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Andrea K. Watson

Trent J. McEvers

Lee-Anne J. Walter

Nathan D. May

Jacob A. Reed

See next page for additional authors

Authors

Andrea K. Watson, Trent J. McEvers, Lee-Anne J. Walter, Nathan D. May, Jacob A. Reed, J. C. MacDonald, Galen E. Erickson, N. Andy Cole, JohnJohn P. Hutcheson, and Ty E. Lawrence

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Mineral composition of serially slaughtered Holstein steers supplemented with zilpaterol hydrochloride

Andrea K. Watson,[†] Trent J. McEvers,[‡] Lee-Anne J. Walter,[‡] Nathan D. May,[‡] Jacob A. Reed,[‡] Jim C. MacDonald,[†] Galen E. Erickson,[†] N. Andy Cole,[||] John P. Hutcheson,[$] and Ty E. Lawrence[‡]

[†]Department of Animal Science, University of Nebraska, Lincoln, NE 68583, USA
[‡]Beef Carcass Research Center, Department of Agricultural Sciences, West Texas A&M University, Canyon, TX 79016, USA
[||]USDA-ARS Conservation and Production Research Laboratory, Bushland, TX 79012, USA
[$]Merck Animal Health, Summit, NJ 07901, USA

Abstract

Calf-fed Holstein steers (n = 115; 449 ± 20 kg) were utilized in a serial harvest experiment. A baseline group of five steers was harvested after 226 d on feed (**DOF**), which was designated day 0. The remaining cattle were assigned randomly to 11 harvest groups, with slaughter every 28 d. Cattle were either not (**CON**) or were fed zilpaterol hydrochloride for 20 d followed by a 3 d withdrawal (**ZH**). There were five steers per treatment in each slaughter group ranging from days 28 to 308. Whole carcasses were divided into lean, bone, internal cavity, hide, and fat trim components. Apparent mineral retention (Ca, P, Mg, K, and S) within the body was calculated as the difference between mineral concentration at slaughter and day 0. Mineral concentration at day 0 was determined from body composition of steers harvested at day 0 multiplied by individual live body weight (**BW**) at day 0. All data were analyzed as a 2 × 11 factorial arrangement with individual animal as the experimental unit. Orthogonal contrasts were used to analyze linear and quadratic contrasts over time (11 slaughter dates). There were no differences in concentration of Ca, P, and Mg in bone tissue as feeding duration increased ($P \geq 0.89$); concentration of K, Mg, and S in lean tissue did fluctuate across DOF ($P < 0.01$). Averaged across treatment and DOF, 99% of Ca, 92% of P, 78% of Mg, and 23% of S present in the body were in bone tissue; 67% of K and 49% of S were in lean tissue. Expressed as gram per day, apparent retention of all minerals decreased linearly across DOF ($P < 0.01$). Expressed relative to empty body weight (**EBW**) gain, apparent Ca, P, and K retention decreased linearly as BW increased ($P < 0.01$) whereas Mg and S increased linearly ($P < 0.01$). Apparent retention of Ca was greater for CON cattle (greater bone fraction) and apparent retention of K was greater for ZH cattle (greater muscle fraction) when expressed relative to EBW gain ($P \leq 0.02$), demonstrating the increase in lean gain by ZH cattle. There were no differences in apparent retention of Ca, P, Mg, K, or S due to treatment ($P \geq 0.14$) or time ($P \geq 0.11$) when expressed relative to protein gain. Apparent retention averaged 14.4 g Ca, 7.5 g P, 0.45 g Mg, 1.3 g K, and 1.0 g S/100 g protein gain. Expressing apparent mineral retention on a protein gain basis minimized effects of rate and type of gain, allowing for better comparison across treatments and time. Feeding zilpaterol hydrochloride did not affect apparent mineral retention when expressed relative to protein gain.

Lay Summary

Mineral requirements for feedlot cattle are largely based on measured mineral concentration in the body at harvest. Fairly extensive research has been done quantifying Ca and P in the body of cattle, but data on Mg, K, and S are sparse. Serial harvest experiments are expensive and labor intensive and therefore not conducted frequently. A group of 115 Holstein steers was fed a finishing diet with serial harvest every 28 d. Two treatments were evaluated, control and cattle fed zilpaterol hydrochloride to increase lean tissue growth. Every 28 d, five steers from each treatment group were harvested with the whole carcass divided into lean, bone, internal cavity, hide, and fat trim components. Apparent mineral retention was calculated as the difference between mineral composition at day 0 (baseline harvest group) and each 28 d harvest group. Averaged across treatment and days on feed, 99% of Ca, 92% of P, 78% of Mg, and 23% of S present in the body were measured in bone tissue; 67% of K and 49% of S were in lean tissue. Apparent retention averaged 14.4 g Ca, 7.5 g P, 0.45 g Mg, 1.3 g K, and 1.0 g S/100 g protein gain.

Key words: calcium, cattle, gain, minerals, phosphorus, requirement

Abbreviations: BW, body weight; DM, dry matter intake; DOF, days on feed; EBF, empty body fat; EBW, empty body weight; ZH, empty body weight

Introduction

Recommendations for Ca and P requirements per unit of gain in the current NASEM (2016) are predominately based on data measuring retention in dairy cattle (Ellenberger et al., 1950). Ellenberger et al. (1950) performed an extensive experiment measuring Ca and P retention of 132 entire carcasses ranging from a 135-d-old fetus to a 12-yr-old cow. Similar data of this magnitude have not been gathered on Mg, K, and S retention. Since the data of Ellenberger et al. (1950) were collected, significant changes in cattle size, feedstuffs commonly used, and mineral analysis procedures have occurred (Karn, 2001; Block et al., 2004; Ekelund et al., 2006). New feed additives may also affect both rate and composition of gain. Zilpaterol hydrochloride (Zilmax; Merck Animal Health, Summit, NJ) is a β-adrenergic agonist that increases lean tissue deposition in feedlot cattle (Avendano-Reyes et al., 2006; Vasconcelos et

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al., 2008; Elam et al., 2009). As feed additives become more commonly used, it is important to understand their effects on both rate and composition of gain to make proper recommendations for mineral requirements. All of these changes suggest a need to re-evaluate retention of minerals within cattle to calculate mineral requirements per unit of gain.

The NRC (1996) and NASEM (2016) report P and Ca retention per unit of protein gain. Because P and Ca are predominately found in bone tissue, it is possible that expressing mineral retention on an alternative basis could better define mineral requirements across treatments, production systems, or rates of gain (Block et al., 2004). The objective of this experiment was to measure apparent Ca, P, Mg, K, and S retention within the whole body of Holstein steers over time with or without the addition of zilpaterol hydrochloride in the diet. An additional objective was to determine an optimal method of expressing mineral retention. Our hypothesis was that feeding zilpaterol hydrochloride would affect mineral retention within the body of cattle and expressing mineral requirements relative to empty body weight (**EBW**) gain would be advantageous.

Materials and Methods

All experimental procedures followed the guidelines described in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (Federation of Animal Science Societies, Savoy, IL) under an IACUC-approved protocol. One hundred and fifteen Holstein steers (449 ± 20 kg BW) were utilized in a serial harvest experiment conducted by the Beef Carcass Research Center, West Texas A&M University (Canyon, TX) conducted from the spring of 2012 to the spring of 2013. Treatments included a control (**CON**) and steers fed zilpaterol hydrochloride (Zilmax) at a rate of 8.33 mg/kg of dietary dry matter (**DM**) for 20 d followed by a 3-d withdrawal immediately preceding harvest (**ZH**). The finishing diet fed during the experiment was identical for CON and ZH steers, except for the addition of zilpaterol hydrochloride. Data from this experiment were presented at the 2014 Plains Nutrition Council Spring Conference and are referenced in Chapter 7 (Minerals) of the NASEM (2016) as Watson et al. (2014). Experimental design, cattle management, feeding behavior, and live growth performance (Walter et al., 2016), skeletal growth and frame size (Reed et al., 2017), harvest yields of non-carcass body components (May et al., 2016a), quality and yield grade outcomes (May et al., 2016b), boxed beef fabrication yields (May et al., 2017), estimated carcass composition via rib dissection (McEvers et al., 2018a), empty body composition (McEvers, 2014), and economic outcomes (McEvers et al., 2018b) have previously been reported from this large project. Cattle were assigned to harvest groups with 10 steers harvested every 28 d. An initial group of five steers was harvested after 226 d on feed (**DOF**) which was considered day 0 as a baseline (June 25, 2012). There were 11 additional groups harvested every 28 d through day 308 (April 29, 2013). Due to multiple outliers (more than 3 SD away from the mean) and high variability, data from slaughter group 6, on day 168, were omitted from the analysis, leaving 10 groups included in the analysis.

Within each of these subsequent harvest groups, cattle were assigned randomly to treatment and fed in pens fitted with GrowSafe bunks (GrowSafe Systems Ltd, Airdrie, AB, Canada). Twenty-eight days prior to harvest, cattle in the current harvest block were reassigned to two separate pens with two GrowSafe nodes per pen. For 5 d after reassignment cattle continued to receive the CON diet, followed by 20 d of ZH supplementation to the ZH treatment cattle and a 3-d withdrawal, for a total of 28 d intensively measured before harvest.

At harvest, carcasses were chilled for 48 h and then fabricated at the West Texas A&M University Meats Lab (Canyon, TX; groups 1 through 6) or Caviness Packing Company (Hereford, TX; groups 7 through 11). A full description of the harvest process is reported by May et al. (2016a). At harvest, carcasses were broken down into five categories: bone, hide, lean, fat, and internal cavity tissues. For the harvest group on day 28, steers had minimal fat deposition and tissues were separated into four categories with no fat separated from other tissues. Each type of tissue was weighed before being ground and sampled. Femurs from the right side of each carcass were used to represent the skeletal portion of the entire empty body with femurs sawn every 1.27 cm (Butcher Boy Band Saw Model B16-F; Selmer, TN) and sawdust sampled. The EBW was calculated as the sum of hot carcass weight, total visceral organs, empty gastrointestinal tract, head, blood, tongue, ears, oxtail, hide, and limbs.

All samples (bone, hide, lean, fat, and internal cavity) were analyzed for Ca, P, Mg, K, S, Na, Fe, and Zn by Servi-Tech Laboratory (Amarillo, TX). For mineral analysis, 0.5 g samples were digested for 1 h at 124°C using 7 mL nitric acid, followed by 15 min using 1 mL hydrogen peroxide, followed by 15 min using 4 mL hydrochloric acid. Samples were then diluted, mixed with an internal standard solution, and filtered before being analyzed using Inductively Coupled Plasma-Atomic Emission Spectroscopy (Huang and Schulte, 1985; Mills and Jones, 1996; US EPA, 1996).

This article will focus on apparent Ca, P, Mg, K, and S retention within the empty body. Diet composition and nutrient analysis are shown in Table 1. Diet composition, including mineral content, was identical between treatments. Daily feed intake including differences between treatments and changes across the feeding period has been described in depth by Walter et al. (2016). Intake of individual minerals was not used to calculate retention in the current experiment, but are reported, and were adequate to support cattle growth, based on current NASEM (2016) recommendations. Analysis of Ca, P, Mg, K, and S was used to calculate apparent mineral retention. Concentrations of Na, Fe, and Zn within the different tissue types are shown, but were not used to calculate mineral retention.

Calculations and statistical analysis

Apparent mineral retention within the body was calculated as the difference between mineral concentration at slaughter and mineral concentration at day 0. Mineral composition at day 0 was calculated from body composition of steers harvested at day 0 (% mineral in different tissues × tissue weight), multiplied by live BW of individual animals at day 0. Live weights were shrunk 4%. Due to the short interval between harvest points (28 d) and no differences in P and Ca concentration of the bone portion over time ($P \geq 0.89$), initial P and Ca composition of the bone fraction was predicted using each steer's mineral composition rather than the average of the day 0 harvested cattle. With no changes over time in concentration of Ca and P in the bone, individual steer data better predicted day 0 composition for that animal, than using day 0 data to

Table 1. Composition and nutrient analysis of finishing diets fed to Holstein steers

Ingredients	% DM basis
Steam flaked corn	70.0
Wheat hay	8.5
Corn Gluten feed	7.0
Cottonseed meal	3.0
Molasses blend	4.0
Fat	2.0
Supplement[1]	5.5
Nutrient	Analysis (± SD)
DM, %	80.22 ± 1.72
Crude protein, % DM	14.60 ± 0.66
Crude fiber, % DM	7.54 ± 1.12
Calcium, % DM	0.62 ± 0.12
Phosphorus, % DM	0.35 ± 0.02
Potassium[2], % DM	0.58
Magnesium[2], % DM	0.16
Sulfur[2], % DM	0.16
NE_m Mcal/kg DM[3]	2.17 ± 0.05
NE_g Mcal/kg DM[3]	1.49 ± 0.33

[1]Supplement contained: 49.01% cottonseed meal, 10.00% rice mill byproduct, 3.20% ammonium sulfate, 3.70% salt, 10.20% urea, 20.55% calcium carbonate (38% Ca), 1.95% potassium chloride (52% K), 0.33% vitamin A (44,092,420 IU/kg), 0.053% vitamin E (275,577,625 IU/kg), 0.001% vitamin D (176,369,680 IU/kg), and 1.00% trace mineral.
[2]Concentration of K, Mg, and S calculated from diet composition and NRC (1996) values for diet ingredients.
[3]Dietary net energy, calculated from Zinn and Shen (1998).

predict individual steer mineral content. This method was not appropriate for other tissues or minerals because changes in mineral content were observed over time ($P < 0.01$). Whole body mineral composition was the sum of individual tissues (lean, bone, internal cavity, fat, and hide). At each harvest, EBW was measured. Apparent mineral retention was calculated on an EBW basis for all cattle and expressed as grams per day, grams per kilogram EBW gain, and grams per 100 g protein gain. Protein gain was measured and reported by McEvers (2014).

All data were analyzed as a 2 × 11 factorial arrangement using the Mixed procedure of SAS (SAS Inst. Inc. Cary, NC). Two treatments (CON and ZH) were analyzed across 11 slaughter dates. Animal was the experimental unit with treatment and DOF (slaughter date) included in the model as fixed effects. All measurements were made on individual animals. Orthogonal contrasts were used to analyze linear and quadratic contrasts over time. Means were considered to be different at the $P < 0.05$ level.

Results and Discussion

Tissue mineral composition

Concentrations of Ca, P, Mg, K, and S within tissues are presented in Table 2. As a % of DM, linear decreases in concentration of Ca were observed in lean and internal cavity tissues ($P \leq 0.04$). There were no differences in concentration of Ca in bone and fat tissue across DOF ($P \geq 0.36$). Averaged across treatments and DOF, 99% of Ca present in the body was in the bone. This is similar to previous estimates that up to 99% of the body's Ca is in skeletal tissue (Ellenberger et al., 1950; Duncan, 1958; Satter et al., 2002).

Concentration of P within lean, hide, internal cavity, and fat tissues decreased linearly as DOF increased ($P < 0.01$). There were no differences in concentration of P in bone tissue across DOF ($P \geq 0.53$). Averaged across treatments and DOF, 92% of P present in the body was in the bone. This is similar to previous estimates that 80% to 90% of total body P is in skeletal tissue (Ellenberger et al., 1950; Duncan, 1958; Satter et al., 2002).

Magnesium concentration within lean tissue increased quadratically across DOF ($P < 0.01$) for both treatments. There were no differences in concentration of Mg within bone tissue across DOF ($P \geq 0.68$). Magnesium was primarily retained in bone tissue which contained 78% of total body Mg. Lean tissue contained an additional 16% of total body Mg. Engels (1981) concluded that 70% of Mg is deposited in the skeleton and the remaining 30% is in the soft tissues.

Concentration of K within lean, hide, and internal cavity tissues increased quadratically across DOF ($P \leq 0.03$). Potassium retention within the lean tissue accounted for 62 and 72% of total body K apparent retention for CON and ZH, respectively.

Concentration of S within lean tissue increased quadratically across DOF ($P < 0.01$) and decreased linearly within bone tissue across DOF ($P < 0.01$). Sulfur retention within lean, bone, and hide tissues accounted for 49%, 23%, and 13% of S apparent retention within the entire body, respectively. The hide had the greatest concentration of S out of all tissues measured, with a linear increase across DOF ($P < 0.01$), going from 0.51% S to 0.87% S on a DM basis, from the first to the last harvest group. This increase in S within the hide portion is likely due to accumulation of S-containing amino acids in the hair coat of animals, especially evident as cattle were housed outdoors with the initial harvest in June and subsequent harvest groups every 28 d until the following April. The numerically greatest concentration was at day 196, 1.03% S, which would have been January 7.

Differences in concentration of minerals due to dietary treatment were minimal, except that lean tissues of ZH cattle had greater concentrations of P, Mg, and K ($P \leq 0.04$) than CON (data not shown). There were no differences in concentration of Ca, P, or Mg in bone tissue due to dietary treatment ($P \geq 0.71$).

Concentrations of Na, Fe, and Zn within each tissue type are shown in Table 3. Both Na and Fe were most concentrated within the hide; the highest concentration of Zn was within the lean tissue. There were no differences due to treatment ($P \geq 0.10$), only Zn concentration varied across DOF ($P = 0.04$). Micro minerals are critical in supporting cattle health and growth although requirements are often difficult to quantify. In particular, Zn is critical for protein synthesis and many nutritionists provide 2 to 3 times more Zn in the diet than the NASEM (2016) lists as required (Messersmith et al., 2021). As technologies to enhance cattle growth are used, such as hormonal implants or β-adrenergic agonists, it is important to understand how these technologies also impact micromineral requirements, especially Zn (Hergenreder et al., 2020). Carmichael et al. (2018) reported a positive correlation ($r = 0.46$) between Zn and N retention suggesting that reporting Zn requirements relative to protein gain is logical. Sodium plays a critical role in the body and requirements are widely met through the addition of salt to most confined cattle diets

Table 2. Mineral composition of tissues from Holstein steers serially harvested during a high concentrate finishing period

Item	Days on feed[1]										SEM	*P*-value[2]		
	28[3]	56	84	112	140	196	224	252	280	308		DOF	Lin	Quad
Calcium, % of tissue DM														
Bone	14.9	16.1	15.9	15.3	15.4	15.9	16.1	15.8	15.9	15.9	0.8	0.89	0.36	0.81
Lean	0.03	0.03	0.03	0.04	0.03	0.03	0.03	0.03	0.03	0.02	0.004	0.14	0.04	0.14
Hide	0.20	0.07	0.10	0.07	0.17	0.13	0.13	0.18	0.11	0.11	0.02	<0.01	0.96	0.70
Internal cavity	0.18	0.11	0.06	0.04	0.03	0.07	0.03	0.04	0.07	0.07	0.02	<0.01	<0.01	<0.01
Fat	—	0.01	0.02	0.01	0.01	0.03	0.01	0.02	0.01	0.01	0.006	0.59	0.61	0.38
Phosphorus, % of tissue DM														
Bone	7.1	7.7	7.5	7.2	7.2	7.5	7.6	7.5	7.4	7.5	0.4	0.91	0.53	0.86
Lean	0.43	0.52	0.52	0.51	0.45	0.48	0.46	0.38	0.41	0.38	0.02	<0.01	<0.01	0.14
Hide	0.09	0.09	0.10	0.09	0.09	0.11	0.11	0.13	0.11	0.10	0.006	<0.01	<0.01	0.21
Internal cavity	0.30	0.22	0.20	0.18	0.13	0.14	0.14	0.10	0.10	0.10	0.009	<0.01	<0.01	<0.01
Fat	—	0.07	0.08	0.06	0.05	0.07	0.05	0.06	0.04	0.05	0.009	<0.01	<0.01	0.76
Magnesium, % of tissue DM														
Bone	0.32	0.35	0.34	0.33	0.33	0.33	0.34	0.33	0.32	0.33	0.02	0.90	0.68	0.85
Lean	0.054	0.065	0.064	0.065	0.056	0.062	0.060	0.047	0.053	0.048	0.004	<0.01	<0.01	<0.01
Hide	0.04	0.02	0.02	0.02	0.04	0.03	0.03	0.05	0.03	0.03	0.004	<0.02	0.02	0.97
Internal cavity	0.07	0.03	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.007	<0.01	<0.01	<0.01
Fat	—	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.0003	0.44	0.99	0.26
Potassium, % of tissue DM														
Bone	0.13	0.12	0.13	0.12	0.12	0.12	0.12	0.14	0.11	0.12	0.009	0.28	0.31	0.86
Lean	0.80	0.98	0.97	0.97	0.86	0.90	0.88	0.70	0.76	0.71	0.05	<0.01	<0.01	<0.01
Hide	0.22	0.18	0.20	0.20	0.22	0.27	0.25	0.26	0.24	0.21	0.01	<0.01	<0.01	0.03
Internal cavity	0.26	0.23	0.24	0.19	0.17	0.15	0.14	0.11	0.11	0.12	0.009	<0.01	<0.01	<0.01
Fat	—	0.10	0.11	0.09	0.08	0.10	0.08	0.10	0.07	0.09	0.006	<0.01	<0.01	0.40
Sulfur, % of tissue DM														
Bone	0.25	0.25	0.25	0.24	0.24	0.23	0.24	0.23	0.23	0.23	0.003	<0.01	<0.01	0.15
Lean	0.44	0.54	0.55	0.52	0.47	0.50	0.48	0.42	0.43	0.39	0.03	<0.01	<0.01	<0.01
Hide	0.51	0.50	0.52	0.58	0.62	1.03	0.96	0.82	0.96	0.87	0.1	<0.01	<0.01	0.12
Internal cavity	0.21	0.15	0.17	0.13	0.11	0.11	0.11	0.08	0.08	0.09	0.01	<0.01	<0.01	<0.01
Fat	—	0.07	0.07	0.06	0.05	0.06	0.05	0.05	0.05	0.05	0.004	<0.01	<0.01	0.18

[1]Mineral composition was measured on five steers/treatment every 28 d, for 11 harvest points. Due to outliers, day 168 data (group 6) were removed. Whole carcasses were divided into bone, lean, hide, internal cavity, and fat components. Combined results for bone, lean, and hide represent a majority of the mineral in the whole body (>60%). All mineral composition data are presented on a DM basis.
[2]DOF = F-test for main effect of days on feed; Lin = linear orthogonal contrast of days on feed; Quad = quadratic orthogonal contrast of days on feed.
[3]For the harvest group on day 28, steers had minimal fat deposition and tissues were separated into four categories (bone, lean, hide, and internal cavity) with no fat separated from other tissues.

Table 3. Mineral composition of tissues from Holstein steers serially harvested during a high concentrate finishing period[1]

	Tissue Type					SEM	*P*-value[2]		
	Bone	Lean	Hide	Internal cavity	Fat		Type	Trt	DOF
Na, %	0.52[b]	0.25[c]	0.77[a]	0.12[d]	0.06[e]	0.03	<0.01	0.56	0.11
Fe, ppm	289.9[b]	99.1b[c]	796.6[a]	130.2[bc]	10.3[c]	136.7	<0.01	0.23	0.13
Zn, ppm	120.3[b]	202.9[a]	57.5[c]	25.1[d]	10.4[d]	12.2	<0.01	0.10	0.04

[1]All mineral concentrations are shown on a DM basis.
[2]*P*-value for main effects of tissue type (Type), treatment (Trt), and days on feed (DOF). The 2-way interactions between these effects were not significant ($P \geq 0.13$). While the 3-way interaction was significant ($P < 0.01$) for Fe and Zn concentration, the biological importance is likely negligible.
[a,b,c,d,e]Means within a row without a common superscript differ ($P < 0.05$).

as well as free choice salt commonly provided to grazing animals (Morris, 1980). Due to supplementation, Na deficiencies are rare and toxic levels are generally only observed when drinking water is contaminated (NASEM, 2016). Iron's role in oxygen transport within the body is critical, but most feedstuffs are adequate in iron, making deficiencies rare for cattle (NASEM, 2016).

Apparent mineral retention

Individual animal intake of each mineral is presented in Table 4. Intake of Ca, P, K, Mg, and S was not different between CON and ZH ($P \geq 0.68$), but did change across DOF ($P < 0.01$), closely following changes in overall dry matter intake (Walter et al., 2016).

There were no differences in Ca apparent retention due to treatment ($P = 0.39$; Table 4) or DOF ($P = 0.11$) when expressed relative to protein gain. There was variation across DOF, but no clear pattern as both linear and quadratic contrasts were not significant ($P \geq 0.43$). Expressed on an EBW gain basis, CON cattle had greater Ca apparent retention ($P = 0.02$) with both treatments linearly decreasing across DOF ($P < 0.01$; Table 5).

Calculating mineral apparent retention relative to EBW gain resulted in no differences due to treatment for P ($P = 0.12$) with a linear decrease over DOF ($P < 0.01$). There were no differences in P apparent retention due to treatment ($P = 0.52$) or DOF ($P = 0.15$) when expressed relative to protein gain. Similar to Ca, there was variation in P apparent retention relative to protein gain across DOF, but no clear pattern as both linear and quadratic contrasts were not significant ($P \geq 0.32$).

Magnesium apparent retention was not affected by treatment ($P \geq 0.56$) regardless of how retention was expressed. When expressed as grams per day, Mg apparent retention linearly decreased ($P < 0.01$) across DOF; however, apparent retention linearly increased when expressed as grams per kilogram EBW gain ($P < 0.01$). There were no differences across DOF when expressed relative to protein gain ($P = 0.34$). Magnesium apparent retention averaged 0.45 g/100 g protein gain for both treatments and all DOF.

Apparent retention of K, expressed as grams per day or grams per kilogram EBW gain, was greater for ZH cattle ($P < 0.01$) with linear decreases across DOF ($P < 0.01$) for both treatments. The interaction between treatment and DOF was significant ($P \leq 0.01$) with ZH cattle having greater decreases in K apparent retention across DOF compared to CON cattle (data not shown). Lean tissue accretion is increased by feeding the β-adrenergic agonist zilpaterol hydrochloride to feedlot cattle (Avendano-Reyes et al., 2006; Vasconcelos et al., 2008; Elam et al., 2009). This increase in lean, in addition to an increase in the concentration of K within the lean, led to greater K apparent retention in ZH cattle compared to CON cattle when expressed as grams per day or grams per kilogram EBW gain. There were no differences across DOF ($P = 0.60$) or treatment ($P = 0.14$) when K apparent retention was expressed relative to protein gain, averaging 1.3 g/100 g protein gain.

Apparent retention of S did not differ by treatment when expressed as grams per day ($P = 0.09$) or relative to EBW gain or protein gain ($P \geq 0.22$). Apparent S retention, expressed as grams per kilogram EBW gain, linearly increased across DOF ($P < 0.01$) and linearly decreased across DOF when expressed as grams per day ($P < 0.01$). However, there were no differences in apparent S retention across DOF when expressed relative to protein gain ($P = 0.57$). Apparent S retention averaged 1.0 g/100 g protein gain for both treatments and all DOF.

Impact of composition of gain

Differences in apparent mineral retention across DOF when expressed as grams per day or grams per kilogram EBW gain were primarily due to changes in tissue weights and not mineral concentration in tissues. Cattle on ZH had a greater percent of EBW as lean, 41.8% compared to 39.7% of EBW for CON ($P < 0.01$). Increasing the amount of lean tissue, without a concurrent increase in bone tissue, reduced Ca retention ($P = 0.02$) and increased K retention ($P < 0.01$) of ZH cattle compared to CON in the current experiment (on an EBW gain basis). Rumsey et al. (1981) studied the effects of the hormonal growth-promotant diethylstilbestrol on mineral retention throughout a finishing period. Both lean and skeletal growth were increased, thus there was greater mineral retention (grams per day of Ca, P, Mg, and K) within cattle receiving an implant, due to increased EBW gain, but no differences in mineral retention per kilogram of gain.

Expressing apparent mineral retention relative to protein gain resulted in no statistical differences due to treatment ($P \geq 0.14$) or DOF ($P \geq 0.11$), thus most of the variation was due to differences in rate and type of gain. Apparent retention averaged 14.4 g of Ca, 7.5 g of P, 0.45 g of Mg, 1.3 g of K, and 1.0 g of S/100 g protein gain. The current NASEM (2016) reports Ca retention as 7.1 g/100 g protein gain and P retention as 3.9 g/100 g protein gain. These values are based on data obtained from the 1940s, primarily measured in Holstein cows. Retention of Mg, K, and S are less readily available in the literature and requirements in the NASEM (2016) for K and S are given as a proportion of the diet, not relative to gain.

Table 4. Main effect of zilpaterol hydrochloride (ZH) inclusion in the finishing diet of Holstein steers on apparent mineral retention within the whole body

Item	CON[1]	ZH	SEM	*P*-value[2]		
				Trt	DOF	Int
DMI, kg/d	9.56	9.49	0.42	0.71	<0.01	0.11
Calcium[3]						
g/d	25.9	24.2	4.0	0.35	<0.01	0.98
g/kg EBW gain	19.4	16.7	2.6	0.02	<0.01	0.45
g/100 g protein gain	15.3	13.5	4.7	0.39	0.11	0.87
Intake, g/d	59.3	58.8	2.6	0.69	<0.01	0.11
Phosphorus						
g/d	13.1	12.8	2.0	0.74	<0.01	0.99
g/kg EBW gain	9.8	8.9	1.3	0.12	<0.01	0.75
g/100 g protein gain	7.8	7.1	2.4	0.52	0.15	0.88
Intake, g/d	33.5	33.2	1.5	0.69	<0.01	0.11
Magnesium						
g/d	0.82	0.84	0.29	0.93	0.01	0.87
g/kg EBW gain	0.18	0.17	0.03	0.56	0.01	0.35
g/100 g protein gain	0.45	0.44	0.15	0.82	0.34	0.91
Intake, g/d	15.3	15.2	0.68	0.68	<0.01	0.11
Potassium						
g/d	2.0	2.8	0.46	< 0.01	<0.01	<0.01
g/kg EBW gain	1.4	2.0	0.36	< 0.01	<0.01	0.01
g/100 g protein gain	1.2	1.4	0.36	0.14	0.60	0.99
Intake, g/d	55.5	55.0	2.5	0.69	<0.01	0.11
Sulfur						
g/d	1.7	2.0	0.44	0.09	0.02	0.16
g/kg EBW gain	0.40	0.43	0.05	0.22	<0.01	0.70
g/100 g protein gain	1.0	1.0	0.30	0.90	0.57	0.87
Intake, g/d	15.3	15.2	0.68	0.68	<0.01	0.11

[1]Treatments consisted of a control group (**CON**) and cattle fed zilpaterol hydrochloride for 20 d followed by a 3-d withdrawal, immediately before harvest (**ZH**).
[2]*P*-values include main effects of treatment (CON or ZH) and days on feed (DOF) and the interaction between treatment and DOF (Int).
[3]Apparent mineral retention was calculated from mineral composition of five steers harvested on day 226 of the finishing period with five steers/treatment every 28 d after that, for 11 more harvest points.

Apparent retention of Ca, P, Mg, K, and S, expressed as grams per day over the entire feeding period, linearly decreased as DOF increased ($P < 0.01$). Regression equations describing apparent Ca, P, Mg, K, and S retention, as grams per day, are below, respectively:

$$y = -0.06(0.01)\ x + 34.4(1.8);\ R^2 = 0.26 \quad (1)$$

$$y = -0.03(0.004)\ x + 18.3(0.9);\ R^2 = 0.31 \quad (2)$$

$$y = -0.003(0.0007)\ x + 1.3(0.1);\ R^2 = 0.14 \quad (3)$$

$$y = -0.008(0.001)\ x + 3.8(0.3);\ R^2 = 0.27 \quad (4)$$

$$y = -0.004(0.001)\ x + 2.5(0.2);\ R^2 = 0.12 \quad (5)$$

The dependent variable (y) is apparent mineral retention and the independent variable (x) is DOF. Standard error of the slope and intercept are displayed in parentheses. The decline was greatest for K (69% decrease) and least for Ca (42% decrease) from the first (28 DOF) to the last (308 DOF) slaughter group. As cattle finish, greater amounts of fat are deposited, resulting in dilution of these minerals, which are primarily found in the skeletal or lean tissues (Rumsey et al., 1981). In the current experiment, fat trim from each carcass contained little mineral, 0.01% Ca, 0.04% P, 0.01% Mg, 0.09% K, and 0.06% S, and the amount of fat trim increased linearly with increasing DOF ($P < 0.01$).

Impact of experimental procedures

Serial slaughter experiments with tissue separation in cattle are challenging and not frequently done. Most data collected focus on energy and protein retention, with few data available on mineral retention (Block et al., 2004). Numerous experiments have calculated apparent mineral retention in metabolism experiments as the difference between total mineral intake and excretion, primarily in dairy cows. There are few examples of whole-body analysis being compared to apparent mineral retention in the same experiment, with unaccounted differences of up to 40% when they have been compared

Table 5. Main effect of days on feed on apparent retention of calcium and phosphorus in the whole body of Holstein steers on a high concentrate finishing diet

Item	Days on feed[1]										SEM	*P*-value[2]		
	28	56	84	112	140	196	224	252	280	308		DOF	Lin	Quad
Calcium														
g/d	34.3	35.5	28.5	29.5	21.5	17.3	21.2	21.2	21.1	19.8	4.0	<0.01	<0.01	0.03
g/kg EBW gain	25.2	20.6	21.8	21.0	16.8	12.8	15.4	14.9	15.4	16.7	2.6	<0.01	<0.01	0.08
g/100 g protein gain	11.3	17.3	16.6	13.7	13.5	10.1	11.5	12.0	13.8	24.7	4.7	0.11	0.43	0.96
Phosphorus														
g/d	18.5	18.4	15.0	15.4	11.1	9.2	10.9	10.8	10.7	9.9	2.0	<0.01	<0.01	0.02
g/kg EBW gain	13.3	10.7	11.4	10.9	8.6	6.8	7.9	7.6	7.8	8.3	1.3	<0.01	<0.01	0.06
g/100 g protein gain	6.2	9.0	8.6	7.1	6.9	5.4	5.9	6.1	7.0	12.3	2.4	0.15	0.32	0.92
Magnesium														
g/d	1.6	1.2	0.84	0.83	0.66	0.60	0.67	0.65	0.62	0.61	0.29	0.01	<0.01	0.01
g/kg EBW gain	0.10	0.14	0.14	0.17	0.16	0.18	0.21	0.21	0.22	0.24	0.03	<0.01	<0.01	0.79
g/100 g protein gain	0.56	0.47	0.43	0.40	0.41	0.35	0.36	0.37	0.41	0.71	0.15	0.34	0.81	0.01
Potassium														
g/d	4.5	3.0	2.8	2.7	2.2	2.1	2.0	1.7	1.7	1.4	0.5	<0.01	<0.01	0.02
g/kg EBW gain	2.9	1.8	2.1	1.9	1.6	1.6	1.5	1.2	1.2	1.1	0.4	<0.01	<0.01	0.26
g/100 g protein gain	1.5	1.5	1.4	1.2	1.2	1.3	1.1	0.92	1.1	1.7	0.4	0.60	0.05	0.72
Sulfur														
g/d	3.0	1.9	2.0	2.0	1.5	1.8	1.8	1.5	1.6	1.3	0.4	0.02	<0.01	0.22
g/kg EBW gain	0.18	0.22	0.34	0.40	0.37	0.55	0.55	0.50	0.54	0.48	0.05	<0.01	<0.01	<0.01
g/100 g protein gain	1.1	1.0	0.97	0.89	0.92	1.1	0.97	0.85	1.0	1.4	0.3	0.57	0.31	0.07

[1]Apparent mineral retention was calculated from mineral composition of five steers harvested on day 226 of the finishing period (designated day 0), followed by five steers/treatment every 28 d, for 11 additional harvest points. Due to outliers, day 168 data (group 6) were removed. Retention was calculated from day 0 for each harvest group.
[2]DOF = F-test for main effect of days on feed; Lin = linear orthogonal contrast of days on feed; Quad = quadratic orthogonal contrast of days on feed.

Table 6. Variation in reported values for mineral retention in cattle

Reference	Cattle Type	Diet	Ca	P	Mg	K	S
Whole body analysis, g/100 g protein gain							
Rumsey et al. (1985)	Hereford steers	70% corn	0.17 to 7.5	0.02 to 0.50	0.20 to 1.40	0.10 to 0.80	—
Whole body analysis, g/d							
Ellenberger et al. (1950)	Dairy cattle mix	Varied dairy	4.2 to 9.4	2.1 to 5.4	—	—	—
Apparent retention, g/d							
Kegley et al. (1991)	Hereford steers	80% corn silage	5.9 to 12.0	0.3 to 4.5	0.4 to 1.4	—	—
Knowlton et al. (2001)	Lactating Holstein cows	33% corn; 26% corn silage	13.0 to 27.8	7.0 to 18.1	−1.1 to 2.3	—	—
Erickson et al. (2000)	Beef steers	33% corn; 20% corn bran	—	6.1 to 6.7	—	—	—
Delaquis and Block (1995)	Dry dairy cows	70% alfalfa haylage	—	—	—	—	7.0 to 8.4

(Duncan, 1958). Values for mineral retention reported in the literature are shown in Table 6. Calcium, P, and K retention in the current experiment, utilizing Holstein steers, were greater than most other reported values; data on K and S retention are very limited. Retention of S measured in the current experiment was below values found in the literature. Magnesium retention measured in the current experiment was within the range reported in literature.

Variation in reported values for mineral retention across experiments is due to differences in age and sex of cattle measured, diets fed, and variation in techniques used to measure mineral retention including serial harvest protocols, sampling error, and mineral assays. The two experiments summarized by Rumsey et al. (1985), using serial harvest techniques to measure mineral retention, reported much lower values for Ca, P, and K retention than the current experiment, or Ca and

Table 7. Main effect of zilpaterol hydrochloride (ZH) inclusion in the finishing diet of Holstein steers on apparent mineral retention within the whole body using only the first four slaughter groups

	CON[1]	ZH	SEM	*P*-value[2]		
				Trt	DOF	Int
Calcium[3]						
g/d	33.4	30.5	5.8	0.48	0.56	0.89
g/kg EBW gain	24.0	20.3	3.7	0.17	0.60	0.44
g/100 g protein gain	17.0	12.5	5.6	0.27	0.69	0.67
Phosphorus						
g/d	17.1	16.5	2.9	0.77	0.49	0.89
g/kg EBW gain	12.2	11.0	1.9	0.39	0.49	0.73
g/100 g protein gain	8.7	6.7	2.8	0.31	0.74	0.71
Magnesium						
g/d	1.1	1.1	0.45	0.94	0.28	0.63
g/kg EBW gain	0.14	0.13	0.04	0.57	0.38	0.33
g/100 g protein gain	0.50	0.42	0.19	0.55	0.84	0.87
Potassium						
g/d	2.5	4.0	0.69	<0.01	0.05	0.01
g/kg EBW gain	1.6	2.7	0.54	0.01	0.20	0.06
g/100 g protein gain	1.3	1.5	0.44	0.55	0.87	0.94
Sulfur						
g/d	1.9	2.6	0.67	0.12	0.30	0.23
g/kg EBW gain	0.25	0.31	0.05	0.11	<0.01	0.54
g/100 g protein gain	1.0	0.93	0.27	0.57	0.92	0.76

[1]Treatments consisted of a control group (**CON**) and cattle fed zilpaterol hydrochloride for 20 d followed by a 3-d withdrawal, immediately before harvest (**ZH**).
[2]*P*-values include main effects of treatment (CON or ZH) and days on feed (DOF) and the interaction between treatment and DOF (Int).
[3]Apparent mineral retention was calculated from mineral composition of five steers harvested on day 226 of the finishing period with five steers/treatment every 28 d after that, for four more harvest points.

P retention reported by Ellenberger et al. (1950). The authors point out the low values for total gain represented by ash in these studies, but reanalyzing all of the samples verified their results. The substantial amount of labor and time required to coordinate serial harvest experiments increases the opportunity for error.

Variation in breeds

When using serial harvest techniques, identifying a statistically similar group of cattle to represent all cattle in the experiment is key to collecting meaningful data. Among experiments, there is also animal-to-animal variation including differences in cattle breed, sex, size, age, and rate of gain. Breed differences, specifically dairy versus beef breeds; impact the bone-to-lean ratio of cattle which can influence overall mineral retention. Breed can also impact mineral metabolism, with differences within both dairy (Du et al., 1996) and beef breeds (Littledike et al., 1995; Ward et al., 1995). In addition, changes to the Holstein breed in the past 70 yr have been substantial and selection for milk production would be expected to influence body composition and mineral requirements for animals. A recent comparison of contemporary Holstein cattle and cattle from an unselected Holstein line since 1964 reported differences in the genome of the two populations affecting milk production, fertility, and immune function (Ma et al., 2019). These changes would also be expected to impact mineral composition of the samples collected by Ellenberger et al. (1950) compared to animals in the current experiment.

Variation in fat and bone tissues

Holstein steers in the current experiment were on feed for up to 534 d and final slaughter groups approached 35% EBF (McEvers, 2014) . Across the last 10 yr, EBF has been steadily increasing in harvested cattle with 28% EBF a common endpoint historically (NRC, 1996), but 31% or greater more common in recent years. Cattle from the 4th slaughter group (112 DOF) averaged 28.7% EBF (McEvers, 2014). Apparent mineral retention at this point was 13.7 g Ca, 7.1 g P, 0.40 g Mg, 1.2 g K, and 0.89 g S/100 g protein gain. Effect of treatment on apparent mineral retention using only the first four slaughter groups is shown in Table 7. The final slaughter group (308 DOF) had especially high retention of minerals when expressed relative to protein gain due to increasing fat deposition and little protein deposition at the end of the finishing period. This demonstrates that Ca, P, Mg, K, and S retention are not purely coupled to protein accretion. Expressing Ca and P requirements relative to skeletal growth may be less variable, as over 90% of these minerals are contained in the bone tissue. However, skeletal growth is not well modeled or easily measured, making requirements relative to skeletal growth not very meaningful. In this dataset, weight of the skeletal tissues linearly increased throughout the experiment from 84 to 119 kg ($P < 0.01$). Cattle were taken to a finishing point beyond typical feedlot cattle (534 DOF and 35% EBF), yet skeletal growth was still occurring. Data collected by Ellenberger et al. (1950) included mature cows (up to 12 yr of age), which may partially explain lower retention of Ca and P in their dataset as skeletal growth was complete.

Variation in sampling procedures

Ellenberger et al. (1950) recovered all bones from each carcass for mineral analysis. They found no large differences in concentration of Ca and P within the skeleton compared to that of one femur, but recommended analyzing the entire carcass to decrease variation in results. Likewise, Duncan (1958) concluded that samples from one bone should not be considered representative of the entire skeleton. Other researchers have reported variable results when using one bone to represent P mineral status, possibly due to variation in P concentration between bones (Shupe et al., 1988; Ternouth, 1990; Geisert et al., 2010). Mineral concentration of bone tissue in the current experiment was predicted from femur samples. Long bones are the last to exhibit Ca and P resorption when animals are fed a diet deficient in Ca or P (Duncan, 1958; Ternouth, 1990). In the current experiment, if concentration of Ca and P of the femur were not representative of the entire carcass, retention could have been over or under-predicted, as bone tissue made up over 90% of entire body Ca and P reservoirs. However, results were very similar to values in the literature for concentration of Ca and P of the bone. Wu et al. (2001) concluded that concentrations of Ca and P within bone ash are relatively constant at 36% for Ca and 17% for P; Geisert et al. (2010) measured phalanx bone ash to be 17.9% P and metacarpal bone ash to be 17.3% P. Current results averaged 36% of bone ash as Ca and 17% as P across both treatments and DOF. Reported measures of Ca in bone range from 15.9% to 23.0% on a DM basis (Ellenberger et al., 1950; Field et al., 1974; Williams et al., 1991; Beighle et al., 1993). Samples from the current experiment averaged 15.7% Ca on a DM basis. Reported measures of P in bone range from 7.5% to 10.9% of DM (Ellenberger et al., 1950; Cohen, 1973; Beighle et al., 1993; Wu et al., 2001). Samples from the current experiment averaged 7.4% P on a DM basis. Mineral assays used to measure Ca and P have evolved since the 1940s when Ellenberger et al. (1950) were collecting data. However, the current experiment had very similar concentrations of Ca and P of the bone as values reported in the literature.

Concentration of minerals within bone tissue, and bone sampling protocols, would have less effect on K and S retention, as a majority of these minerals are deposited in lean tissue. Magnesium retention could be highly impacted by bone sampling protocol, but Mg retention measured in the current experiment was consistent with values found in the literature.

Mineral requirements

Maintenance requirements are very difficult to measure, especially in young, growing calves where growth and maintenance are not easily separated (Hansard et al., 1957; Tillman et al., 1959). Digestibility of minerals can be variable depending on feed source, amount in the diet, interactions with other minerals in the diet, and age of animals (Hansard et al., 1957; Karn, 2001; Ekelund et al., 2006). Availability of minerals potentially has a larger influence on amount of minerals required in the diet than any other factor (NRC, 2001). Mineral requirements for gain, which were measured as apparent retention of minerals in the current experiment, are used in conjunction with availability to calculate amount of minerals to be included in the diet.

Measures of Ca and P retention in the whole body from the current experiment were greater than requirement for gain values currently used by the NASEM (2016), suggesting that animals' ability to retain or store Ca and P is greater than requirements to support growth. Geisert et al. (2010) observed a tendency (P = 0.07) for a linear increase in phalanx bone P of feedlot heifers, as P in the diet increased from 0.10% to 0.38% of diet DM, but no differences in concentration of P within metacarpal bones. Cohen (1973) observed linear increases in bone P of yearling beef steers, as pasture P content increased. However, cattle in the Cohen (1973) experiment were grazing pastures that were most likely not meeting P requirements (0.04 to 0.11% P) when they observed linear increases in bone P as pasture P content increased. They hypothesized that the relationship would have become a sigmoid curve had P supplements been provided. Based on the diet fed and NASEM (2016) P requirements, cattle in the current experiment were not P deficient, and most likely had not been P deficient at any point prior to the experiment, suggesting that retention of these minerals is equal to gain requirements. When cattle have been fed at levels below requirements, due to large requirements such as a lactating cow, or due to poor quality feed, a period of bone repair may follow with increased Ca and P retention, when requirements subside or amount available is increased (Ekelund et al., 2006). These periods are not typical of cattle that have been fed adequate amounts of mineral, and may not be indicative of normal requirements. At present, the definition of requirement for gain is the amount of minerals retained in the body, regardless of whether there is a performance response to inclusion in the diet.

Conclusions

Calculating apparent mineral retention relative to protein gain (g/100 g protein gain) resulted in no statistical differences due to treatment ($P \geq 0.14$) or DOF ($P \geq 0.11$). These data suggest that in the current experiment, protein gain and mineral retention were closely related, and expressing mineral retention on a protein gain basis minimized differences due to rate and type of gain, resulting in more uniform recommendations. This appears to be true for cattle on high concentrate finishing diets during a normal feeding period. Cattle in the current experiment were on feed for an extended period of time and as cattle fattened (up to 35% EBF), mineral retention relative to protein gain was quite high, due to decreasing amounts of protein accretion. Discrepancies between this and previous mineral retention work could be due to differences in methods used to analyze minerals, animals' ability to retain more minerals than required to support maximum growth performance, differences in metabolism of minerals by different breeds, or ongoing skeletal growth throughout the experiment. The NASEM (2016) recommendations for mineral requirements for gain are based on the assumption that retention is equal to the gain requirement. No differences in apparent Ca, P, Mg, K, and S retention relative to protein gain due to zilpaterol hydrochloride supplementation suggest current mineral requirement recommendations are adequate and separate mineral requirements for cattle fed β-adrenergic agonists are not required.

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

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