

# Influence of Live Weight Gain and Calcium Salts of Conjugated Linoleic Acid on Growth Performance and Immune Function of Growing Cattle

*H. Flórez-Díaz<sup>1</sup>, E.B. Kegley<sup>1</sup>, G.F. Erf<sup>2</sup>, D.L. Kreider<sup>1</sup>, K.P. Coffey<sup>1</sup>, N.D. Luchini<sup>3</sup>, and S.L. Krumpelman<sup>1</sup>*

## Story in Brief

Forty-eight crossbred beef calves (initial BW = 772 lb) were used to determine the effects of live weight gain and conjugated linoleic acid (CLA) on immunity and growth performance during a 56-d study. Calves were blocked by weight, stratified by gender, and assigned to 16 pens. Pens within each block were assigned randomly to 1 of 4 diets arranged as a 2 x 2 factorial. Main effects were rate of gain (diets formulated for calves to gain 1.5 [L] or 3.0 [H] lb/d), and fatty acid source (4% Ca salts of palm oil [PO] or 4% Ca salts of CLA). Feeding CLA tended to increase ( $P = 0.07$ ) ADG from d 1 to 56, and decrease ( $P = 0.06$ ) F/G from d 29 to 56 in cattle fed L diets, and tended to decrease the percentage of monocytes ( $P = 0.09$ ) and eosinophils ( $P = 0.06$ ) in H diets. Total white blood cell ( $P = 0.02$ ) and lymphocyte concentrations ( $P = 0.05$ ) were greater in L vs. H diets. Feeding CLA decreased the proliferation of peripheral blood mononuclear cells (PBMC) stimulated with phytohemagglutinin (PHA;  $P = 0.04$ ) or concanavalin A ( $P = 0.03$ ) in H but not in L diets. Responses of PBMC to PHA were lower ( $P = 0.003$ ) in L than in H diets. Monocyte/macrophage phagocytosis was not affected by CLA or rate of gain ( $P > 0.22$ ). In conclusion, CLA may increase growth performance and modulate immunity in growing cattle but responses depend on time and rate of growth.

## Introduction

Research utilizing different intake levels of concentrate or forage based diets have demonstrated that growing programs in beef cattle can affect body composition and future feedlot performance. Conjugated linoleic acid (CLA), a mixture of positional and geometric isomers of the omega-6 essential fatty acid linoleic acid, is produced by ruminal biohydrogenation (Belury, 2002). Supplemental CLA increased feed efficiency (Chin et al., 1994), decreased subcutaneous fat thickness, and increased lean tissue in steers (Gassman et al., 2000). Several studies in animals and humans have also reported effects of CLA on immunity. Conjugated linoleic acid supplementation resulted in increased T-cell proliferation and enhanced production of interleukin 2 (IL-2), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and immunoglobulin (Ig) A, IgM, and IgG (Bontempo et al., 2004). The combination of feeding for different rates of weight gain and CLA supplementation may result in alterations in growth performance and immune function of growing beef cattle. The objective of this research was to determine the effects of feeding total mixed diets for 2 rates of live weight gain and supplemental Ca salts of CLA isomers on immune response and growth performance of cattle.

## Experimental Procedures

Forty-eight crossbred beef heifer ( $n = 12$ ) and steer ( $n = 36$ ) calves (13 to 14 mo of age,  $772 \pm 67.9$  lb initial BW) of predominantly Angus breeding were obtained from the University of Arkansas cow-calf facility in Savoy for a 56-d growing trial. Animals were stratified by body weight (4 blocks) within gender and assigned randomly to pens with 3 animals per pen and 4 pens per dietary treatment (12 animals per dietary treatment). Dietary treatments (Table 1) were assigned in a completely randomized design with a 2 x 2 factorial arrangement. The 4 diets were: low live weight gain (1.5 lb/d) with 4% rumen-protected Ca salts of palm fatty acid

distillate (LPFA; EnerGII, Virtus Nutrition, LLC, Corcoran, Calif.); low live weight gain with 4% rumen-protected Ca salts of mixed isomers of CLA (LCLA; Virtus Nutrition, LLC); high live weight gain (3.0 lb/d) with 4% Ca salts of palm fatty acid distillate (HPFA); and high live weight gain with 4% Ca salts of mixed isomers of CLA (HCLA). Diets were balanced to obtain the desired rates of gain according to model 1 NRC net energy equations (NRC, 1996). Fat supplements were mixed with the concentrates and offered as part of the total ration to experimental animals. Diets were formulated to result in the desired rates of gain when feeding a constant amount of DM (17.5 lb/d) and were fed once daily at approximately 0800 h. Calves were adapted to the diets over a 15-d period, by increasing the proportion of concentrate fed by 20% every 3 d until the desired ration composition was reached.

Animals were weighed on d 0, 14, 28, 42, and 56. On d 0, 28, and 56, jugular blood samples were collected from 24 steers (6/treatment) to determine proportions and concentration of total white blood cells, as well as to determine lymphocyte proliferation responses to phytohemagglutinin (PHA; Sigma Chemical Co., St. Louis, Mo.), concanavalin A (CONA; Sigma Chemical Co.) and pokeweed mitogen (PWM; Sigma Chemical Co.), and the phagocytic ability of harvested monocytes/macrophages.

In order to determine the effect of dietary treatments on the immune function of calves, concentrations (number of cells/ $\mu$ L) of total white blood cells (WBC), and differential WBC (lymphocytes, neutrophils, monocytes, eosinophils, basophils) were analyzed on an automated hematology analyzer (Cell-Dyn 3500 system, Abbott Laboratories, Abbot Park, Ill.) standardized for analysis of bovine blood. For the in vitro proliferation of peripheral blood mononuclear cell (PBMC) response, polymorphonuclear cells were isolated from whole blood by density gradient centrifugation, then resuspended in cell culture medium (RPMI 1640) supplemented with 10% fetal bovine serum at  $2 \times 10^6$  cells/mL and plated in triplicate in 96-well round-bottom plates with  $2 \times 10^5$  cells/well. Mitogens used were phytohemagglutinin (PHA; Sigma Chemical Co.), pokeweed mitogen (PWM; Sigma Chemical Co.), and concanavalin A

<sup>1</sup> Department of Animal Science, Fayetteville

<sup>2</sup> Department of Poultry Science, Fayetteville

<sup>3</sup> NutriScience Technologies, Inc., Fairlawn, Ohio

(ConA; Sigma Chemical Co.). Mitogens were diluted in RPMI medium (Sigma Chemical Co.) and administered in 50- $\mu$ L aliquots/well at a concentration of 5, 15, and 20  $\mu$ g/mL, respectively. Cultures not stimulated with mitogen received 50  $\mu$ L of medium/well in place of mitogen. Cell cultures were incubated for 48 h at 39.2°C and 5% CO<sub>2</sub>. After this time, 1  $\mu$ Ci of tritiated-thymidine was added to each well and cultures incubated for an additional 18 h. Cells were harvested on glass fiber mats and the mats were transferred to scintillation vials, then the radioactivity (cpm) was measured on a liquid scintillation analyzer. Differences in cpm of stimulated (mitogens) and control (medium only) were calculated by subtracting the arithmetic mean of cpm from triplicate control cultures from the arithmetic mean of cpm from the corresponding stimulated cultures. The results were referred to as “ $\Delta$  cpm”. Stimulation indices (SI) were calculated by dividing the mean counts per minute (cpm) of the triplicate mitogen-stimulated cultures by the mean cpm of the unstimulated control cultures.

Monocyte/macrophage phagocytosis of pig red blood cells (PRBC) was measured by diluting  $2 \times 10^6$  isolated PBMC/mL in Leibovitz's L-15/McCoy's Hahn medium. A glass cover slip was added to each well of a six-well plate and 2 mL of cell suspension was added to each well in duplicate for each sample. Cells were incubated for 8 h at 102.6°F and 5% CO<sub>2</sub>. Following the 8-h incubation period, medium from each well was removed and replaced by 2 mL of fresh LM Hahn medium. Plates were incubated for an additional 24 h at 102.6°F and 5% CO<sub>2</sub>. Following incubation, 2 mL of a 5% PRBC suspension were added to each well. Plates were incubated with PRBC for 8 h at 102.6°F and 5% CO<sub>2</sub>. After this time, cover slips were removed and stained. Phagocytic ability of monocytes/macrophages was determined by observing 200 monocytes/macrophages per coverslip and recording the number of monocytes/macrophages that had phagocytosed PRBC and the number of PRBC that had been engulfed per monocyte/macrophage.

Data from growth performance and monocyte/macrophage phagocytosis were analyzed using the GLM procedure of SAS (SAS Inst., Inc., Cary, N.C.). The model included the effects of fat source, rate of weight gain, block, and their interactions. Blood cell proportions and concentrations and lymphocyte blastogenesis were evaluated using the MIXED procedure of SAS. Fixed effects included fat source, rate of gain, block, time, and the fat source  $\times$  rate of gain interaction. Pen was used as the experimental unit.

## Results and Discussion

The ADG (1.9 lb/d) of cattle fed the high-energy diets (HPFA and HCLA) was greater ( $P = 0.002$ ) than the ADG (1.4 lb/d) of animals fed the low energy diets (LPFA and LCLA) from d 1 to 56 of the growing phase. In addition, the F/G of HPFA and HCLA groups (9.7) was less ( $P = 0.02$ ) than the F/G (14.6) for cattle in the LPFA and LCLA groups from d 1 to 56.

The predicted growth rate for animals fed the high-energy diet was greater than that observed in the present experiment (3 vs. 1.9 lb/d), but in animals fed the low energy diet the predicted growth was similar to the actual growth rate (1.5 vs. 1.4 lb/d). This may be the result of a constant feed intake during the period of experimentation without adjusting the intake to meet the increasing energy needs for maintenance as the animals grew. In addition, calves were adapted to the diets over a 15-d period, and performance during this period negatively impacted the overall ADG of calves fed the high-energy diet.

Calcium salts of CLA tended to increase ADG from d 1 to 56 (fat source  $\times$  rate of gain interaction,  $P = 0.07$ , Table 2) and decreased F/G from d 29 to 56 (fat source  $\times$  rate of gain interaction,  $P = 0.06$ ) when supplemented to animals with a low rate of live weight gain, but had no impact when supplemented to animals with high rate of gain. Effect of CLA on ADG and efficiency has been reported previously in beef cattle. Angus  $\times$  Hereford heifers supplemented with diets with 2% of Ca salts of CLA had greater ADG and gain:feed was improved compared to those fed diets with 4% corn oil or control diets (Gillis et al., 2004). The mechanisms by which CLA may affect ADG and F/G in low rate of weight gain but not in high rate of weight gain animals are not clear. However, these results may be related to changes in the energy partitioning and balance between the groups. Supplementation for 9-wk with CLA in dairy cows during the transition period and early lactation induced a progressive reduction in milk fat (11 to 21%) relative to the control group, and the decrease was significant by the third week of lactation (Castañeda-Gutiérrez et al., 2005). Consistent with the reduction in milk fat, experiments supplementing CLA in early lactation cows have often reported an increase in milk yield (Bernal-Santos et al., 2003); however, this is not always observed (Castañeda-Gutiérrez et al., 2005). The milk yield response in the early lactation studies has been attributed to the greater availability of energy caused by reduction of milk energy output in a physiological state where synthesis activity in the mammary gland is up-regulated (Bernal-Santos et al., 2003).

Supplementation with CLA decreased the concentrations of circulating WBC ( $P = 0.02$ ) and monocytes ( $P = 0.04$ ). Animals fed for low growth rate (LPFA and LCLA) had greater WBC concentrations ( $P = 0.02$ ) and total circulating lymphocytes ( $P = 0.05$ ) compared to animals with a high rate of growth (HPFA and HCLA).

Supplementation with CLA tended to decrease the percentages of monocytes ( $P = 0.09$ ) and eosinophils ( $P = 0.06$ ) as well as the eosinophil concentration in animals fed for high rate of growth, but had no effect in animals with a low rate of growth (fat source  $\times$  gain interaction,  $P = 0.08$ ). There was a time effect on monocyte ( $P < 0.001$ ) and eosinophil ( $P = 0.03$ ) concentrations, and on the percentage of monocytes ( $P = 0.002$ ) and eosinophils ( $P = 0.02$ ). These values decreased from d 1 to 56 (data not shown). Diet or rate of gain did not affect neutrophil and basophil concentrations, the numbers of neutrophils, lymphocytes, and basophils, or the neutrophil:lymphocyte ratio (Table 3).

The lower concentration of leukocytes and in particular of monocytes in animals supplemented with CLA may indicate lower stimulation of the phagocytic, humoral, and cellular immune activity in these animals. Monocytes and macrophages produce pro-inflammatory cytokines (IL-1, IL-6 and TNF- $\alpha$ ) that play an important role in immunoregulation. To attenuate the negative effects of proinflammatory cytokines, CLA can be supplemented in the diet. Conjugated linoleic acid has been shown to have anti-inflammatory activities in humans, and domestic and laboratory animals by reducing the release of pro-inflammatory cytokines, or by stimulating the production of anti-inflammatory cytokines.

Conversely, the greater WBC and monocyte concentrations in LPFA and LCLA groups may suggest greater cellular and humoral immune activity in these individuals. The decrease in eosinophil concentrations and percentage of monocytes and eosinophils associated with CLA in the high rate of growth group, but not in the low rate of growth group may indicate that CLA decreased the inflammatory response differently based on energy content of the diet. Bassaganya-Riera et al. (2001) reported that CLA supplementation to early weaned pigs preceding a disease challenge prevented

disease associated growth suppression.

Supplementation with CLA with the high rate of growth diet decreased the stimulation index (SI) of PBMC stimulated with PHA ( $P = 0.04$ ) and ConA ( $P = 0.03$ ) but had no effect when fed with the low rate of growth diet (fat x gain interaction). Animals with a high growth rate had greater ( $P = 0.003$ ) proliferative responses to PHA at d 28 and 56 compared with animals with a low rate of growth. There was no effect of CLA or rate of growth on the proliferative response of unstimulated or PWM stimulated cells (Table 4).

The effects of CLA on lymphocyte proliferation in animals with a high growth rate compared to animals with a low rate of growth may indicate a differential effect of CLA based on diet energy level. In contrast with these results, research reported in humans, and laboratory and domestic animals demonstrated an increase in lymphocyte proliferation of individuals supplemented with CLA (Nugent et al., 2005).

### Implications

Results of this experiment present evidence that controlling growth rate and feeding calcium salts of conjugated linoleic acid in growing beef cattle affected growth performance and immune function. Future studies are needed to clarify the consequences of restricting rate of growth and supplementing conjugated linoleic acid during the growing phase on the performance of finishing cattle.

### Acknowledgments

The authors would like to express their sincere appreciation to P. Hornsby, G. Carte, and T. Davis for the management and care of experimental animals.

### Literature Cited

- Bassaganya-Riera, J.R., et al. 2001. *J. Nutr.* 131:2370-2377.  
 Belury, M.A. 2002. *Annu. Rev. Nutr.* 22:505-531.  
 Bernal-Santos, G., et al. 2003. *J. Dairy Sci.* 86:3218-3228.  
 Bontempo, V.D., et al. 2004. *J. Nutr.* 134:817-824.  
 Castañeda-Gutiérrez, E., et al. 2005. *J. Dairy Sci.* 88:1078-1089.  
 Chin, S.F., et al. 1994. *J. Nutr.* 124:2344-2349.  
 Gassman, K.J., et al. 2000. *J. Anim. Sci.* 78 (Suppl. 1):275-276. (Abstr.)  
 Gillis, M.H., et al. 2004. *J. Anim. Sci.* 82:851-859.  
 NRC. 1996. *Nutrients Requirements of Beef Cattle*. 7th ed. Natl. Acad. Sci.  
 Nugent, A.P., et al. 2005. *Eur. J. Clin. Nutr.* 59:742-750.

**Table 1. Ingredient composition of experimental diets fed.**

Item	Treatments <sup>a</sup>			
	Low weight gain		High weight gain	
	LPFA	LCLA	HPFA	HCLA
	% of DM			
Ingredient				
Corn, cracked	2.86	2.75	45.42	45.31
Cottonseed hulls	50.86	50.86	10	10
Soybean meal	9.94	9.94	6.86	6.86
Wheat middlings	5.71	5.71	9.14	9.14
Bermudagrass hay	24	24	20.57	20.57
Molasses, cane	1.43	1.43	2	2
Dicalcium phosphate	0.4	0.4	-	-
Limestone	0.06	0.06	0.58	0.58
Salt	0.29	0.29	0.29	0.29
Urea	0.29	0.29	0.98	0.98
Ca salts of palm oil	4	-	4	-
Ca-CLA	-	4.11	-	4.11
Monensin	+	+	+	+
Vitamin premix	+	+	+	+
Trace mineral premix	+	+	+	+

<sup>a</sup>LPFA = low rate of gain with Ca-salts of palm oil; LCLA = low rate of gain with Ca-salts of CLA; HPFA = high rate of gain with Ca-salts of palm oil; HCLA = high rate of gain with Ca-salts of CLA.

**Table 2. Influence of live weight gain and Ca salts of conjugated linoleic acid (CLA) supplementation on ADG and feed/gain.**

Item	Treatments <sup>a</sup>				SEM	<i>P</i> ≤ <sup>b</sup>		
	Low weight gain		High weight gain			Fat	Gain	Fat x Gain
	LPFA	LCLA	HPFA	HCLA				
d 1 to 28								
ADG, lb	0.66	0.93	1.14	1.07	0.08	0.29	0.03	0.11
F/G	26.92	18.81	15.35	16.38	3.97	0.98	0.05	0.40
d 29 to 56								
ADG, lb	1.98	2.08	2.74	2.66	0.04	0.76	0.001	0.13
F/G	9.31	8.63	6.57	6.88	0.16	0.34	0.001	0.06
d 1 to 56								
ADG, lb	1.32	1.51	1.94	1.87	0.05	0.32	0.002	0.07
F/G	16.49	12.65	9.99	9.35	1.04	0.22	0.02	0.12

<sup>a</sup>LPFA = low rate of gain with Ca-salts of palm oil; LCLA = low rate of gain with Ca-salts of CLA; HPFA = high rate of gain with Ca-salts of palm oil; HCLA = high rate of gain with Ca-salts of CLA.

<sup>b</sup>Fat = main effect of fat supplementation, Ca-salts of palm oil vs. Ca-salts of CLA; Gain = main effect of rate of growth, low vs. high; Fat x Gain = interaction of fat supplementation and rate of growth.

**Table 3. Influence of live weight gain and Ca salts of conjugated linoleic acid (CLA) supplementation on differential leukocyte counts (total cells and percentage of white blood cells [WBC]).**

Item	Treatments <sup>a</sup>				SEM	<i>P</i> ≤ <sup>b</sup>		
	Low weight gain		High weight gain			Fat	Gain	Fat x Gain
	LPFA	LCLA	HPFA	HCLA				
Leucocyte count, total cells × 10 <sup>9</sup> /μL								
WBC	11.8	9.7	9.7	9.1	0.46	0.02	0.02	0.14
Neutrophils	2.5	2.1	2.4	2.1	0.17	0.13	0.87	0.70
Lymphocytes	7.9	6.2	5.9	6.0	0.50	0.14	0.05	0.11
Monocytes	0.78	0.72	0.85	0.68	0.04	0.04	0.80	0.29
Eosinophils	0.51	0.51	0.52	0.23	0.07	0.08	0.08	0.08
Basophils	0.08	0.08	0.08	0.06	0.009	0.13	0.41	0.49
Leukocyte, %								
Neutrophils	21.5	22.2	25.4	24.2	1.74	0.89	0.12	0.69
Lymphocytes	66.3	63.6	59.2	65.0	2.82	0.60	0.32	0.17
Monocytes	6.9	7.7	9.1	7.7	0.57	0.58	0.09	0.09
Eosinophils	4.5	5.6	5.4	2.5	0.93	0.38	0.26	0.06
Basophils	0.74	0.82	0.78	0.69	0.09	0.95	0.61	0.33
N:L <sup>c</sup> ratio	0.35	0.38	0.48	0.40	0.04	0.58	0.11	0.26

<sup>a</sup>LPFA = low rate of gain with Ca-salts of palm oil; LCLA = low rate of gain with Ca-salts of CLA; HPFA = high rate of gain with Ca-salts of palm oil; HCLA = high rate of gain with Ca-salts of CLA.

<sup>b</sup>Fat = main effect of fat supplementation, Ca-salts of palm oil vs. Ca-salts of CLA; Gain = main effect of rate of growth, low vs. high; Fat x Gain = interaction of fat supplementation and rate of growth.

<sup>c</sup>Neutrophil:lymphocyte ratio

**Table 4. Influence of live body weight gain and Ca salts of conjugated linoleic acid (CLA) supplementation on lymphocyte blastogenic response and macrophage phagocytosis<sup>a</sup>**

Item	Treatments <sup>b</sup>				SEM	<i>P</i> ≤ <sup>c</sup>		
	Low weight gain		High weight gain			Fat	Gain	Fat x Gain
	LPFA	LCLA	HPFA	HCLA				
Lymphocyte proliferation, cpm × 10 <sup>3</sup>								
Unstimulated	0.29	0.26	0.24	0.28	0.02	0.86	0.50	0.11
PHA	104	99	131	117	6.3	0.16	0.003	0.49
PWM	60	50	59	52	8.0	0.32	0.95	0.90
ConA	170	178	176	168	9.6	0.97	0.80	0.44
Lymphocyte proliferation, stimulation index <sup>d</sup>								
PHA	424	466	647	460	49.7	0.17	0.05	0.04
PWM	218	234	311	207	34.4	0.23	0.36	0.11
ConA	700	835	868	662	67.1	0.60	0.97	0.03
Monocyte/macrophage phagocytosis <sup>e</sup>								
% Phagocytic	6.8	10.6	6.4	10.6	3.00	0.22	0.95	0.95
Average PRBC	1.2	1.4	1.3	1.3	0.10	0.33	0.72	0.58

<sup>a</sup>Peripheral blood samples were taken from 24 animals on d 1, 28, and 56 to determine the proliferative response of lymphocytes administered phytohemagglutinin (PHA, 5 μg/mL), pokeweed mitogen (PWM, 15 μg/mL), and concanavalin A (ConA, 20 μg/mL). Values are means of 4 pens representing each dietary treatment, and are displayed as counts per minute (cpm).

<sup>b</sup>LPFA = low rate of gain with Ca-salts of palm oil; LCLA = low rate of gain with Ca-salts of CLA; HPFA = high rate of gain with Ca-salts of palm oil; HCLA = high rate of gain with Ca-salts of CLA.

<sup>c</sup>Fat = main effect of fat supplementation, Ca-salts of palm oil vs. Ca-salts of CLA; Gain = main effect of rate of growth, low vs. high; Fat x Gain = interaction of fat supplementation and rate of growth.

<sup>d</sup>Stimulation indices were calculated by dividing the mean cpm of the triplicate wells containing mitogens by the mean cpm of the wells (unstimulated) containing complete medium only.

<sup>e</sup>Peripheral blood was taken from 24 animals on d 56 to determine the percentage of phagocytic monocyte/macrophages and average number of pig red blood cells (PRBC) phagocytosed by monocyte/macrophages. Values are means of 4 pens representing each dietary treatment.