Effect of Zinc Source and Level on Finishing Cattle Performance, Carcass Characteristics, and Adipocyte Differentiation

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Story in Brief

The objective of the present experiments was to determine the effects of zinc source and level on feedlot performance and carcass characteristics, and to determine the effects of zinc source and level on growth and differentiation of primary adipocytes in cell culture. For performance response variables, overall body weight, average daily gain, dry matter intake, and feed efficiency did not differ among treatments. Although not statistically significant, when steers were fed 90 ppm Zn, marbling score was 4.0% greater and 12th-rib fat was 21.8% lower for steers fed organic compared with inorganic Zn. In vitro, results suggest that soluble Zn methionine decreased expression of peroxisome proliferator-activated receptor-γ and stearoyl CoA desaturase in cell cultures of stromal-vascular cells isolated from intramuscular and subcutaneous fat depots compared with inorganic Zn. However, more time intervals between 0 and 24 h and more Zn concentrations between 0 and 5 µM should be evaluated before conclusions are drawn.

Key Words: Adipogenesis, Carcass Quality, Cattle, Feedlot Performance, Zinc

Introduction

Due to the potential for increased bioavailability from amino acid complexed Zn (Engle et al., 1997), addition of organic sources of Zn to feedlot cattle diets seems favorable. Greene et al. (1988) reported higher marbling scores and carcass quality for steers fed Zn methionine (360 mg/kg of Zn) compared with steers fed ZnO (360 mg/kg of Zn) or steers fed a control diet (82 mg/kg of Zn). The increase in intramuscular fat deposition with Zn methionine supplementation was mirrored by increased KPH and external fat (Greene et al., 1988). McBeth et al. (2002) recently fed 336 crossbred steers to determine the effects of Zn level and source on feedlot performance and carcass merit. Thirty ppm of added Availa®Zn resulted in similar or greater performance compared with 30 ppm of added ZINPRO® Zn methionine, and numerically greater improvements in performance were observed when 60 ppm of added Availa Zn was added to the basal diet compared with 60 ppm of added ZnSO4. When 120 ppm of total dietary Zn was fed, 50:50 ZnSO4:AvailaZn resulted in 1.6% greater daily gain, 2.7% lower DMI, 4.2% improved efficiency, and 28.2% more choice carcasses than when 120 ppm from ZnSO4 was fed. Adding 30 ppm of Zn methionine to the control diet (60 ppm Zn from ZnSO4) resulted in similar carcass advantages; however, feedlot performance was numerically lower compared with feeding 90 ppm total dietary Zn from ZnSO4. These results suggested increases in daily gain and DMI with increasing supplemental Zn. In addition, numeric advantages in feedlot performance and carcass traits were observed when a combination of Availa Zn and ZnSO4 were fed.

Research regarding the effects of Zn on fat metabolism and adipogenesis in ruminants is limited. Tanaka et al. (2001) reported increases in the specific activity of glycerol phosphate dehydrogenase with the addition of 1 µM of Zn in adipocyte culture with or without the addition

of insulin to the medium. Similarly, the same researchers reported that addition of fattened cattle serum to the growth medium increased the Zn concentration of the medium from 1 µM to 3 µM suggesting that Zn concentration in the medium reflects the adipogenic activity in cattle serum. Murine and in vitro models have also indicated that Zn has the ability to enhance adipogenesis. Shisheva et al. (1992) reported that addition of Zn to both growth media and differentiation media increased the incorporation of glucose into lipids in vitro and illustrated this effect both independent of insulin and additively with insulin. It has been demonstrated in ruminants that insulin sensitivity is decreased with age and carcass fat accumulation (McCann et al., 1986; Eisemann et al., 1997). This relationship between insulin sensitivity and Zn could offer some explanation into the mechanism of increased fat deposition with Zn supplementation.

The purpose of the present experiments was to determine the effects of zinc source and level on feedlot performance and carcass characteristics, and to determine the effects of zinc source and level on growth and differentiation of bovine stromal-vascular cells.

Materials and Methods

Experiment 1. One hundred sixty crossbred steers (avg initial BW = approximately 320 kg) were delivered to the Willard Sparks Beef Research Center near Stillwater, OK on March 14, 2003. On arrival, steers were individually weighed and ear tagged. On the following day, steers were horn tipped as needed, implanted with Revalor-S (Hoechst Roussel Vet, Clinton, NJ), vaccinated with IBR-PI3-BVD-BRSV (Titanium 5, Intervet, Millsboro, DE), vaccinated with a seven-way clostridial preparation (Vision 7, Intervet, Millsboro, DE), and treated for control of external and internal parasites (Ivomec-Plus injectable, Merial, Duluth, GA). Steers were blocked by initial BW into eight weight blocks. Body weight (unshrunk) taken on the day of arrival (d 0) was considered initial weight. Within block steers were assigned randomly to 4 pens (5 steers/pen; 8 pens/treatment).

Treatments included: 1) 60 ppm ZnSO4 (control); 2) control plus 30 ppm ZnSO4; 3) control plus 30 ppm Zn®Methionine; and 4) control plus 30 ppm Availa®Zn. The basal diet included (DM basis) rolled corn (76.5%), ground alfalfa (10.0%), molasses (4.0%), yellow grease (2.0%), and a pelleted supplement (7.5%), and was formulated to meet or exceed NRC (1996) nutrient requirements. Monensin (33 mg/kg of diet) and tylosin (11 mg/kg of diet) were fed. Steers were gradually adapted to the final 90% concentrate diet by offering 65, 75, and 85% concentrate diets for 7 d each. Feed refused was weighed at 28-d intervals and as needed (e.g., following inclement weather). In addition, diet and ingredient samples were collected, and DM samples were composited by 28-d periods, allowed to air dry, and ground in a Wiley mill to pass a 1-mm screen. Diet samples were analyzed for N, starch, ash (AOAC, 1996), ADF (Goering and Van Soest, 1970) and Zn. Interim unshrunk BW was determined at 28-d intervals. Steers were slaughtered at a commercial facility. Hot carcass weight, external fat, internal fat, longissimus muscle area, marbling score, yield grade and quality grade were determined.

Data for BW, DMI, ADG, feed efficiency and normally distributed carcass characteristics were analyzed as a randomized complete block design using the Proc Mixed procedure of SAS Release 8.02 (SAS Institute Inc., Cary, NC). The model included treatment, and block was included as a random variable. Pen served as the experimental unit. Pre-planned comparisons

were: 1) Zn level (60 vs 90 ppm); 2) inorganic vs organic Zn; and 3) Zn Methionine vs Availa Zn.

Experiment 2. Our goal was to determine if Zn would increase the expression of genes involved with fat metabolism in cultured stromal-vascular cells harvested from subcutaneous and intramuscular adipocytes. Intramuscular and subcutaneous adipose tissues were collected from steers harvested at the Food and Agricultural Products Center and placed in sterile Hanks balanced salt solution containing 250 ng/mL amphotericin B and 0.2 mg/mL gentamycin for transportation to the laboratory (Ohyama et al., 1998). In the lab, tissue from each depot was digested in Hanks balanced salt solution containing 1 mg/mL Type I collagenase for 1 h at 38ºC with shaking at 170 cycles/min. Subcutaneous adipose tissue was minced into sections of approximately 1 mm^2 with scissors before incubation in the digestion solution. Following digestion, the cell suspensions were filtered through 250-µm nylon mesh to remove undigested tissue and large aggregates of cells. The filtrate was centrifuged at 700 x g for 5 min and decanted to separate floating adipocytes from the pellet of stromal-vascular cells. The cell pellet was resuspended and washed three times with DMEM containing 10% FBS, 100 U/mL penicillin, 100 µg/mL streptomycin, 250 ng/mL amphotericin B, and 0.2 mg/mL gentamycin (growth medium; Ohyama et al., 1998).

The stromal-vascular cells were seeded on 12-well (22 mm diameter) tissue culture plates at a density of 1 x 104 cells/cm^2. Cells were incubated in the growth medium at 37ºC under a humidified atmosphere of 95% air and 5% CO_2. After reaching confluence, the growth medium was replaced with the differentiation medium composed of 1:1 (vol:vol) mixture of DMEM/Ham's F12 media containing 10 µg/mL bovine transferrin, 17 µM biotin, 10 µg/mL bovine insulin, 100 U/mL penicillin, and 100 µg/mL streptomycin with or without an inorganic or organic Zn concentration of 5 µM. Cells were cultured in these differentiation media for 0, 12 or 24 h. Total cell extracts were collected and RNA extracted with 4 mL of TRIzol™ reagent (Gibco, Grand Island, NY).

We used a PCR-based assay to determine mRNA expression of peroxisome proliferator-activated receptor-γ (PPARγ) and stearoyl CoA desaturase (SCD-1). Expression of these genes was evaluated by quantitative RT-PCR utilizing the fluorescent reporter and 5' exonuclease assay system (TaqMan®, PE Biosystems, Foster City, CA). Briefly, reverse transcription of total RNA and PCR amplification were performed using the TaqMan® One-Step RT-PCR Master Mix Reagents Kit, TaqMan® fluorescent probe, and sequence detection primers (PE Biosystems). TaqMan® probe specific for target was designed to contain a fluorescent 5' reporter dye (TET) and 3' quencher dye (TAMRA). Each one-step RT-PCR reaction (25 µL) contained 2X Master Mix without uracil-N-glycosylase (12.5 µL), 40X MultiScribe® and RNAse Inhibitor Mix (0.625 µl), target forward primer (200 nM), target reverse primer (200 nM), and the fluorescent labeled target probe (200 nM) designed from the mRNA sequence for bovine PPARγ and SCD-1. The PCR amplification was carried out in the ABI PRISM® 7700 Sequence Detection System (PE Biosystems). Thermal cycling conditions were 50°C for 2 min, 95°C for 10 min followed by 40 repetitive cycles of 95°C for 15 sec and 60°C for 1 min. As a normalization control for RNA loading, parallel reactions in the same multiwell plate were performed using 18S ribosomal RNA as target (18S Ribosomal Control Kit, PE Biosystems).

Data were analyzed as a complete randomized design using the Mixed procedure of SAS. The model included terms for fat depot, Zn source, Zn level, time, and the appropriate interactions.

Results and Discussion

Experiment 1. Effect of Zn source and level on feedlot cattle performance is shown in Table 1. Overall (d 0 through 139) body weight, average daily gain, dry matter intake, and feed efficiency did not differ among treatments. From d 85 to 112, average daily gain was greater (P=.07) for steers fed 60 ppm Zn compared with 90 ppm Zn. From day 113 to 139, steers fed Zn methionine had greater (P=.005) average daily gain than steers fed Availa Zn. Steers fed Availa Zn were more (P=.01) efficient than steers fed Zn methionine from d 85 to 112, whereas steers fed Zn methionine were more (P=.05) efficient than steers fed Availa Zn from d 113 to 139. Overall, steers fed 90 ppm of organic Zn were 2.6% more (P=.16) efficient than steers fed inorganic Zn.

Effects of Zn source and level on carcass characteristics is shown in Table 2. Hot carcass weight, marbling score, 12th-rib fat depth, longissimus muscle area, % kidney, pelvic and heart fat, and USDA Yield Grade did not differ among treatments. Although not statistically significant, when steers were fed 90 ppm Zn, marbling score was 4.0% greater and 12th-rib fat was 21.8% lower for steers fed organic compared with inorganic Zn.

Experiment 2. In cultured adipocytes, appearance of PPARγ is followed by expression of many genes representing the mature adipocyte phenotype. Because recent research indicates that Zn may promote adipogenesis (Tanaka et al., 2001), we hypothesized that zinc might stimulate the expression of PPARγ and subsequently genes commonly found in mature adipocytes (e.g., SCD-1, which catalyzes the rate-limiting step in cellular synthesis of monounsaturated fatty acids such as oleate [C18:1] and palmitoleate [C16:1]). Based on work in feedlot cattle, we also hypothesized that differences might occur between inorganic and organic sources of Zn. Figure 1 shows the effect of time, Zn source, and Zn level on fold expression of PPARγ from subcutaneous and intramuscular fat cells grown in primary culture (time x Zn source x Zn level interaction, P<.05). Inorganic Zn did not affect fold expression of PPARγ at 12 or 24 h. However, 5 μM of organic Zn decreased expression of PPARγ at both 12 and 24 h. Expression of PPARγ was decreased at 24 h compared with 12 h of incubation. At 12 h, expression of SCD-1 was increased fourfold compared with no Zn, whereas organic Zn decreased expression of SCD-1 approximately twofold (Figure 2). A similar response was observed at 24 h, although expression of SCD-1 was decreased at 24 h when both sources and levels of Zn were included in the differentiation media. These results suggest that soluble Zn methionine decreased expression of PPARγ and SCD-1 in cell cultures of stromal-vascular cells isolated from intramuscular and subcutaneous fat depots. However, more time intervals between 0 and 24 h and more Zn concentrations between 0 and 5 μM should be evaluated before conclusions are drawn.

Table 1. Effects of zinc level and source on feedlot cattle performance

Item	60 ppm ZnSO4	60 ppm ZnSO4			SEM	Contrasts		
		30 ppm ZnSO4	30 ppm Availa Zn	30 ppm Zn Met		60 vs 90 ppm	Inorganic vs Organic	Availa Zn vs Zn Methionine
BW								
Initial	786	783	783	783	14.7	--	--	--
d 139	1312	1297	1297	1317	22.5	.47	.84	.19
Daily gain, lb								
d 0 – 28	4.10	3.96	4.08	3.90	.23	.61	.85	.56
d 29 – 56	4.35	4.41	4.44	4.36	.22	.83	.91	.78
d 57 – 84	3.98	3.91	3.74	4.08	.22	.79	.88	.28
d 85 – 112	3.90	3.34	3.64	3.52	.18	.07	.82	.65
d 113 – 139	2.49	2.74	2.46	3.20	.17	.14	.23	.005
d 0 – 139	3.79	3.70	3.70	3.84	.08	.58	.74	.17
DM intake, lb/d								
d 0 – 28	22.3	21.8	21.6	21.7	.70	.30	.44	.84
d 29 – 56	22.1	22.2	21.8	21.9	.74	.85	.59	.90
d 57 – 84	23.9	23.0	22.8	23.2	.56	.11	.39	.54
d 85 – 112	24.2	23.8	22.9	23.8	.57	.29	.24	.20
d 113 – 139	24.5	24.7	24.5	25.4	.52	.47	.44	.22
d 0 – 139	23.4	23.1	22.7	23.2	.49	.34	.41	.32
Feed:Gain, lb/lb								
d 0 – 28	5.53	5.63	5.31	5.63	.22	.96	.60	.30
d 29 – 56	5.14	5.14	4.94	5.05	.21	.70	.50	.71
d 57 – 84	6.21	5.92	6.25	5.73	.32	.51	.82	.25
d 85 – 112	6.24	7.18	6.44	6.82	.24	.30	.25	.01
d 113 – 139	10.63	9.21	10.57	7.97	.89	.19	.47	.05

d 0 – 139	6.17	6.26	6.14	6.05	.09	.84	.16	.40

Table 2. Effects of zinc level and source on carcass merit

		60 ppm ZnSO4				Contrasts		
Item	60 ppm ZnSO4	30 ppm ZnSO4	30 ppm Availa Zn	30 ppm Zn Met	SEM	60 vs. 90 ppm	Inorganic vs Organic	Availa Zn vs Zn Methionine
Hot carcass wt., lbs	832	833	837	844	15.0	.54	.35	.60
Marbling a	395	374	382	396	27.0	.72	.87	.71
12th-rib fat, in.	.56	.67	.54	.55	.05	.64	.16	.95
Ribeye area, in2	14.03	13.59	14.29	13.96	.40	.84	.39	.54
KPH, %	1.93	1.97	2.00	1.94	.06	.62	.75	.52
Yield grade	2.98	3.40	2.87	2.98	.20	.62	.20	.70

a300 = slight, 400 = small

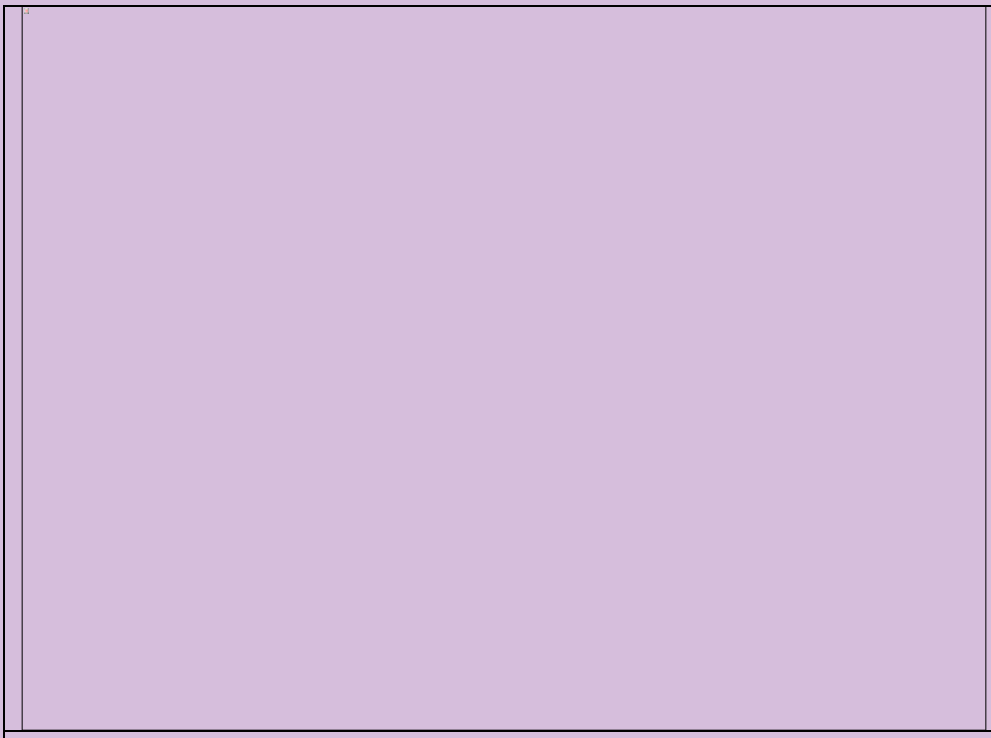

Figure 1. Effect of time after dosing (12 or 24 h), Zn source (organic or inorganic) and Zn level (0 or 5 μM) on fold differences in PPARγ gene expression in subcutaneous and intramuscular primary cell cultures

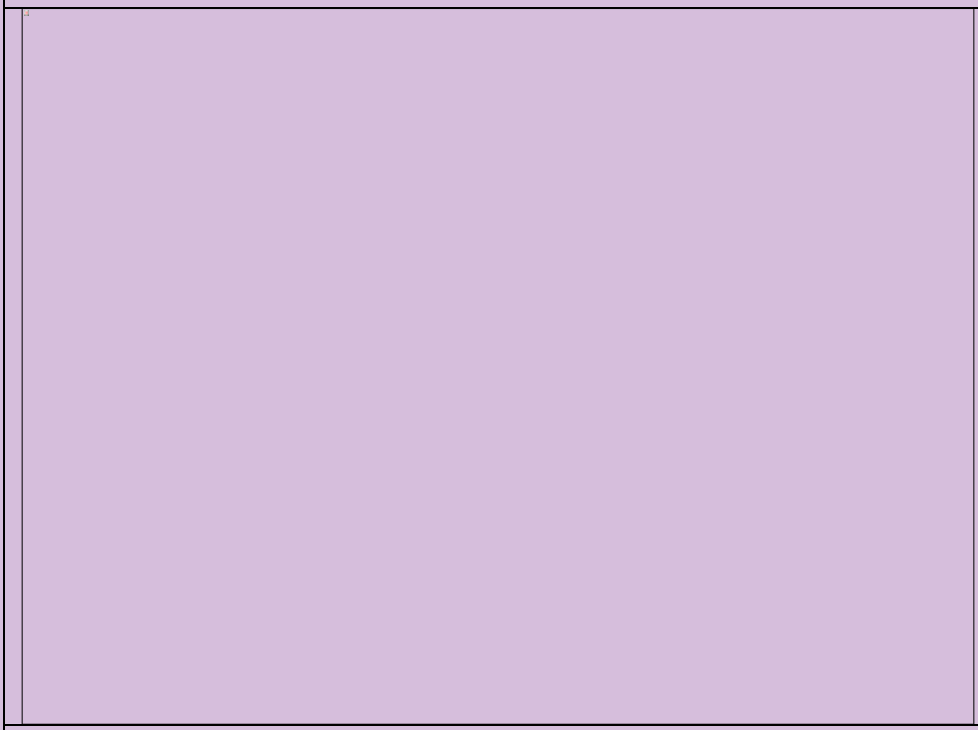

Figure 2. Effect of time after dosing (12 or 24 h), Zn source (organic or inorganic) and Zn level (0 or 5 μM) on fold differences in stearoyl-CoA desaturase gene expression in subcutaneous and

intramuscular primary cell cultures

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