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IMPORTANCE OF DIFFERENT LEVELS OF POTASSIUM IN THE DIET ON WHOLE-BODY ^{40}K COUNT AND MUSCLE AND BLOOD POTASSIUM [1]

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Summary

THE influence of three levels (1.31, 1.03 and 0.29% K) of dietary potassium on ^{40}K count, blood serum and muscle tissue potassium was determined using 36 crossbred Angus-Hereford steers. A balanced 3 x 3 latin square experimental design was utilized which allowed estimates of carry-over effects of diets to be evaluated. Diets were fed for 2-week periods and appropriate samples and measurements were collected at 14-day intervals over a 60-day period. Measurements were made on steers unshrunk and after 24 hr. off feed and water.

Carry-over effect means were nonsignificant for each trait. The steers on the high potassium diet had 447.5 higher unshrunk ^{40}K counts per minute and 218 higher shrunk counts per minute than steers on the low potassium diet ($P<.01$). The steers on the high potassium diets had higher blood serum and muscle potassium concentrations than those on low potassium diets although these differences were not significant.

Animal to animal variation in potassium concentration was an important source of variation in this study. It appears tissue potassium concentrations are not constant between animals and that this variation could be a limiting factor to the precision of estimating lean in live animals by ^{40}K counting. These data indicate that the primary influence of dietary potassium to ^{40}K counting is the effect on potassium content of the gastrointestinal tract contents.

Introduction

Anderson (1959) first proposed ^{40}K content of live animals as a nondestructive index of lean. Subsequent reports of the relationship between whole-body potassium and fat-free lean have been somewhat variable. The exact cause for much of this variation has not been resolved, however, 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 source of potassium in the body.

Recent research with cattle at this station has shown correlations of 0.80 and 0.87 between ^{40}K content of live animals and pounds of fat-free lean (McLellan, 1970; Frahm, Walters and McLellan, 1971). However, in this work all animals were fed the same diet and handled as similarly as possible prior to being ^{40}K counted.

Information is needed concerning the importance of level of potassium in the diet on the precision of estimates of lean in live animals as determined by whole-body ^{40}K measurements. If dietary potassium is an important source of variation, estimates of lean in live animals would depend on the level of potassium in the diet fed prior to ^{40}K evaluation. This would indicate the necessity of developing equations which predict the pounds of fat-free lean in a live animal from the animal's ^{40}K count with animals fed a standard diet of known potassium content. Consequently, this standard diet would need to be fed to all animals in a group before comparisons among these animals could be made by ^{40}K measurements.

The purpose of this study was to investigate the importance of three levels of dietary potassium on whole-body ^{40}K count and blood serum and muscle potassium concentration in steers.

Materials and Methods

Three levels of dietary potassium were evaluated with 36 Angus-Hereford crossbred steers by randomly assigning each steer to a row of one of 12 3 x 3 latin squares. Columns of the squares were 2-week feeding periods and treatments were the three diets shown in table 1. Average potassium concentrations of the diets, as determined by atomic absorption

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TABLE 1. 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%.

spectrophotometry, were 1.31%, 1.03% and 0.29% for diets A, B and C, respectively.

Prior to being placed on treatment the steers were all placed on the same diet (a mixture of one-half diet A and one-half diet C) for a 2-week adjustment period. The steers were hand fed twice daily to maximum feed consumption. Diet B was prepared at each feeding by hand mixing 772 g of pre-weighed KCl to each 45.4 kg of diet C.

At the end of each of the four 2-week feeding periods (adjustment period and the three periods of the design) the steers were trucked to the Oklahoma State University Live Animal Evaluation Center (approximately 1.6 km) where all data were collected. The steers remained at the evaluation center for 2 days before being returned to the Oklahoma State University Beef Center and placed on the designated treatment of the next period. At the end of each experimental period the steers were weighed and ^{40}K counted unshrunk and again after being held off feed and water for 24 hours. Blood samples were also collected after 24 hr. shrink in each period.

The ^{40}K counter used was the Nuclear Electronic ^{40}K counter described by Frahm *et al.* (1971). The counting procedure was also as described by these authors.

Blood samples were taken by puncturing the jugular vein and collecting approximately 10 ml of blood in polyethylene centrifuge tubes. The serum was separated from the whole blood and analyzed for potassium.

In the third experimental period only, samples of muscle were taken from each steer and analyzed for potassium content. Two 5 g samples, one dorsal and one ventral, were surgically removed from the 10th rib area of the right *longissimus* muscle of each steer. Potassium analysis of feed, muscle and blood serum was determined in duplicate using a Perkin-Elmer Model 303 spectrophotometer equipped with a digital concentration readout.

A difficulty encountered with an experiment of this type is that the measurement of the direct effect of a treatment in any experimental period is confounded with any carry-over effect of the treatment in the preceding period. This difficulty was overcome by balancing the latin squares so that in any experimental period one-third of the steers received each diet. For instance, six of the steers received the treatment sequence ABC, six the sequence BCA and six the sequence CAB. With this arrangement, the three diets are equally represented in each experimental period. Six steers received each of the six possible sequences of treatments.

Means, analyses of variance and estimates of carry-over effects of diets from one period to the next were determined for all traits except muscle potassium concentration by methods described by Cochran, Autrey and Cannon (1941) and by H. L. 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 diet being received in that period, the diet received the previous period and experimental error. Utilizing this model, each trait was analyzed for the presence of carry-over effects. Carry-over effect mean squares for each treatment were small in relation to the error mean squares (table 2) indicating no evidence carry-over effects existed, thus the data were reanalyzed assuming carry-over effects from one period to the next to be negligible. This analysis simplified to the general analysis of a latin square experiment where the performance of any steer in any period is expressed as a function of the effects of the steer, the period, the diet received in that period and experimental error.

In the chemical analysis of blood serum potassium, all samples taken from a group of steers on any day were analyzed at the same time. The effect of groups was added to the model for analysis of these data as a measure of the technical variation (measurement variation attributable to technique of sampling and analysis) associated with the potassium analysis of blood serum. The steers within each treatment were assigned to groups at random so that four steers from each of treatments A, B and C were in each group. For

TABLE 2. MEAN SQUARES FOR CHARACTERISTICS UTILIZING CARRY-OVER AND NON CARRY-OVER MODEL

Source of variation	df[a]	Steer wt kg		Net ^{40}K Counts/min.		Blood serum K, ppm
		Unshrunk	Shrunk	Unshrunk	Shrunk	
		Carry-over effects present				
Group	.. (2)					5410.8
Square	11 (9)	3381.3	3873.2	4483718	3530057	2108.0
Row (Steers in square)	24	5193.7	4539.4	2136919	1788121	2144.8
Period	2	3583.5	2325.6	6478495	1308049	7814.6
Group X Period	.. (4)					2072.2
Square X Period	22 (18)	68.6	31.8	69670	44781	476.1
Adj. treatment direct eff.	2	447.0	518.3	1135901	143871	851.3
Carry-over Effect	2	40.2	21.4	11342	5726	133.0
Square X Treatment	22 (18)	65.8	31.1	99114	51218	540.6
Adj. Direct Eff. X Group	.. (4)					873.5
Carry-over Eff. X Group	.. (4)					1094.8
Error	22 (18)	56.2	35.2	91896	109962	662.9
		Carry-over effects negligible				
Group	.. (2)					5410.8**
Square	11 (9)	3381.3**	3873.2**	4483718**	3530057**	2108.0**
Row (Steers in square)	24	5193.7**	4599.4**	2136919**	1788121**	2144.8**
Period	2	4145.9**	2325.6**	6478495**	1308049**	7814.6**
Group X Period	.. (4)					2072.2*
Treatment	2	699.5**	697.2**	1859561**	433940**	1134.8
Group X Treatment	0 (4)					1135.8
Error	68 (60)	62.9	32.4	84671	66803	581.3

[a] df in parenthesis apply only to blood serum potassium.
* P<.05.
** P<.01.

all traits other than blood serum potassium it was felt the effect of groups was a random variable whose variation is included in that due to latin squares.

For all traits the interaction of squares with periods and treatments was small and non-significant and assumed to be nonexistent. In the second analysis, assuming carry-over effects negligible, these interaction sum of squares were pooled with error sum of squares to obtain an error mean square for tests of significance.

Muscle potassium was analyzed as a split-plot design where rations were the main-plot treatments and animals the main-plots. Subplots were the position of the *longissimus* muscle (dorsal or ventral) from which samples were removed.

In the analyses of weight and ^{40}K count, unshrunk and shrunk data were analyzed separately. All possible comparisons among treatment means were made for all traits by LSD.

Results and Discussion

The average unshrunk and shrunk steer weights and respective standard deviations at the end of period 1 (adjustment period) were 393.7 kg (SD=40.8) and 371.1 kg (SD=30.5). The average weight gain per steer per 2-week period for diets A, B and C measured from unshrunk weights was 4.2, 14.9 and 12.5 kg, respectively, and measured from shrunk weights was 3.1, 12.0 and 12.9 kg, respectively. Because of these differences it became necessary to adjust ^{40}K count treatment means for weight.

Potassium-40 count means for the steers on each treatment were adjusted for differences in treatment weight means by regression methods. The regression of ^{40}K count on weight was calculated for each treatment from both unshrunk and shrunk data. The differences between the regression coefficients were not significant. Thus the corrected sums of squares and cross products for each treatment were pooled to obtain an estimate of the relationship between ^{40}K count and weight. The pooling was done separately for unshrunk and shrunk data. The pooled coefficients were then used to adjust the mean unshrunk and shrunk net ^{40}K count for each treatment to the overall mean unshrunk and shrunk weight of 414.6 and 391.7 kg, respectively. The pooled unshrunk and shrunk regression coefficients of ^{40}K count on weight were 14.75 ± 1.92 and 12.41 ± 1.76 counts per minute (cpm) per kilogram, respectively.

The analyses of variance for the traits analyzed for carry-over and non carry-over

effects are presented in table 2. Carry-over effect mean squares were small and nonsignificant for all traits. All carry-over effect means except two (unshrunk net ^{40}K count for diet B and C) were smaller than the associated standard errors and no carry-over effect mean was significantly different from zero. It appears that the diet fed in any 2-week period did not affect the weight, net ^{40}K count or blood serum potassium concentration in the following period. This could be interpreted to mean that, within the range of dietary potassium levels studied, feeding a standard diet for a 2-week period prior to ^{40}K counting would allow ^{40}K comparisons to be made among cattle within a group free from the effects of previous diets these animals may have been receiving.

In the second analysis, ignoring carry-over effects, the effect of steers and period were significant ($P<.01$) for all traits. Since an equal number of animals received each treatment in each period, the effect of period on weight and net ^{40}K count can be interpreted to represent the growth of animals over time, plus other possible time effects which may have existed. Because of the method of handling all blood serum samples from any one period together for potassium analysis, the effect of periods on blood serum potassium cannot be interpreted to have any biological meaning. This source of variation coupled with the significant group mean square and significant group x period interaction indicates that measurement error was a large source of variation in potassium analysis. The standard error of duplicate analysis was 18.15 parts per million with a CV of 9.5%.

The effect of rows in the analysis of variance (table 2) was highly significant. This effect is difficult to interpret. It compares the average measurements of steers in rows of the squares. With respect to ^{40}K count, some of this variation is undoubtedly due to differences in steer weight, as shown by a significant effect of rows on 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. The significant effect of rows on blood serum potassium would indicate that blood serum potassium concentrations are not constant between animals. Potassium concentrations ranged from 133 to 306 ppm and the overall standard deviation calculated from the mean of duplicate analysis was 18.57 ppm with a CV of 9.7%. The standard deviation of 0.44 gm K/kg wet tissue for muscle potassium, calculated from the mean of the two samples extracted from each animal, also suggests there is considerable variation between animals in potassium concentration. This value is higher than that reported by Lohman, Ball and Norton (1970) of 0.14 gm K/kg, however, the CV of 15.5% is in agreement with that of 17.2% reported by Ward, Johnson and Tyler (1967). Some of this variation could be caused by differences in fat content of the muscle samples.

The effect of diets were significant ($P<.01$) for weight and ^{40}K count both unshrunk and shrunk. Those steers receiving the highest potassium diet (diet A) always had higher ^{40}K counts but gained less than those on the other diets. Thus, it was felt necessary to make all ^{40}K count treatment comparisons at a uniform weight and net ^{40}K count means were adjusted to the overall mean weights as described above. The effect of diet was nonsignificant for muscle or blood serum potassium.

Unshrunk and shrunk weight adjusted net ^{40}K count means and blood serum and muscle potassium concentration means for each treatment are presented in table 3. The mean unshrunk net ^{40}K count for steers on diet A was 146.6 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.

TABLE 3. TREATMENT MEANS AND STANDARD ERRORS FOR EACH TRAIT

Treatment	No.	^{40}K Count/Min.		Bl. Serum K	Muscle K[c]
		Unshrunk[a]	Shrunk[b]	ppm	g/kg
A	36	14105.4±48.5	13254.7±43.1	196.1±4.32	2.93±0.24
B	36	13958.9±48.5	13121.2±43.1	189.3±4.32	2.64±0.24
C	36	13657.9±48.5	13036.7±43.1	188.0±4.32	2.90±0.24
Avg	108	.....	.....	191.1±2.49	2.82±0.14

[a] All means are adjusted to a common weight of 415 kilograms.
[b] All means are adjusted to a common weight of 319.7 kilograms.
[c] Each mean is the average of 12 steers per treatment in period 3.

The mean ^{40}K cpm for steers on diet B was 301 cpm higher ($P<.01$) than the mean for steers on diet C. Differences between treatment means were not as large after 24 hr. shrink. The mean of steers receiving diet A was 133.5 cpm higher ($P<.05$) than the mean of steers receiving diet B and 218 cpm greater ($P<.01$) than the mean of steers receiving diet C. There was a nonsignificant difference of 84.5 cpm between means for diets B and C.

Measurements of ^{40}K for each diet were made on each steer. Means should make comparisons of the same steers at a uniform weight, and the average fat-free lean of 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 *et al.* (1971) developed with yearling Angus bulls weighing an average of 384.6 kilograms. The equation, $\hat{Y}=34.25+.00668$ (cpm), resulted in estimates of average fat-free lean of 121.5, 120.5 and 120.0 kg for steers on diets A, B and C, respectively, based on shrunk measurements. These differences are not large considering the effects of diet were highly significant. Differences would be greater based on unshrunk measurements; however, prediction equations utilizing unshrunk ^{40}K count are unavailable.

These data provide evidence that a fat-free lean prediction equation should be developed while cattle are receiving a standard diet of known potassium content. It also appears desirable to feed this diet to cattle for about two weeks prior to ^{40}K evaluation. This would give more confidence that the predictions and associated standard errors of estimates do apply to those cattle being evaluated. These results are in agreement with those reported by Lohman *et al.* (1966), who reported that steers fed a high roughage diet averaged 3% higher whole-body potassium than those fed a low roughage diet.

The effects of diet on blood serum and muscle potassium were not significant although there was a tendency for steers on high potassium diets to have higher blood serum and muscle potassium concentrations. Lohman and Norton (1968) also 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. Clark *et al.* (1970), however, reported a higher ($P<.05$) level of potassium in the fat-free dry matter of the total carcass when cattle received only corn silage for the entire feeding period compared to cattle fed a full feed of corn the last half of the feeding trial, 1.07% *vs.* 1.04%. In a second trial, however, no differences were found.

The position of muscle removal did not significantly affect potassium concentration. The mean for samples excised ventrally was 2.86 ± 0.87 g K/kg wet tissue as compared to a mean of 2.79 ± 0.87 g K/kg for samples excised dorsally. Measurement variation was also a large source of variation in the analysis of muscle potassium. The standard error of duplicate analysis was 0.322 g K/kg. The CV of 11.1% based on the mean of duplicates is in agreement with that of 9.6% reported by Lohman, Dieter and Norton (1970). These authors discussed the limitations of atomic absorption spectrophotometry to estimate potassium in lean.

For every measurement of potassium, the highest mean was associated with diet A, the diet with the highest potassium concentration and with the exception of muscle potassium, lowest means were associated with diet C, the low potassium diet. The simple correlation between muscle and blood serum potassium, calculated from the average concentrations of the 36 steers in the third experimental period (only period in which muscle samples were taken), was 0.85 ($P<.01$). Because diet significantly affected whole-body measurements of potassium but not blood serum or muscle potassium, these data provide evidence that the primary influence of dietary potassium is on the potassium concentration of the gastrointestinal tract contents and not on the potassium concentration of intracellular fluids.

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