

ANALYSIS OF VARIANCE FOR MUSCLE TISSUE POTASSIUM CONCENTRATIONS

| Source of Variation | df | MS |
|---|---|---|
| Total[a] | 127 | |
| Animals (whole plots) | 35 | 0.762 |
| Groups | 2 | 1.315 |
| Rations | 2 | 1.263 |
| Ration x Group | 4 | 0.240 |
| Error[b] A | 27 | 0.761 |
| Sub-plot | 92 | |
| Position | 1 | 0.181 |
| Position x Group | 2 | 0.314 |
| Position x Ration | 2 | 0.144 |
| Position x Ration x Group | 4 | 0.210 |
| Error[c] B | 19 | 0.295 |
| Sampling Error | 64 | 0.104 |

[a]Sixteen degrees of freedom were lost in total degrees of freedom and sub-plot error to missing observations.

[b]Error A and Ration x Group were pooled for testing the effects of ration.

[c]The interactions of position with group and ration were pooled for testing the effects of position.

# VITA

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Master of Science

Thesis: THE INFLUENCE OF DIFFERENT LEVELS OF DIETARY POTASSIUM ON WHOLE-BODY $^{40}K$ COUNT, BLOOD SERUM AND MUSCLE POTASSIUM CONCENTRATION IN STEERS

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