MINERAL STUDIES IN ALL BARLEY RATIONS FED TO RUMINANTS

By

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INTRODUCTION

Previous investigations at the Oklahoma Station with steam rolled barley supplemented with soybean meal, calcium and vitamin A indicated that the addition of dehydrated alfalfa meal, molasses or a trace mineral mixture containing cobalt, copper, iron, zinc, manganese and iodine improved gains whereas cobalt or cobalt, copper and iron gave a lesser response. Later results by Renbarger (1964) demonstrated that supplemental cobalt, zinc and iron improved average daily gain and feed efficiency as much as a cobalt, zinc, iron, copper, manganese and iodine combination, or a combination of dehydrated alfalfa meal and molasses, two rich sources of trace minerals. These results combined with those of five earlier trials, indicate that barley is probably deficient in cobalt, marginal in zinc and adequate in iron, copper, manganese and iodine. Cattle experiments were conducted to determine the effects of supplementation of all barley fattening rations with cobalt and zinc alone and in combination. Effects of supplemental zinc in an all barley fattening ration for sheep fed different levels of calcium were also determined.

LITERATURE REVIEW

Barley, which ranks fourth in importance as a grain crop in the United States, is the most widely cultivated of the cereals throughout the world (Morrison, 1956). Recent investigations have demonstrated that rolled barley properly supplemented with protein, minerals and vitamin A can constitute the entire feedlot ration (Embry et al., 1965; Guerin et al., 1959; Thomas and Myers, 1961). Whetzal and Embry (1962 and 1963) concluded that it was not necessary to provide additional protein in an all-barley ration where the barley was of good quality and when supplemented with minerals and vitamin A. Oltjen et al. (1959) have shown that the response to trace minerals by fattening cattle depends on the grain being fed; trace mineral additions to milo rations were without effect on gain or feed efficiency, but that similar fortification of fattening rations containing corn produced a highly significant increase in rate of gain.

There were some questions regarding the need for buffering activity since Matrone et al. (1959a, b) reported that satisfactory lamb growth could be obtained by the addition of 11 percent sodium and potassium bicarbonate to purified diets; however, this level of cation supplementation proved to be too high for calves fed an all concentrate diet (Wise et al., 1961). Nicholson et al. (1963) reported an increase in feed intake but no significant change in average daily gain, when three percent sodium bicarbonate was added to a ration based on rolled barley.

Wise et al. (1965) reported that beef cattle may be finished successfully on all concentrate rations and that the advisability of using rumen buffering agents is questionable. Because the roughage which normally is a good source of trace minerals has been removed from the all concentrate ration, more critical data is needed on the advantages of adding trace minerals to this type of ration.

Previous experimental evidence and chemical analysis of the barley for trace mineral content has shown that barley is deficient in cobalt and zinc; therefore, this discussion will be limited to these two minerals.

Cobalt

Recently cobalt has been added to the list of mineral elements that are essential for animal growth and health. Australian workers, investigating the causes of a certain peculiar wasting disease known by different names throughout the world, found the first conclusive evidence that cobalt was a dietary essential (Underwood and Filmer, 1935 and Marston, 1935). Previous to this it was thought that the disease could be alleviated by massive doses of limonite, an iron compound (Filmer, 1933). Its potency was later found to be due to the cobalt which it contained.

Cobalt has been shown to be important to the ruminant since the demonstration of its presence in vitamin B_{12}. Smith, (1948) and Rickes et al., (1948) suggested that this vitamin might be the functional form of cobalt in ruminant metabolism. Cobalt as a constituent of vitamin B_{12} makes up about four percent of the weight of the vitamin. Several lines of evidence earlier indicated that cobalt functioned in some manner associated with the ruminant stomach. There were early reports

indicating that non-ruminants, horses and rabbits behaved normally on pastures that were cobalt deficient for cattle and sheep. Laboratory animals, rats (Houk et al., 1946) and rabbits and guinea pigs (Thompson and Ellis, 1947), failed to develop a cobalt deficiency even though fed diets containing much less cobalt than cobalt deficient pastures. Further emphasis was put on the importance of cobalt as a dietary essential for ruminants with the discovery that the pathway of propionic acid metabolism is via succinic acid through an intermediate, methylmalonic acid (Flavin et al., 1957; Beck et al., 1957; Barker et al., 1958 and Lenhyel et al., 1960). It has been shown that the coenzyme form of vitamin B_{12} is required for the metabolism of methylmalonate to succinate and leads to some understanding of why ruminants have such high vitamin B_{12} requirements (Tillman, 1961). Thus cobalt, through vitamin B_{12}, is required for energy reactions typical of ruminants only. Some suggestion has been made that cobalt may be involved in hemopoieses but (Cartwright, 1955) suggests that the only physiological hemopoietic function for cobalt is as a constituent of vitamin B_{12}.

Marston and Lee (1949) have stated that cobalt exerts its influence on the ruminant primarily either in the lumen of the alimentary canal or when passing through its wall. An important function of cobalt in the ruminant might thus concern the nature and activity of the mixed populations of microorganisms which normally flourish within the paunch and which play a fundamentally important part in the nutrition of the ruminant. A study by Gall et al. (1949) clearly demonstrated that in cobalt deficient lambs the rumen bacteria were reduced from a control level of 54 billion per gram of dry material to 30 billion; the principal types of bacteria were in lesser amounts than in comparable cobalt-fed

lambs and mixed cultures of bacteria obtained from cobalt deficient lambs failed to grow beyond dilutions of 10^{-9}; whereas, cultures from cobalt supplemented lambs grew even at dilutions of 10^{-11}. It is evident that a shortage of cobalt significantly affects rumen bacteria--their total numbers, types present and cultural qualities.

Several workers have shown the value of supplemental cobalt in increasing gains. Becker et al. (1951), investigating metabolism of cobalt in lambs, found that the addition of one mg. of cobalt per day to a deficient ration improved gains in ten days and that these animals were more efficient in digesting the ether extract and NFE fractions of the rations. Chapman et al. (1964) showed that Brahman-Angus heifers when supplemented with eight mg. cobalt per head per day gained an average of 36 pounds more over a 279 day period than those not supplemented. In another report Chapman and Kidder (1964) found that cobalt supplementation decreased weight loss in Hereford cows and improved gains of Brahman steers. Bentley et al. (1954), feeding a ration composed of ground ear corn and timothy hay, found that cobalt significantly increased average daily gain and also increased ground ear corn consumption 32.5 percent. Thomas et al. (1964), feeding cattle a combination of trace minerals in a ration consisting of 80 percent steam rolled barley, found that trace minerals improved gains; cobalt was fed at a level of ten mg. per head per day.

Cobalt deficiency has been observed in ruminants, particularly cattle and sheep, in many areas of the world. Marston (1952) stated that the deficiency is characterized in young animals by a seriously retarded growth rate, unthriftiness and anemia after only a short time on cobalt deficient pasture. In adult animals he observed loss of

appetite and condition, anemia; followed by emaciation and lethargy. The blood volume and concentration of protein in plasma are materially reduced and the oxygen carrying capacity falls to 30 percent of normal or lower. Underwood (1962) reported that the body of a severly affected animal presents, on autopsy, a picture of extreme emaciation often with a total absence of body fat. The liver is fatty and the spleen hemosiderized. The red blood cell numbers and blood hemoglobin levels are always low in affected animals in the field. Maynard and Loosli (1962) stated that there also appears to be a lowering of the vitamin B_{12} content of the blood.

The only certain diagnosis of cobalt deficiency rests upon the response of the animal to cobalt feeding. Gall (1949) showed that cobalt deficient sheep had no response to injected cobalt and that sheep fed cobalt at a level of one mg. per head per day recovered. This would indicate that microbial activity in the rumen is essential. This is true as rumen microorganisms have been shown to concentrate cobalt and synthesize vitamin B_{12}. The possibility also exists that the bacterial metabolic processes during which vitamin B_{12} is synthesized are important for the maintenance of good ruminal activity (Bentley, 1954). To be effective the supplement of cobalt must be administered frequently (Marston, 1952). Cartwright (1955) stated that synthesis of vitamin B_{12} in the rumen cannot take place in the absence of cobalt and that parenteral administration of about five micrograms of vitamin B_{12} per day was effective in alleviating deficiency symptoms. Sherman et al. (1960) showed that a cobalt oxide pellet weighing 20 grams corrected cobalt deficiency and concentration of vitamin B_{12} in liver held its consistancy for eight months. Administration of the heavy pellet

is thought to be advantageous due to its slow release of the mineral over a long period of time. Ruminants appear to utilize cobalt solely as an integral part of vitamin B_{12} and are completely dependent upon the activities of microorganisms within the rumen for their supply of this vitamin (White et al., 1964 and Underwood, 1959). Cobalt deficiency in ruminants is therefore essentially a vitamin B_{12} deficiency.

Zinc

The zinc content of the body is only approximately three mg. percent. The highest concentrations are found in the epidermal tissues, such as skin, hair and wool, but traces occur in the bones, muscles, blood and various organs. Early work showed that zinc was an essential mineral for plants and that many areas of zinc-deficient soils were revealed throughout the world. Until the recent evidence (Legg and Sears, 1960) indicated that there may be some adverse effects to ruminants grazing on zinc deficient pastures zinc deficiency was not considered a problem in the nutrition of ruminants.

One of the important functions of zinc is as a constituent of the enzyme carbonic anhydrase, which is found in the red blood cells, the pancreas and the mucosal lining of the stomach. Carbonic anhydrase is involved in carbon dioxide elimination and may be concerned with HCl formation in the border cells of the stomach. Zinc is also a constituent of crystalline insulin isolated from the animal pancreas and may play a role in carbohydrate metabolism. Other enzymes including pancreatic carboxypeptidase, alcohol dehydrogenase, glutamic dehydrogenase and lactic dehydrogenase contain zinc as a structural and functional component (Tillman, 1961). Chang et al. (1961) showed that it is also a constituent of DPN and/or TPN containing enzymes with which it functions.

Evidence presented earlier shows that zinc is a dietary essential for ruminants. Ott et al. (1964), feeding zinc in a deficient ration for young lambs, found that zinc increased both average daily gain and feed consumption. He also found that lambs fed zinc and phytic acid in combination were significantly more efficient in feed conversion, however, zinc supplementation improved feed efficiency more than phytic acid alone. Smith et al. (1964a) showed that the addition of one or two grams of zinc per head per day to fattening cattle fed a corn, soybean meal ration significantly improved growth and provided feed savings of three percent.

Zinc deficiency was first described in swine (Kerncamp and Ferrin, 1953) as a dermatitis designated as parakeratosis. The demonstration by Tucker and Salmon (1955) that zinc supplementation cures and prevents parakeratosis in swine has been amplified and confirmed by many workers (Luecke et al., 1956; Lewis et al., 1956; Hoekstra et al., 1956; Stevenson and Earle, 1956; Lewis et al., 1957a; Lewis et al., 1957b and Conrad and Beeson, 1957). From these investigations it is apparent that (a) signs of zinc deficiency, namely, sub-normal growth, inappetence, poor feed efficiency and parakeratotic skin lesions, can arise in growing pigs fed rations containing 30-40 ppm zinc or less, (b) these symptoms can be completely overcome or prevented by zinc supplements at the rate of 40-100 ppm zinc and partially overcome by smaller supplements, and (c) in most cases the severity of the symptoms increases as the calcium content of the diet increases (Underwood, 1959). Analyses of foodstuffs for zinc are not adequate to indicate the value of the feeds as dietary sources of this element. For example soybean, sesame and peanut meals all contain significant quantities of zinc, yet this is unavailable when ingested (White et al., 1964).

Only as recently as 1960 did Miller and Miller (1960) first describe the actual zinc deficiency symptoms in ruminants. Miller and Miller (1962) described these deficiency symptoms in dairy calves: zinc content and carbonic anhydrase activity of the blood was significantly lower; skin of the deficient calves exhibited parakeratosis; the papillae of the rumen showed excessive over growth, a moderate keratin formation and retention of nuclei. They found that the addition of 260 ppm zinc increased the weekly feed intake and rate of gain. Further deficiency symptoms in sheep were described by Ott et al. (1964) as being: anorexia, depraved appetite (eating wool), decreased growth, reduced feed efficiency, loose wool, swollen hocks, red, wrinkled skin and open lesions of the skin above the hoof and around the eyes; tissue changes, including decreased tissue zinc and parakeratotic lesions of the skin. The deficiency was completely alleviated by 100 mg. zinc per kg. of ration.

Some work has been done to attempt to elucidate if a zinc deficiency is aggravated in ruminants by the addition of calcium. Wise et al. (1963) reported that ruminants can tolerate wide calcium-phosphorus ratios, up to 7 or 8:1, without serious effects. Smith et al. (1964b) supplemented three levels of zinc, 0.5 gm., 1.0 gm. and 1.5 gm., per head per day with two levels of calcium, 0.25 percent and 0.5 percent. Results indicated that neither zinc nor calcium had an effect on growth performance and apparently there was no interaction; however, zinc supplementation increased serum and hair zinc while calcium decreased both. Fontenot et al. (1964) fed calcium-phosphorus ratios of 1:1, 2:1, 4:1 and 8:1 with and without the addition of 100 mg. of supplemental zinc per head per day and observed that (1) gain

and feed efficiency were depressed at ratios of 4:1 and 8:1 without supplemental zinc, and (2) that with supplemental zinc, calcium had no effect on gain and feed efficiency.

EXPERIMENTAL PROCEDURE

Cattle

Trial I

Sixty grade Hereford steers averaging about 15 months of age and weighing an average of 328 kilograms were allotted by weight and grade into four similar groups of 15 per group. Treatments were assigned at random to each group and the steers were divided into groups of five and randomly allotted to pens providing three replications per treatment. Experimental design and ration treatments are given in Table I. The animals were self-fed in pens with dirt floors. No bedding was provided and the animals had free access to water and salt. Fifty percent cottonseed hulls were added to all rations initially, but were gradually withdrawn at weekly intervals until all hulls were removed by the end of the fifth week. The rations contained 11.5 percent crude protein, 0.40 percent calcium and 0.40 percent phosphorus. No other minerals were available to the cattle except those supplied in the ration. Details of ration composition are shown in Table II. A summary of the chemical analyses (Sandell, 1959), of the barleys used, for cobalt and zinc content are shown in Table III.

Initial and final weights were preceded by a 16-hour shrink away from feed and water. Steers were weighed at 28 day intervals throughout the course of the trial. The experiment was initiated on May 5, 1964 and continued for 136 days.

TABLE I

EXPERIMENTAL DESIGN - TRIAL I

	Treatment			
	A Basal	B Basal & 3 mg. Cobalt[a]	C Basal & 300 mg. Zinc[a]	D Basal & 3 mg. Cobalt[a] 300 mg. Zinc
Lots[b]	2, 6, 10	4, 8, 12	1, 5, 9	3, 7, 11

[a]Supplemental cobalt and zinc as mg./hd./day.
[b]Five animals per lot.

TABLE II

COMPOSITION OF THE BASAL RATION - TRIAL I

Supplement[a]	1.6 lb./head/day
Steam rolled barley	ad libitum

[a]Composition: Soybean meal - 93.6 percent, calcium carbonate - 6.2 percent, 1 gm. Quadrex per lb., potency 20,000 I.U./gm.

TABLE III

COBALT AND ZINC CONTENT OF BARLEY

Year	Parts Per Million (ppm)	
	Cobalt	Zinc
1964	< 0.02	33
1965	< 0.03	29

Response criteria were rate of gain, feed consumption, feed efficiency; slaughter data, which included dressing percentage, carcass grade, marbling score, fat thickness and rib eye area. Other criteria were volatile fatty acid (VFA) production, hematocrit and hemoglobin. Rumen fluid samples were obtained using the technique of Raun and Burroughs (1962), on one day of the last week of the trial, for VFA and pH determinations. Immediately after collection pH determinations were made, microbial action in the fluid was stopped by the addition of mercuric chloride and the samples were stored to be prepared later for VFA determination using the method of Erwin et al. (1961). An Aerograph Hy-Fi Model A-600-B gas chromatograph using nitrogen as the carrier gas was utilized for separation and determination of each acid. Blood samples were also collected and the final day of the trial and were analyzed for percentage packed cells using a micro-hematocrit and gram percentage hemoglobin using the cyanomethemoglobin method of Cannon (1958).

Trial II

Thirty-two Angus and 28 Angus-Hereford crossbred yearling steers weighing an average of 296 kilograms were allotted by weight and grade into 12 lots. There were five animals per lot and the experimental design was a randomized block with three blocks by breed and four treatments in each block providing three replications per treatment. Experimental design and ration treatments are given in Table IV. Details of ration composition are shown in Table V. The purpose of this trial was to determine the effects of increasing cobalt level alone and in combination with zinc.

TABLE IV

EXPERIMENTAL DESIGN - TRIAL II

Block	Treatment A 3 mg. Cobalt[a]	B 6 mg. Cobalt[a]	C 3 mg. Cobalt[a] & 300 mg. Zinc	D 6 mg. Cobalt[a] & 300 mg. Zinc
Angus lots[b]	11	10	4	3
Crossbred lots	6	9	5	7
Mixed lots[c]	1	2	8	12

[a]Supplemental cobalt and zinc as mg./head/day.
[b]Five animals per lot.
[c]Three Angus and two crossbreds per lot.

TABLE V

COMPOSITION OF THE RATIONS - TRIAL II

Supplement[a]	0.6 lb./head/day
Steam rolled barley	ad libitum

[a]Percentage composition:
Ration A - $CoCO_3$, 0.0022; $CaCO_3$, 32.14; Quadrex (20,000 I.U./gm.), 0.55; soybean meal, 67.31.
Ration B - $CoCO_3$, 0.0044; $CaCO_3$, 32.14; Quadrex, 0.55; soybean meal, 67.31.
Ration C - $CoCO_3$, 0.0022; $CaCO_3$, 32.14; $ZnSO_4 \cdot 7\ H_2O$, 0.48; Quadrex, 0.55; soybean meal, 66.83.
Ration D - $CoCO_3$, 0.0044; $CaCO_3$, 32.14; $ZnSO_4 \cdot 7\ H_2O$, 0.48; Quadrex, 0.55; soybean meal, 66.83.

The steers were placed on feed May 6, 1965 and the trial continued for 130 days. All other details of experimental procedure were identical to those outlined in trial I.

Sheep

Trial III

Twenty-four crossbred lambs, 16 ewes and eight wethers, averaging 29.5 kilograms were randomly allotted, within sex, to each of four treatments providing six animals per treatment. The lambs were individually fed indoors on slatted floors and were fed twice a day to maximum consumption with water being supplied ad libitum. Initially, 50 percent cottonseed hulls were added to the rations and gradually withdrawn until the animals were on full feed at the end of one week. Ration compositions are shown in Table VI. Each ration was supplemented with a trace mineral premix at a level of 0.5 percent. The composition of the premixes is shown in Table VII. The purpose of this trial was to determine the effects of adding zinc in two different calcium-phosphorus ratios.

The lambs were placed on feed March 13, 1965 and the trial continued for 56 days. Weights, without shrinking, were taken at 14-day intervals during the trial, but initial and final weights were preceded by a 16-hour shrink away from feed and water.

Response criteria were rate of gain, feed efficiency and feed consumption. Blood samples were collected on the final day of the trial and were subjected to analyses for hematocrit and gram percentage hemoglobin.

TABLE VI

RATION COMPOSITIONS - TRIAL III

	Percentage of Ration			
	Ration A	Ration B	Ration C	Ration D
Steam rolled barley	96.7	96.7	95.5	95.5
Soybean meal	1.4	1.4	1.6	1.6
Calcium carbonate	0.9	0.9	1.9	1.9
Trace mineral premix	0.5[a]	0.5[b]	0.5[a]	0.5[b]
Salt	0.5	0.5	0.5	0.5
Vitamin A[c]	Added to supply 1500 I.U./lb. of ration			
Percentages of:				
Crude protein	11.58	11.55	11.55	11.53
Calcium	.41	.41	.79	.79
Phosphorus	.40	.40	.40	.40
Calcium:phosphorus ratio	1:1	1:1	2:1	2:1

[a]Trace mineral premix without zinc.

[b]Trace mineral premix with supplemental zinc to supply 100 mg./head/day.

[c]Quadrex 20,000 I.U./gm.

TABLE VII

COMPOSITION OF TRACE MINERAL PREMIXES

Mineral Carrier	PPM in Total Ration	Percentage in Premix
Premix Without Zinc		
$CoCO_3$	0.3	0.01210
KI	0.3	0.00786
$CuCO_3$	5.0	0.19400
$MnSO_4 \cdot H_2O$	15.0	0.92300
$FeSO_4$	50.0	2.72000
	Soybean meal	96.14300
Premix With Zinc		
Same as above		3.85700
$ZnSO_4 \cdot 7\ H_2O$	100.0	8.79000
	Soybean meal	86.35300

Trial IV

Thirty crossbred wether lambs with an average weight of 33 kilograms were randomly allotted to six groups of five lambs per treatment. Treatments in this 70-day growth trial consisted of three different calcium-phosphorus ratios, with and without the addition of zinc in a factorial arrangement of treatments. Details of ration composition are shown in Table VIII. Trace mineral premixes are as outlined in Table VII. The ration was modified by the addition of two percent molasses to function as a carrier for the mineral premix and to increase palatability. The lambs were provided with feed and water free choice. All other experimental procedure was as outlined in trial III.

TABLE VIII

RATION COMPOSITIONS - TRIAL IV

Ingredient	Percentage of Ration		
	Rations A & D	Rations B & E	Rations C & F
Steam rolled barley	94.55	93.30	90.70
Soybean meal	1.60	1.85	2.50
Calcium carbonate	0.85	1.85	3.80
Trace mineral premix[a]	0.50	0.50	0.50
Molasses	2.00	2.00	2.00
Salt	0.50	0.50	0.50
Vitamin A[b]	Added to supply 1500 I.U./lb. of ration		
Percentages of:			
Crude protein	11.50	11.49	11.53
Calcium	.39	.77	1.52
Phosphorus	.39	.39	.38
Calcium:phosphorus ratio	1:1	2:1	4:1

[a]Premix for rations A, B, & C contains no supplemental zinc, premix for rations D, E, & F contains supplemental zinc to supply 100 mg./head/day.

[b]Quadrex 20,000 I.U./gm.

RESULTS

Cattle

Trial I

Results of this trial are shown in Table IX. Supplemental cobalt alone or in combination with zinc apparently increased gains and feed consumption but these differences only approached significance ($P < .10$). Zinc supplementation did not affect gains or feed consumption. Both cobalt and zinc supplementation tended to reduce feed efficiency but these differences were not significant ($P > .05$); the combination of cobalt and zinc had no effect upon response criteria.

Supplemental cobalt alone improved carcass grade ($P < .01$), rib eye area ($P < .01$) and marbling score ($P < .05$), but supplemental zinc did not affect ($P > .05$) any of these response criteria; the combination of cobalt and zinc also improved these criteria and in the case of rib eye area, differences between cobalt alone and the combination were significant ($P < .05$). Neither dressing percentage nor fat thickness was affected by any treatment.

Supplemental zinc lowered ($P < .05$) ruminal acetate, propionate and total VFA levels while cobalt had no ($P > .05$) effect. None of the treatments affected ($P > .05$) acetate/propionate ratio, ruminal fluid butyrate or hematocrit.

TABLE IX

EXPERIMENTAL RESULTS - TRIAL I

Item	Treatments				Standard Error of Treatment Means ($S_{\bar{x}}$)
	Basal	Basal & 3 mg. Cobalt	Basal & 300 mg. Zinc	Basal & 3 mg. Cobalt 300 mg. Zinc	
No. of steers	15	15	15	15	
Av. weights, kg.					
Initial	326	327	329	330	
Final	461	469	465	476	
Av. daily gain, kg.	.99	1.04	.99	1.07	.01
Av. daily feed intake, kg.	8.6	9.4	9.2	9.1	.04
Feed/100 kg. gain	868	906	920	857	2.50
Carcass grade[a]	8.9	9.5**	8.9	9.5**	.07
Marbling score[b]	11.6	12.8*	11.9	12.6*	.15
Dressing percentage	60.7	60.2	60.6	60.8	.06
Rib eye area, $cm.^2$	70.2	71.0**	69.8	73.9**	.33
Fat thickness, cm.	2.4	2.3	2.4	2.4	.05
Volatile fatty acids[c]					
C_2	55.5	68.7	45.7*	44.7*	3.01
C_3	46.3	60.4	38.7*	32.3*	3.40
C_4	11.1	9.0	7.1	7.1	.95
Total	113.2	138.1	91.4*	84.1*	6.61
C_2/C_3	1.27	1.25	1.24	1.44	.17

[a]8 = av. good; 9 = high good; 10 = low choice.
[b]11 = slight; 12 = slight +; 13 = small -.
[c]Millimoles/liter of rumen fluid.

*P < .05.
**P < .01.

Trial II

Results of this trial are shown in Table X. Differences between treatments were small and insignificant ($P>.05$) except for the depression ($P<.05$) in marbling score when cattle were fed six mg. of cobalt and 300 mg. of zinc daily; however, there appeared to be a trend toward improvement in gains, carcass grade, marbling score, dressing percentage and fat thickness when the low level of cobalt plus zinc were fed, with some depression noted when cobalt level was increased. Treatment had no effect on VFA production or blood values.

Discussion

These results are discussed in connection with the analyses of the feeds, which are shown in Table III. Cobalt requirements of beef cattle are 0.06 to 0.10 ppm (NRC, 1964) while that of zinc is not well defined. Smith et al. (1964a) found that 100 ppm of supplemental zinc increased gains of beef cattle; however, in more recent work (Smith et al., 1964b) a level of 20 ppm zinc met their requirement. As the barley contained about 0.02 ppm cobalt and from 29 to 33 ppm zinc it is apparent that cobalt was a limiting nutrient while zinc is questionable. Since the calcium levels of the rations (0.35 percent to 0.40 percent) were not excessive in these trials, levels of zinc in barleys used were perhaps adequate or nearly adequate which could lend explanation to a somewhat variable response obtained from supplemental zinc. The results of the two trials support the idea that cobalt was limiting while indicating that additional zinc was not needed.

TABLE X

EXPERIMENTAL RESULTS - TRIAL II

Item	Treatments				Standard Error of Treatment Means ($S_{\bar{x}}$)
	3 mg. Cobalt	6 mg. Cobalt	3 mg. Cobalt 300 mg. Zinc	6 mg. Cobalt 300 mg. Zinc	
No. of steers	15	15	15	15	
Av. weights, kg.					
Initial	293	297	295	298	
Final	431	437	437	433	
Av. daily gain, kg.	1.05	1.08	1.09	1.04	.02
Av. daily feed intake, kg.	9.1	9.5	9.3	8.7	.09
Feed/100 kg. gain	862	883	850	841	2.70
Carcass grade[a]	10.1	10.1	10.5	9.9	.13
Marbling score[b]	15.1	15.6	16.1	14.9*	.38
Dressing percentage	61.1	61.6	62.1	60.4	.23
Rib eye area, $cm.^2$	70.1	73.3	71.1	69.2	.12
Fat thickness, cm.	1.50	1.53	1.63	1.56	.04
Volatile fatty acids[c]					
C_2	43.2	44.2	37.0	43.4	1.50
C_3	35.1	36.1	33.1	31.6	1.72
C_4	7.5	6.9	5.0	11.0	.77
Total	85.9	87.2	75.0	86.0	3.20
C_2/C_3	1.23	1.25	1.12	1.50	.08

[a] 9 = high good; 10 = low choice; 11 = av. choice.

[b] 14 = small; 15 = small +; 16 = modest -.

[c] Millimoles/liter of rumen fluid.

*P < .05.

Dietary cobalt can be used to synthesize vitamin B_{12} in the rumen (White et al., 1964 and Underwood, 1959). As vitamin B_{12} is involved as an important cofactor in the utilization of propionic acid (Tillman, 1961) cobalt supplementation in rations deficient in this element would be expected to improve appetite and gains (Becker and Smith, 1951; Bentley et al., 1954; Thomas et al., 1964; and Chapman and Kidder, 1964) with no improvement in feed efficiency (Stewart, 1953); results of trial I, thus support these observations. In the second trial the increased dietary level of cobalt gave no further increase. Actually, the requirement of the steers for cobalt should have been met by the addition of one mg. per day, thus even the lowest level supplied more than three times as much as needed and no further increase should be expected. The highest level of cobalt was well below the toxic level (Underwood, 1962) of this element.

The effect of cobalt supplementation upon carcass traits must also be considered in connection with the effect of a deficiency of vitamin B_{12} on total energy intake and the utilization of propionic acid. As cobalt increased energy consumption of animals fed a fattening ration, it would be logical to expect carcass traits to be improved. As propionate can be used as the carbon skeleton for amino acid synthesis, it is not surprising that rib eye area was increased by additional cobalt.

Founder, liver abcesses and abnormal rumens are often associated with high concentrate rations. In these trials, due to the fact steers were carefully and slowly brought on to full feed, no cases of founder were noted. Of 120 head slaughtered only one abcessed liver was noted

and approximately 50 percent of the rumens examined had varying degrees and types of abnormalities.

Sheep

Trial III

Results of this trial are shown in Table XI. Although not significant, the addition of zinc apparently improved gains, feed efficiency and feed consumption while decreasing ($P < .01$) the hematocrit. There was an interaction ($P < .05$) between the calcium:phosphorus ratio and zinc on hematocrit: the addition of zinc increased the hematocrit when a 1:1 ratio was fed, but caused a decrease when the ratio was increased to 2:1. Hemoglobin was apparently decreased by the addition of zinc, but the differences were not significant.

Trial IV

Results of this trial are shown in Table XII. Zinc additions increased ($P < .01$) gains and decreased the hematocrit ($P < .05$) and hemoglobin level ($P < .025$). Also feed consumption and feed efficiencies apparently were improved, but the differences were not significant.

Discussion

The analyses of barley (Table III) indicated that the samples used in these trials contained about 30 ppm, a level considered by many to be adequate (Smith, 1964b). In non-ruminants, the requirement for zinc is increased (Lewis, 1957a) when the level of ration calcium is increased. The results of the two trials in the present experiment

TABLE XI

EXPERIMENTAL RESULTS - TRIAL III

Item	Treatment				Standard Error of Treatment Means ($S_{\bar{x}}$)
	1:1[a]	2:1[a]	1:1[a] & 100 mg. Zinc	2:1[a] & 100 mg. Zinc	
No. of sheep	6	6	6	6	
Av. weights, kg.					
Initial	34	37	35	36	
Final	38	41	40	43	
Av. daily gain, kg.	.18	.17	.20	.21	.01
Feed cons./day, kg.	1.1	1.2	1.2	1.3	.09
Feed/gain, kg.	6.6	7.7	6.0	6.2	.16
Hematocrit	32.3	37.9	33.2**	30.9**	.51
Hemoglobin, gm. %	13.7	13.9	12.7	12.7	.38

[a]Calcium/phosphorus ratio.
**P< .01.

TABLE XII

EXPERIMENTAL RESULTS - TRIAL IV

Item	Treatments						Standard Error of Treatment Means ($S_{\bar{x}}$)
	1:1[a]	2:1[a]	4:1[a]	1:1[a] & 100 mg. Zinc	2:1[a] & 100 mg. Zinc	4:1[a] & 100 mg. Zinc	
No. of sheep	5	5	5	5	5	5	
Av. weights, kg.							
Initial	33	33	34	33	31	33	
Final	41	41	39	42	41	43	
Av. daily gain, kg.	.12	.11	.10	.13**	.14**	.14**	.01
Feed cons./day, kg.	.84	.96	.84	.95	.99	1.01	.08
Feed/gain, kg.	7.4	8.8	10.2	8.3	7.8	7.5	.18
Hematocrit	37.8	35.2	41.0	35.9*	33.9*	33.9*	.76
Hemoglobin, gm. %	13.4	12.7	14.0	13.2*	12.1*	12.2*	.18

[a]Calcium/phosphorus ratios.
*$P < .05$.
**$P < .01$.

indicate that calcium level exerts an influence on the zinc requirement of ruminants: increased calcium levels in both trials decreased feed consumption, gains and feed efficiency, but zinc additions improved these response criteria at all calcium:phosphorus ratios. Also, the effect of the added zinc appeared to increase as the ratio widened. These results tend to agree with those of Fontenot (1964).

No explanation is offered for the significant interaction noted between calcium:phosphorus ratio and zinc level on the hematocrit in trial III; however, no interaction was found in trial IV, thus it may have been a chance occurrence. Additional zinc decreased the hematocrit and hemoglobin levels in both trials and the effect was increased with increasing calcium level. No explanation for these observations are offered except to point out that excess zinc interferes (Underwood, 1962) with the utilization of copper, iron, phosphorus and sulfur. The role of copper and iron in hematopoiesis is well known and needs no further discussion here. Sulfur and phosphorus, of course, would be involved in hematopoiesis only in an indirect manner and would have a bearing only under certain conditions. Since calcium interferes directly with zinc metabolism, (Cabell and Earle, 1964) one would expect that the effect of zinc on hematocrit and hemoglobin with increasing calcium levels would have been opposite of that observed. It is difficult to explain these results on the basis of calcium interference on other minerals. Excess calcium only interferes with the utilization of phosphorus, iodine, magnesium and manganese in addition to zinc, and none of these minerals are directly involved in hematopoiesis. Thus, the reasons why additional zinc is more effacious in decreasing the

hematocrit and hemoglobin levels when the level of calcium is high remains obscure and more research in this area is indicated.

SUMMARY

Two growth trials, involving 120 steers were conducted to determine the separate and combined effects of cobalt and zinc supplementation of all barley fattening rations on performance, carcass traits, VFA production and blood values. In trial I the addition of three mg. of cobalt per head per day tended to improve weight gain and feed consumption, and significantly improved carcass grade, marbling score and rib eye area. The addition of 300 mg. of zinc per head per day had no effect on performance or carcass traits; however, a significant depression in rumen fluid acetate, propionate and total VFA production was noted. The addition of three mg. of cobalt in combination with 300 mg. zinc significantly increased rib eye area over cobalt alone.

In trial II addition of six mg. of cobalt per head per day had no advantage over three mg. of cobalt. When six mg. of cobalt were fed in combination with 300 mg. zinc a significant depression in marbling score occurred. There appeared to be a trend toward improvement in gains, carcass grade, marbling score, dressing percentage and fat thickness when the low level of cobalt plus zinc were fed. Treatment had no effect on VFA production or blood values.

Also, two growth trials, involving 54 lambs were conducted to determine the effects of zinc supplementation upon calcium level supplied in all barley fattening rations. In trial III the addition of 100 mg. of zinc per head per day to rations containing 1:1 and 2:1 calcium:

phosphorus ratios tended to improve gains, feed efficiency and feed consumption while hematocrit values were decreased significantly. There was a significant interaction between calcium:phosphorus ratio and zinc on hematocrit.

In trial IV 100 mg. of zinc per head per day was added to rations containing 1:1, 2:1 and 4:1 calcium:phosphorus ratios. The addition of zinc at all levels significantly improved gains and decreased hematocrit and hemoglobin levels. Feed consumption and feed efficiencies were again improved by the addition of zinc although the improvements were not significant.

LITERATURE CITED

Barker, H. A., H. Weissbach and R. D. Smyth. 1958. A coenzyme containing psuedovitamin B_{12}. Proc. Nat'l. Acad. Sci. 44:1093.

Beck, W. S., M. Flavin and S. Ochoa. 1957. Metabolism of propionic acid in animal tissues. III. Formation of succinate. J. Biol. Chem. 229:997.

Becker, D. E. and S. E. Smith. 1951. The metabolism of cobalt in lambs. J. Nutr. 43:87.

Bentley, O. G., M. Moinuddin, T. V. Hershberger, E. W. Klosterman and A. L. Moxon. 1954. The effect of trace minerals on growth performance and vitamin B_{12} synthesis of steers. J. Animal Sci. 13: 789.

Cabell, C. A. and J. P. Earle. 1964. Calcium and phosphorus levels and utilization of dietary zinc. J. Animal Sci. 23:869. (Abstr.).

Cartwright, G. E. (M. D.). 1955. The relationship of copper, cobalt and other trace minerals to hemopoieses. Am. J. Clinical Nutr. 3:11.

Chang, M. L., R. M. Forbes, H. M. Scott and B. C. Johnson. 1961. The effect of zinc deficiency on the content of pyridine nucleotide in chick and rat livers. Fed. Proc. 20:296.

Conrad, J. H. and W. M. Beeson. 1957. Effect of calcium level on trace minerals on the response of young pigs to unidentified growth factors. J. Animal Sci. 16:589.

Embry, L. B., A. E. Dittman and F. W. Whetzal. 1965. Value of various levels of hay with rolled barley for finishing cattle. Mimeo. S. Dak. Animal Sci. Ser. 65-3.

Filmer, J. F. 1933. Enzootic Marasmus of cattle and sheep. Australian Vet. J. 9:163.

Flavin, M. and S. Ochoa. 1957. Metabolism of propionic acid in animal tissues. I. Enzymatic conversion of propionate to succinate. J. Biol. Chem. 229:965.

Fontenot, J. P., R. F. Miller and N. O. Price. 1964. Effects of calcium level and zinc supplementation of fattening lamb rations. J. Animal Sci. 23:874. (Abstr.).

Gall, L. S., S. E. Smith, D. E. Becker, C. N. Stark and L. K. Loosli. 1949. Rumen bacteria in cobalt deficient sheep. Science. 109:468.

Geurin, H. B., J. L. Williamson, J. C. Thompson, H. L. Wilcke and R. M. Bethke. 1959. Rolled common barley serves as both grain and roughage for fattening steers. J. Animal Sci. 18:1489.

Hoekstra, W. G., P. K. Lewis, Jr., P. H. Phillips and G. H. Grummer. 1956. The relationship of parakeratosis, supplemental calcium and zinc to the zinc content of certain body components of swine. J. Animal Sci. 15:752.

Houk, A. E. H., A. W. Thomas and H. C. Sherman. 1946. Some interrelationships of dietary iron, copper and cobalt in metabolism. J. Nutr. 31:609.

Kerncamp, H. C. H. and E. F. Ferrin. 1953. Parakeratosis in swine. JAVMA. 123:217.

Legg, S. P. and L. Sears. 1960. Zinc sulfate treatment of parakeratosis in cattle. Nature 186:1061.

Lenhyel, P., R. Mazumder and S. Ochoa. 1960. Mammalian methylmalonyl isomerase and vitamin B_{12} coenzymes. Proc. Nat'l. Acad. Sci. 46: 1312.

Luecke, R. W., J. A. Hoefer, W. S. Brammell and F. Thorp, Jr. 1956. Mineral interrelationships in parakeratosis of swine. J. Animal Sci. 15:347.

Lewis, P. K., Jr., W. G. Hoekstra, R. H. Grummer and P. H. Phillips. 1956. The effect of certain nutritional factors including calcium, phosphorus and zinc on parakeratosis in swine. J. Animal Sci. 15:741.

Lewis, P. K., Jr., W. G. Hoekstra and R. H. Grummer. 1957a. Restricted calcium feeding versus zinc supplementation for the control of parakeratosis in swine. J. Animal Sci. 16:578.

Lewis, P. K., Jr., R. H. Grummer and W. G. Hoekstra. 1957b. The effect of method of feeding upon the susceptability of the pig to parakeratosis. J. Animal Sci. 16:927.

Marston, H. R. 1935. J. Council Sci. and Indus, Res. Australia. 8:111, 293, as cited by Maynard, L. A. and J. K. Loosli. 1962. Animal Nutrition. McGraw-Hill Book Co., Inc. New York, New York.

Marston, H. R. and H. J. Lee. 1949. Primary site of the action of cobalt in ruminants. Nature 164:529.

Marston, H. R. 1952. Cobalt, copper and molybdenum in the nutrition of animals and plants. Physiol. Revs. 32:66.

Matrone, G., H. A. Ramsey and G. H. Wise. 1959a. Effect of volatile fatty acids, sodium and potassium bicarbonate in purified diets for ruminants. Proc. Soc. Exp. Biol. Med. 100:8.

Matrone, G., H. A. Ramsey and G. H. Wise. 1959b. Role of sodium and potassium cations in volatile fatty acid metabolism of ruminants. Proc. Cornell Nutr. Conf. for Feed Manufacturers, p. 36.

Maynard, L. A. and J. K. Loosli. 1962. Animal Nutrition. McGraw-Hill Book Co., Inc., New York, New York.

Miller, J. K. and W. J. Miller. 1960. Development of zinc deficiency in Holstein calves fed a purified diet. J. Dairy Sci. 43:1854.

Miller, J. K. and W. J. Miller. 1962. Experimental zinc deficiency and recovery of calves. J. Nutr. 76:467.

Morrison, F. B. 1959. Feeds and Feeding. The Morrison Publishing Co., Clinton, Iowa.

N. R. C. 1964. Nutrient requirements of domestic animals. IV. Nutrient requirements of beef cattle. National Research Council, Washington, D. C.

Nicholson, J. W. G., H. M. Cunningham and D. W. Friend. 1963. Effect of adding buffers to all concentrate rations on feedlot performance of steers, ration digestibility and intra-rumen environment. J. Animal Sci. 22:368.

Oltjen, R. R., E. F. Smith, B. A. Koch and F. H. Baker. 1959. The value of supplemental trace minerals in cattle fattening rations. J. Animal Sci. 18:1196.

Ott, E. A., W. H. Smith, M. Stob and W. M. Beeson. 1964. Zinc deficiency syndrome in the young lamb. J. Nutr. 82:41.

Renbarger, R., L. S. Pope and G. Waller. 1964. Trace mineral supplements to "all barley" rations for fattening steers. Okla. Agr. Expt. Sta. Mis. Pub. MP-75, p. 68.

Rickes, E. L., N. G. Brink, F. R. Koniusky, T. R. Wood and F. Folkers. 1948. Vitamin B_{12}, a cobalt complex. Science 108:134.

Sandell, E. B. 1959. Colorimetric determination of traces of metals. (3rd ed.). Interscience Pub. Inc., New York, New York.

Sherman, K. D., A. K. Sutherland, M. W. O'Hallonan, J. M. Rourke and B. L. Mundy. 1960. The correction of cobalt or vitamin B_{12} deficiency in cattle by cobalt pellet therapy. Am.J. of Vet. Res. 20:79.

Smith, E. L. 1948. Presence of cobalt in the anti-pernicious anemia factor. Nature 162:144.

Smith, W. H., T. W. Perry, R. B. Harrington, H. E. Parker, M. T. Mohler and W. M. Beeson. 1964a. Effect of zinc, cobalt and injectable iron on the performance of finishing steers. Purdue Res. Prog. Rpt. 109.

Smith, W. H., T. W. Perry, M. T. Mohler, R. B. Harrington and W. M. Beeson. 1964b. Performance of steers fed three levels of zinc with two levels of calcium. J. Animal Sci. 23:893. (Abstr.).

Stevenson, J. W. and I. P. Earle. 1956. Studies on parakeratosis in swine. J. Animal Sci. 15:1038.

Stewart, J. 1953. The effects of cobalt deficiency on the appetite of lambs. Brit. J. Nutr. 7:231.

Thomas, O. O. and L. L. Myers. 1961. Steam or dry rolled barley in high concentrate rations for fattening beef steers. J. Animal Sci. 20:953. (Abstr.).

Thomas. O. O., J. L. Clark, J. J. Metz, Jr. and L. G. Young. 1964. Effect of trace mineral additions to beef cattle fattening rations. J. Animal Sci. 23:894. (Abstr.).

Thompson, J. F. and G. H. Ellis. 1947. Is cobalt a dietary essential for the rabbit? J. Nutr. 34:121.

Tillman, A. D. 1961. Minerals in livestock nutrition and metabolism. Proc. Midwest Feed Mfg. Assn. Symposium, Kansas City, Missouri.

Tucker, H. F. and W. D. Salmon. 1955. Parakeratosis or zinc deficiency in the pig. Proc. Soc. Exptl. Biol. Med. 88:613.

Underwood, E. J. and J. F. Filmer. 1935. The determination of the biologically potent element (cobalt) in limonite. Australian. Vet. J. 11:84.

Underwood, E. J. 1959. Mineral metabolism. Ann. Rev. Biochem. 28:499.

Underwood, E. J. 1962. Trace Elements in Human and Animal Nutrition. Academic Press Inc., New York, New York.

Whetzal, F. W. and L. B. Embry. 1962. Value of protein supplement in barley rations for fattening cattle. A. H. Mimeo Series. 62-18. S. Dak. State. College.

Whetzal, F. W. and L. B. Embry. 1963. Value of a protein supplement in barley rations for fattening cattle. A. H. Mimeo Series. 63-18. S. Dak. State College.

White, A., P. Handler and E. L. Smith. 1964. Principles of Biochemistry. McGraw-Hill Book Co., New York, New York.

Wise, M. B., T. N. Blumer, G. Matrone and E. R. Barrick. 1961. Investigations on the feeding of all concentrate rations to beef cattle. J. Animal Sci. 20:561.

Wise, M. B., A. L. Ordoveza and E. R. Barrick. 1963. Influence of variations in dietary calcium:phosphorus ratio on performance and blood constituents of calves. J. Nutr. 79:79.

Wise, M. B., T. N. Blumer, H. B. Craig and E. R. Barrick. 1965. Influence of rumen buffering agents and hay on performance and carcass characteristics of steers fed all-concentrate rations. J. Animal Sci. 24:83.

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