THESIS

REPLACING DIETARY CARBOHYDRATE WITH CALCIUM SALTS OF FATTY ACIDS AND THE EFFECTS TO FINISHING LAMB FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS

Submitted by

Jill L. Seabrook

Department of Animal Sciences

In partial fulfillment of the requirements

for the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Spring 2010

COLORADO STATE UNIVERSITY

January 20, 2010

WE HEREBY RECOMMEND THAT THE THESIS, PREPARED UNDER OUR SUPERVISION BY JILL L. SEABROOK, ENTITLED: REPLACING DIETARY CARBOHYDRATE WITH CALCIUM SALTS OF FATTY ACIDS AND THE EFFECTS TO FINISHING LAMB FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

Graduate Committee

Robert J. Callan

Terry E. Engle

Advisor: R. Kraig Peel

Department Head: William Wailes

ABSTRACT OF THESIS

REPLACING DIETARY CARBOHYDRATE WITH CALCIUM SALTS OF FATTY ACIDS AND THE EFFECTS TO FINISHING LAMB FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS

The objective of this study was to investigate the performance and physiological effects imposed on finishing lamb (n = 60, BW $\bar{x}$ = 41.6 ± 1.4 kg) feedlot performance and carcass characteristics by replacing dietary carbohydrate with calcium salts of fatty acids (CSFA). Upon arrival, lambs were weighed on 2 consecutive d and randomly assigned to 1 of 4 dietary concentrations of CSFA. Treatments included 1) 0% CSFA (Control); 2) 4% CFSA; 3) 7% CSFA and 4) 11% CSFA on a DM basis. Diets were formulated to be isoenergetic (TDN basis) and isonitrogenous (DIP and UIP) and consisted of 75% concentrate, and 25% roughage (corn silage). Ration TDN was kept similar between treatments; rations with less corn had a higher concentration of CSFA-pellet. Intake was controlled to balance TDN per kilogram of BW across treatments; all treatments met 2006 NRC requirements for growing lambs [18]. Lambs were weighed and bled every 14 ± 2 d. Blood samples were analyzed for glucose, lactate and insulin concentrations. On d 61, lambs were transported and slaughtered at a commercial abattoir. Hot carcass weight (HCW) was determined at the time of slaughter, and longissimus muscle (LM), liver and

subcutaneous adipose tissue samples were collected at slaughter and snap-frozen for later analysis of FA composition. Longissimus muscle area (LMA) and back fat (BF) carcass measurements were determined after 48 h storage at 0°C chill. There was a treatment by time interaction for overall ADG ($P = 0.01$). Lambs receiving 11% CSFA had lower ADG, lighter HCW ($P < 0.001$) and smaller LMA ($P < 0.01$) than all other treatments. Control lambs had greater ($P < 0.05$) BF than lambs receiving 11% CSFA, but had similar BF to lambs receiving 4 and 7% CSFA. There was no treatment effect on dressing percentage. There was a treatment by time interaction observed for blood glucose ($P = 0.02$); lambs fed the control diet had a tendency to have higher blood glucose concentrations. Blood insulin values were not different ($P = 0.36$) between treatments, and insulin to glucose ratios were similar among treatments. Overall blood lactate levels had a tendency to be lower as dietary CSFA concentration increased, although not significantly ($P = 0.11$). Fatty acid profiles for LM, liver and subcutaneous adipose were similar across treatments. Overall, the data suggest that CSFA can be used to replace a carbohydrate source such as corn; at rates up to 7% DMI before performance is negatively affected.

Jill L. Seabrook
Department of Animal Sciences
Colorado State University
Fort Collins, CO 80523
Spring 2010

ACKNOWLEDGEMENTS

I would like to thank my committee members whose support and encouragement were much appreciated: Dr. Callan, who took the time to review my work; Dr. Engle, whose door was rarely closed- thank you for making time to answer my questions and encouraging me to stay the course; Dr. Peel, for trusting in my abilities and giving me the freedom to pursue my interests.

I would also like to thank the students and professors in the departments of Animal Sciences and Statistics whose work made this project possible. In particular, I would like to extend thanks to Dr. Chapman whose time and patience helped me refine my statistical analyses.

TABLE OF CONTENTS

INTRODUCTION

Finishing programs are designed to achieve a degree of fatness and to maximize the rate of gain within a predetermined period of time. Since energy provided by forage is limited by the bulk volume limitations of the rumen, finishing rations rely on the inclusion of concentrates to achieve optimal rates of gain and fat deposition [5]. Compared to forage, grains are energy dense due to high starch content and are consequently an important component in feedlot rations. Ruminants convert high-starch concentrates into the glucose precursor, propionate via microbial fermentation, which is the primary substrate for gluconeogenesis in ruminants [10, 13, 29]. The use of fat as an energy source in ruminant diets is limited by the ruminal capacity for hydrogenation; concentrations above 5% typically are inhibitory to microbial function causing a decrease in nutrient absorption and a reduction in DMI [1, 11, 21]. The development of specialty fats: calcium salts of fatty acids (CSFA), hydrogenated fats and encapsulated fats, has made it possible to increase lipid supplementation without impacting rumen function [12].

The recent expansion of domestic ethanol production provided impetus for seeking alternative commodities for feedlot rations [17, 28]. Diversion of corn into ethanol production has increased demand, impacted crop acreage allocation, commodity price and availability [26, 30]. Finding viable alternatives to corn would improve market stability by expanding and diversifying options for feedlot ration formulation [17, 28]. Calcium salts of fatty acids (CSFA) are readily available and easily incorporated into

ruminant diets; their affordability will depend largely upon duration and amount of supplementation.

The objective of this study was to determine the physiological and performance effects caused by the isoenergetic replacement of flaked corn with CSFA in finishing lamb rations, in order to determine if CSFA is a viable alternative for corn as an energy source.

MATERIALS & METHODS

ANIMALS

The animals used for this project were managed in accordance with an approved Colorado State University Animal Care and Use Committee protocol (2008). The experiment consisted of 60 recently weaned (6 - 8 mo) crossbred (Western white-face x Suffolk) lambs from a single local supplier. There were 30 wether lambs and 30 ewe lambs. Upon arrival, all lambs were tagged for identification, weighed, vaccinated (per manufacturer recommendation) with the first of a two-injection vaccine series for tetanus, and overeating disease caused by Clostridium perfringens types C and D (Bar Vac CD/T, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO), and dewormed with 200μg per kg of BW ivermectin solution (Ivomec Sheep Drench, Merial, Iselin, NJ). For a 14 d acclimation period, lambs were housed in two 10 x 30-m pens in an open-sided barn and gradually switched from a ration of grass hay to corn silage. At the conclusion of the 14 d acclimation period, lambs received a second injection of CD/T and were weighed on 2 consecutive d prior to feeding (initial individual BW = 41.4 ± 5.7 kg). Lambs were randomly distributed into 4 treatment groups, blocking by sex and BW and then randomly assigned to one of 5 treatment-allotted 2 x 13-m pens in an open-sided barn. The resulting design consisted of 4 treatment groups and 20 pens, with each treatment

consisting of 5 pens housing 3 lambs apiece. The resulting initial average pen weight after randomization was 41.4 ± 2.0 kg. Sex was considered a nuisance variable so each experimental pen was a random assortment of sex.

DIETS

Four total mixed rations (TMR) were designed with increasing rates of calcium salts of fatty acids (CSFA) (EnerG II, Virtus Nutrition, LLC., Corcoran, CA). The rations consisted of corn silage, CSFA-supplemented pellet, and flaked corn. Calcium salts of fatty acids were incorporated into 4 custom alfalfa-based pellets at a rate calculated to compensate for discrepancies in TDN caused by treatment determined inclusion rates of flaked corn in the TMR (Table 1). All rations were formulated to be isoenergetic (TDN basis) and isonitrogenous (degradable and undegradable intake protein), and to meet or exceed NRC (2006) requirements for growing lambs [18]. Beginning on October 18, 2008 lambs were fed once daily at 0700 with ad libitum access to water and mineral (Ranch-O-Min 7 Sheep Mineral, RanchWay Feeds, Fort Collins, CO). From d 0 - 7, lambs were gradually adjusted to experimental levels of CSFA-supplemented pellet and flaked corn and pair-fed (TDN) thereafter for 54 days. Feed was initially offered at a daily rate of 0.04 kg of TDN (AF basis) per kilogram of pen weight, but was increased every 2 d until trace amounts of uneaten feed remained at the subsequent feeding. Pair-feeding was given priority, so the pen with the lowest DMI limited upward adjustments for all other pens. Trace feed was used as an indication that lambs were being fed ad libitum to maximize the rate of average daily gain. Lambs were weighed at 14 ± 2 d intervals prior to feeding but with unrestricted access to water and the data were used to

recalculate feed rate to accommodate for changes in pen weight. Final BW was determined by averaging individual BW values obtained on the 2 final weigh days.

EXPERIMENTAL PROCEDURES

Live carcass measurements were made using an Aloka SSD-500V ultrasound console and 3.5 MHz 17 cm linear array transducer (Aloka CO., LTD, Tokyo Japan) on days -1, 30 and 60. Ultrasonography images were collected from a shorn section on the left side of the animal between ribs 12 and 13. Backfat thickness and longissimuss muscle area (LMA) were determined from the analysis of images using Ovine Image Analysis software application (Designer Genes Technologies, Inc., Harrison, AR).

Approximately 30 mL of blood were collected via jugular venipuncture on d -1, 16, 30, 44 and 59. Blood was collected using silicone-coated red-top tubes and potassium oxalate/sodium fluoride grey-top tubes (BD Vacutainer, Franklin Lakes, NJ). Blood collected in red-top tubes was kept at ambient temperature for 30 min before being transferred to ice; blood collected in other tubes was stored on ice directly after collection. Blood solids were separated by centrifuge (IEC Centra GP8, Thermo Fisher Scientific Inc., Waltham, MA) at 1,500 x g for 15 min at 25°C after which fluid tissue was transferred into cryogenic tubes and stored at -80°C. Potassium oxalate/sodium fluoride treated plasma samples were transferred to 4°C for 24 h or until fluid, and analyzed in duplicate for plasma lactate and glucose concentrations using a YSI 2700 Select Biochemistry Analyzer (YSI Inc., Yellow Springs, OH). Silicone-coated red-top treated serum samples were analyzed for insulin concentration using Ovine Insulin ELISA kit and manufacturer recommended procedure (Mercodia, Uppsala, Sweden).

On day 62, lambs were transported 56 km to a commercial packing company (Innovative Foods, LLC., Evans, CO) where they were humanely slaughtered via captive bolt and exsanguination. Tissue samples were collected and snap-frozen in liquid nitrogen: Subcutaneous and retroperitoneal adipose tissues were collected from the torso, medial to the elbow joints; skeletal muscle tissue was collected from the left forelimb (including M. extensor carpi radialis (longus and brevis), M. extensor digitorum communis and M. extensor carpi ulnaris); liver tissue was collected from the right anatomical lobe. Hot carcass weight was determined after hot-water spray washing and before sample collection of adipose and muscle tissues. After 48 h at 0°C carcasses were cross-sectioned between ribs 12 and 13; circumferences of both LM were traced on chromatography paper, and BF measurements were made at the lateral three-quarter point over the LM. Longissimus muscle area (LMA) was calculated from tracings using Meatscan Image Analyzer software application (AEW Consulting, Lincoln, NE).

Duplicate 5-g samples of adipose, muscle and liver tissue were sectioned and oven-dried at 100°C for 24 h. Moisture content of dried samples was determined by weight loss after cooling in a desiccation chamber at 25°C for 4 to 6 h. Total lipids were extracted in duplicate from subcutaneous adipose, muscle and liver tissue samples according to the procedures of Folch et al (1957). Folch-extracted samples were transmethylated according to the method of Park and Goins (1994) and evaporated on a rotary evaporator under a gentle stream of nitrogen at 25°C. Dried fatty acid methyl esters were reconstituted with 2 µL of hexane and analyzed by an Agilent 6890 Series gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with a flame ionization detector and a 100 m x 0.25 mm (i.d.), fused silica capillary column (SP-2560, 0.2 µm

film thickness, Supelco, Bellefonte, PA). A triacylglycerol of tridecanoic acid (13:0, 1.0 mg) was used as the internal standard. Oven temperature was maintained at 175°C for 40 min, and then increased to 240°C at 10°C/min. Injector and flame-ionization detector temperatures were 245°C. Helium was the carrier gas at a split ratio of 50:1 and a constant flow rate of 0.8 mL/min. Fatty acid peaks were recorded and integrated using GC ChemStation software (version A.09.03, Agilent Technologies). Retention times were compared with known FAME standards to identify individual FA (Nu-Chek Prep, Inc., Elysian, MN, and Matreya Inc., Pleasant Gap, PA).

STATISTICAL ANALYSES

All analyses were made using the SAS statistical software application (SAS Inst. Inc., Cary, NC). The experimental unit was pen, unless otherwise specified, and α was set at 0.05.

Biochemical parameters glucose and lactate were analyzed using the MIXED procedure, REML method and repeated measures covariance structure AR(1). The model included fixed effects of treatment, pen, and day; random effects included pen nested within treatment. Due to the normal fluctuations inherent in cyclical biological and metabolic systems, the repeated measures approach was preferred over a covariate model in order to include intermediate sample fluctuation details within the analyses of overall trends. Day zero data were analyzed using the GLM procedure to confirm that initial lactate and glucose concentrations did not differ among treatments ($P = 0.78$ and 0.66 respectively).

Blood concentrations of insulin were analyzed using the MIXED procedure and REML method for a covariate model; GLM analysis was used to determine that initial day zero

values did not differ among treatments (P = 0.67). Analyses using the MIXED procedure and REML method for a covariate model were also conducted for the following carcass and performance parameters: LMA, BF, fatty acid composition, hot carcass weight, ADG, feed efficiency (G:F), dressing percentage and DMI. The model included fixed effects of treatment and pen, and for ADG period; random effects included pens nested within treatment.

RESULTS & DISCUSSION

This experiment was designed to explore the effects and viability of exchanging a carbohydrate-based energy source such as corn, with calcium salts of fatty acid (CSFA) within a ruminant feedlot setting. The high energy density inherent to CSFA resulted in a staggered decrease in feed volume and percent nitrogen-free extract (NFE) as a larger proportion of corn was replaced. Treatments were designed to test the dose response of CSFA on ruminant feedlot performance and carcass characteristics, not to distinguish whether high CSFA inclusion in conjunction with low NFE was responsible for observed effects, or if one was singly responsible for observed effects.

PERFORMANCE

The experimental data indicate that lamb feedlot performance is not significantly affected until CSFA inclusion reaches 7% of TMR.

Treatment was a source of variation in overall ADG (Table 3). There was no significant difference in overall ADG between the lambs being fed the control diet, and those fed either 4 or 7% CSFA ($P = 0.61$, 0.13 respectively), but lambs receiving 11% CSFA had lower overall ADG than all other treatments ($P = 0.03$, in relation to 7%). There was a treatment by time interaction on ADG (Table 4). Lambs fed control, 7% and 11%

treatment rations followed a similar growth curve; with rate of gain accelerating through the first period, peaking during second period and tapering at different degrees to establish overall ADG. Lambs fed 4% CSFA gained at a slower rate, with the rate of daily gain peaking more modestly later in the second period. While the growth curve pattern was different for lambs fed 4% CSFA, overall ADG for lambs fed 4% CSFA did not differ from lambs fed the control or 7% CSFA treatments. Compensatory growth projections made using the overall rates ADG suggest that lambs being fed 4, 7 and 11% CSFA would require additional days on feed to reach the average final weight of 54 kg achieved by the control lambs (5, 19 and 150 days respectively). Differences in ADG manifested in different final BW. Treatment, and treatment by time interaction were sources of variation in BW (Table 2). Initial BW at d 0 did not differ ($P = 0.57$) but by d 16, lambs fed 7 and 11% CSFA were lighter than lambs fed the control diet ($P = 0.03$, < 0.01 respectively). On d 30, lambs fed 11% CSFA were lighter than all other lambs ($P < 0.01$) and remained significantly lighter through trial completion resulting in lower hot carcass weights at slaughter.

Treatments differed in feed efficiency (Table 3). Comprehensive experimental treatment means ± 0.04 kg of G:F efficiency were sequentially lower with increasing CSFA inclusion: 0.13, 0.12, 0.10, and 0.04 respectively.

CARCASS

Few differences were observed in carcass characteristics between control lambs and those fed 4 or 7% CSFA (TABLE 5). The greatest variance was observed in carcasses from lambs fed 11% CSFA: the average hot carcass weight (HCW) was lighter overall and

yield grade was lower (P = 0.005). While the lambs fed 11% CSFA had lighter HCW, the dressing percent did not differ among treatments (53.6%, 53.8%, 54.8% and 52.8%, P = 0.41). Treatments differed in carcass LMA; lambs fed 11% had smaller LMA than lambs in the other treatment groups. Ultrasound LMA and BF measurements did not differ between treatment groups on day 0 (P = 0.40 and 0.30 respectively). Percent of HCW contributing to LMA did not differ between treatments, and it was concluded that the smaller carcass LMA observed in lambs fed 11% CSFA resulted from a general and proportional retardation of adolescent anabolic growth.

TISSUE PARAMETERS

The percent inclusion of C6 – C24 fatty acids (FA) in subcutaneous adipose, skeletal muscle and liver tissue samples was quantified and compared (Table 7). Skeletal muscle FA composition did not differ between treatments, but treatment was a significant source of variation for subcutaneous adipose and liver FA tissue composition (Table 8). Subcutaneous adipose levels of 9-cis,12-cis-linoleic acid (LA) and liver tissue concentrations of (all-cis)-5,8,11,14,17-eicosapentaenoic acid (EPA) were highest in lambs fed 4% CSFA. Lambs fed 7% CSFA had higher concentrations of odd-chain fatty acid tridecanoic acid (C13) than all other lambs.

In ruminants unsaturated fatty acids are hydrogenated before being absorbed, resulting in tissue depots comprised predominantly of stearic acid. Branched, unsaturated and odd-chain FA that are incorporated into milk, muscle and adipose tissues typically result from the digestion and absorption of rumen microorganisms [19]. In 2007, Or-Rashid et al suggested that the profile of the resident microbial population contributed to the extent to

which unsaturated FA were incorporated into tissues, with the presence and quantity of protozoa being the largest influence. Since rumen content sampling was performed as a part of this trial, Or-Rashid's conclusion could not be validated.

While odd-chain fatty acids are oxidized in same way as even-chain fatty acids, they result in propionyl-coenzyme A, which must be converted by methylmalonyl-CoA mutase before entering the citric acid cycle as succinyl-CoA [27]. In 2008, Gotoh, et al found that the metabolic rate of odd-chain FA was slower than that of even-chain FA. The data was interpreted to suggest that in mammalian species, even-chain FA may be favored as a substrate for β-oxidation, resulting in the accumulation of odd-chain FA in tissue stores. Since rumen-inert fats such as CSFA bypass ruminal hydrolysis, supplementation could also lead to alternative FA deposition and subsequent tissue profiles. Although odd-chain FA were not a direct component of the CSFA product used, absorption of FA other than stearic acid from the small intestine may have resulted in alternative FA metabolic pathways and subsequent deposition profiles.

Blood concentrations of insulin and lactate did not differ between treatments, however there was a borderline trend toward lower lactate in lambs fed 7 and 11% CSFA. This may have resulted from increased gluconeogenic conversion of lactate to glucose.

Biochemical measurements of blood glucose did not deviate beyond normal physiological ranges (2.4 - 4.5 mmol/L [15]), but a treatment by time interaction was observed among treatments (Table 6). Most significant were the lowered blood glucose concentrations observed in d 16 samples from lambs fed 11% CSFA. However, the difference did not perpetuate through to successive sample days. Blood glucose

concentrations in control lambs were consistently higher than other treatment groups, but all treatments followed similar patterns of blood glucose fluctuation throughout the experiment (Figure 3). It is accepted that rumen volatile fatty acid profiles high in propionate are more gluconeogenic than those high in acetate [10, 13]. High starch grains, such as corn, produce fatty acid profiles higher in propionate, resulting in higher blood glucose levels and improved growth performance [10, 13]. However, given the stringency and complexities of the physiological control mechanisms regulating glucose homeostasis, it is likely that other factors within the endocrine complex contributed to the depressed growth observed in the lambs fed the 11% diet [14]. Protein and adipose accretion associated with somatic growth are regulated by the somatotropic axis; a complex feedback mechanism including growth hormone, somatomedins, insulin, leptin, glucocorticoids and thyroid hormones [25]. This hormone axis is responsive to nutrient intake, and changes in nutritional status have been shown to alter the circulating concentrations of these hormones [9, 14, 25]. Experiments with dietary fat have induced alterations to concentration and pulsatility of these hormones in a number of species: Caton, et al (2007) found that rats fed a low-carbohydrate, high-fat diet grew at a slower rate than controls and attributed this to lower circulating levels of growth hormone and leptin. The Caton article summarized the findings of Cappon (1993) and Penman (1981) whose experiments demonstrated elevated levels of plasma somatostatin, a growth hormone inhibitor, in subjects fed high-fat diets. In addition to the effects observed from the daily consumption of a high-fat diet, discrete incidences of fat consumption and spikes in serum fatty acid concentrations have altered the characteristics of subsequent somatotropic hormone secretion [14]: Galassetti et al (2006) found that exercise-induced

release of growth hormone was attenuated in children who had previously consumed a high-fat meal. While extrapolating the underlying biological mechanisms responsible for the observed differences in growth among treatments was beyond the scope of this experiment, these data would be worth pursuing in future research.

CONCLUSIONS

An animal's growth potential is determined by a complex combination of genetic, nutritional and environmental factors; each of these factors affects basic physiological and endocrine mechanisms ultimately contributing to a resulting phenotype [9, 25]. For agricultural animal production systems, understanding how the application of a given nutritional regimen affects productivity helps facilitate effective decision-making, which ultimately leads to increased efficiency and profitability. This experiment demonstrates that CSFA can be used to replace carbohydrates, such as corn, in lamb feedlot rations at a rates of up to 7% TMR before causing a reduction in performance or carcass quality. Additional research would be valuable to determine if alternate concentrations of dietary protein, carbohydrate, or vitamin and mineral supplements could positively influence the supplementation rate. Currently, the price of corn is not high enough to warrant its substitution with CSFA; this estimation will vary as prices fluctuate. Additionally, the substitution of corn for CSFA may require a greater number of days on feed to achieve finishing weight, so desirability of such a substitution will hinge upon the price of corn in conjunction with daily operational expenses. Follow-up research is needed to isolate the

effects of CSFA supplementation from percent NFE, to determine the intrinsic biological mechanisms, and to determine if treatment responses are the same across ruminant species.

REFERENCES

1. Bauchart D., D. Gruffat, D. Durand. 1996. Lipid absorption and hepatic metabolism in ruminants. Proceedings of the Nutrition Society. 55(1B):39-47.
2. Brethour J.R. 1992. The repeatability and accuracy of ultrasound in measuring backfat of cattle. J. Animal Science 70:1039-1044.
3. Cappon J.P., E. Ipp, J.A. Brasel and D.M. Cooper. 1993. Acute effects of high fat and high glucose meals on growth hormone response to exercise. J. Clinical Endocrinology and Metabolism. 76(6):1418-22.
4. Caton S.J., B. Yinglong, L. Burget, L.J. Spangler, M.H. Tschöp and M. Bidlingmaie. 2006. Low-carbohydrate high-fat diets: Regulation of energy balance and body weight regain in rats. Obesity. 17(2):283–289.
5. Corah L.R. 2008. ASAS Centennial Paper: Development of a corn-based beef industry. J. Animal Science 86(12):3635-9.
6. Folch J., Lees M. and G.H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biological Chemistry 226(1):497-509.
7. Galassetti P., J. Larson, K. Iwanaga, S.L. Salsberg, A. Eliakim and A. Pontello. 2006. Effect of a High-Fat Meal on the Growth Hormone Response to Exercise in Children. J. Pediatric Endocrinology and Metabolism. 19(6):777-86.

8. Gotoh, N., K. Moroda, H. Watanab, K. Yoshinaga, M. Tanaka, H. Mizobe, K. Ichioka, S. Tokairin and S. Wada. 2008. Metabolism of odd-numbered fatty acids and even-numbered fatty acids in mouse. J. of Oleo Science. 57(5):293-9.
9. Hossner K.L. 2005. Hormonal Regulation of Farm Animal Growth. Oxfordshire, UK. CABI Publishing.
10. Huntington G.B. 1997. Starch utilization by ruminants: from basics to bunk. J. Animal Science 75(3):852-67.
11. Jenkins T.C. 1994. Regulation of lipid metabolism in the rumen. J. Nutrition. 124(8 Suppl):1372S-1376G.
12. Jenkins T.C. 1993. Lipid metabolism in the rumen. J. Dairy Science. 76(12):3851-63.
13. McDonald P., et al 2002. Animal Nutrition. Essex, England. Pearson Education Limited.
14. McDowell, G.H. 1983. Hormonal control of glucose homeostasis in ruminants. The Proceedings of the Nutrition Society. 42(2):149-67.
15. Merck Veterinary Online Manual. 2008. http://www.merckvetmanual.com/. Merck & Co., Inc. Whitehouse Station NJ, USA.
16. Nappo F., K. Esposito, M. Cioffi, G. Giugliano, A.M. Molinari, G. Paolisso, E. Marfella and D. Giugliano. 2002. Postprandial Endothelial Activation in Healthy Subjects and in Type 2 Diabetic Patients: Role of Fat and Carbohydrate Meals. J. American College of Cardiology. 39:1145–1150.
17. National Agriculture Statistic Services. 2009. Inventory numbers and values of all cattle and calves, number of operations and size group estimates by class, state and U.S. 1972-2009.

18. NRC. 2006. Nutrient Requirements of Small Ruminants. First ed. Natl. Acad. Press. Washington, DC, USA.

19. Or-Rashid M.M., N.E. Odongo and B.W. McBride. 2007. Fatty acid composition of ruminal bacteria and protozoa, with emphasis on conjugated linoleic acid, vaccenic acid, and odd-chain and branched-chain fatty acids. J. Animal Science. 85(5):1228-34.

20. Peairs A.T. and J.W. Rankin. 2008. Inflammatory response to a high-fat, low-carbohydrate weight loss diet: effect of antioxidants. Obesity. 16(7):1573-8.

21. Palmquist D.L. 1994. The role of dietary lipids in efficiency of ruminants. J. Nutrtion. 124(8 Suppl):1377S-1382S.

22. Park P.W. and R.E. Goins. 1994. In situ preparation of fatty acid methyl esters for analysis of fatty acid composition in food. J. Food Sci. 59:1262–1266

23. Penman E., J.A. Wass, S. Medbak, L. Morgan, J.M. Lewis, G.M. Besser and L.H. Rees. 1981. Response of circulating immunoreactive somatostatin to nutritional stimuli in normal subjects. Gastroenterology. 81(4):692-9.

24. Rankin J.W. and A.D. Turpyn. 2007. Low carbohydrate, high fat diet increases C-reactive protein during weight loss. J. American College of Nutrition. 26(2):163-9.

25. Renaville, R., M. Hammadi and D. Portetelle. 2002. Role of the somatotropic axis in the mammalian metabolism. Domestic Animal Endocrinology. 23(2002)351–360.

26. Renewable Fuels Association. 2009. Ethanol Industry Statistics. http://www.ethanolrfa.org. Washington, DC, USA.

27. Sbaï, D., C. Narcy, G.N. Thompson, A. Mariotti, F. Poggi, S.M. Saudubray and J.L. Bresson. 1994. Contribution of odd-chain fatty acid oxidation to propionate

production in disorders of propionate metabolism. American Journal of Clinical Nutrition. 59(6):1332-7.

28. USDA Economic Research Service. 2009. Briefing Room: Corn. www.ers.usda.gov. United States Department of Agriculture. Washington, DC, USA.
29. Vasconcelos J.T., J.E. Sawyer, L.O. Tedeschi, F.T. McCollum and L.W. Greene. 2009. Effects of different growing diets on performance, carcass characteristics, insulin sensitivity, and accretion of intramuscular and subcutaneous adipose tissue of feedlot cattle. J. Animal Science 87(4):1540-7.
30. Westcott P.C. 2007. Ethanol Expansion in the United States: How will the agricultural sector adjust? USDA Economic Research Service. Outlook Report No. (FDS-07D-01) 20.

APPENDIX A: TABLES AND FIGURES

TABLE 1. TOTAL MIXED RATION ENERGY ANALYSES AND CHEMICAL COMPOSITION ON A DRY MATTER BASIS.

	Treatment			
Item (%)	**CTRL**	**4%**	**7%**	**11%**
Corn Silage	14%	14%	14%	17%
Flaked Corn	26%	23%	15%	0%
CSFA-Pellet	36%	40%	47%	58%
Alfalfa	50.2%	41.9%	34.7%	37.6%
Soybean meal	31.4%	32.8%	30.9%	27.4%
Wheat Middlings	9.8%	8.8%	7.5%	6.1%
Bypass fat (EnerG II®)	0.0%	9.1%	15.4%	19.6%
Fat (non bypass)	5.8%	4.9%	3.5%	2.2%
Lime (38% Calcium)	1.6%	-	-	-
Sheep Premix	0.5%	0.5%	0.5%	0.5%
Ammonium Chloride	0.8%	0.7%	0.7%	0.8%
Ameirbond 2X (Pellet Binder-Lignin Sulfonate)	-	-	1.2%	1.3%
Bentonite	-	1.3%	5.5%	4.7%
NFE	30%	28%	24%	16%
CFat	2%	5%	8%	11%
TDN	57%	60%	61%	59%
CP	12%	12%	12%	12%
DIP	9%	9%	9%	10%
UIP	5%	5%	5%	4%
Ca	0.7%	0.8%	1.3%	1.9%
P	0.3%	0.3%	0.2%	0.2%

TABLE 2. TREATMENT, AND TREATMENT X TIME INTERACTIONS ON BODY WEIGHT.

	Treatment					P-value		
	CTRL	**4%**	**7%**	**11%**	**SEM**	**TRT**	**Time**	**TRT*Time**
μ	49.2[a]	48.3[a,b]	46.9[b]	43.7[c]	0.48	0.001	0.001	<0.001
d 16	43.8[a]	42.8[a,b]	41.9[b,c]	40.5[c]	0.48			
d 30	47.8[a]	46.6[a]	46.7[a]	43.8[b]	0.66			
d 44	51.0[a]	50.8[a]	48.3[b]	45.4[c]	0.63			
d 59	54.2[a]	53.0[a]	50.7[b]	45.2[c]	0.55			

[a, b, c] Means within rows without common superscripts differ ($P < 0.05$).

FIGURE 1. BODY WEIGHT (KG) OVER TIME BY TREATMENT.

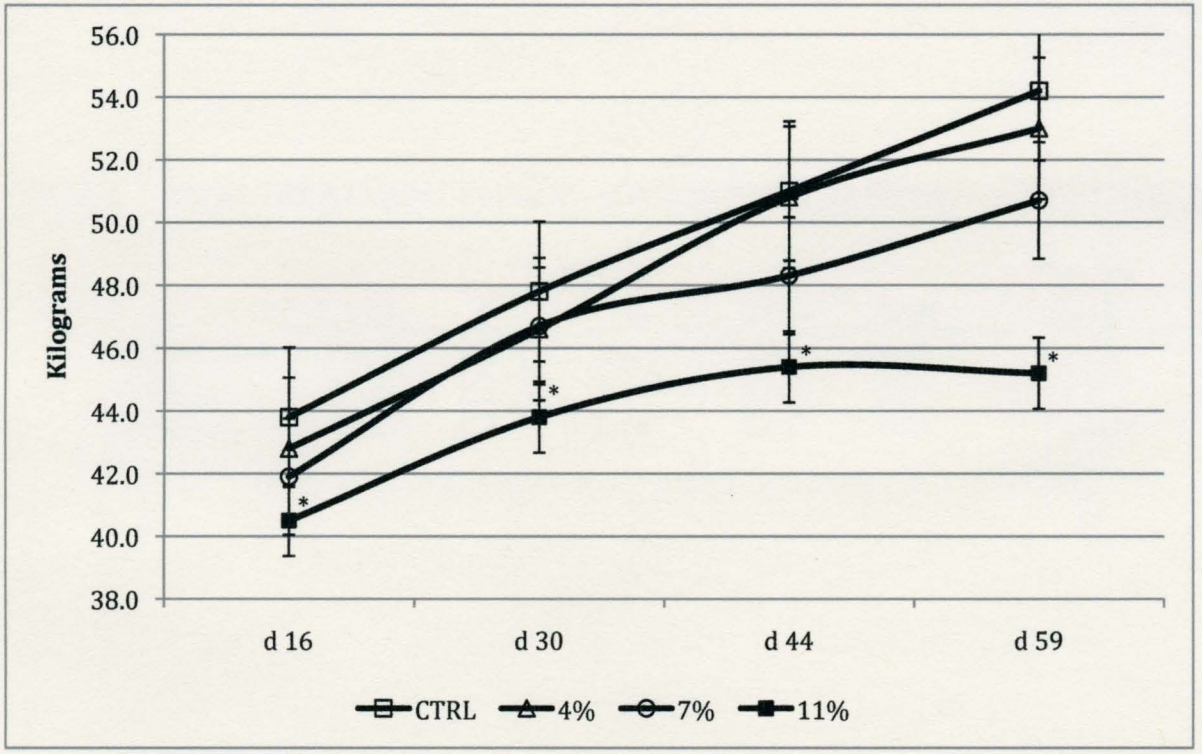


TABLE 1. MAIN TREATMENT EFFECTS ON FEEDLOT PERFORMANCE PARAMETERS.

	Treatment					P-value
	CTRL	**4%**	**7%**	**11%**	**SEM**	**TRT**
Pen DMI (kg)	301.7[a]	281.8[b]	263.5[c]	259.2[c]	4.80	< 0.001
ADG (kg/d)	0.22[a]	0.20[a]	0.16[a]	0.06[b]	0.01	< 0.001
G:F (kg)	0.13[a]	0.12[a]	0.10[b]	0.04[c]	0.01	< 0.001

[a, b, c] Means within rows without common superscripts differ ($P < 0.05$).

TABLE 2. AVERAGE DAILY GAIN TREATMENT BY PERIOD INTERACTIONS.

	Treatment					P-value		
	CTRL	**4%**	**7%**	**11%**	**SEM**	**TRT**	**Time**	**TRT*Time**
μ	0.22^{a}	0.20^{a}	0.16^{a}	0.06^{b}	0.01	<0.0001	<0.0001	0.003
Period 1	0.17^{a}	$0.10^{a,b}$	0.02^{b}	$(0.08)^{c}$	0.02			
Period 2	0.30^{a}	0.29^{a}	0.33^{a}	0.25^{a}	0.02			
Period 3	0.21^{a}	0.28^{a}	0.11^{b}	0.10^{b}	0.01			
Period 4	0.23^{a}	0.16^{a}	0.18^{a}	$(0.03)^{b}$	0.01			

$^{a, b, c}$ Means within rows without common superscripts differ ($P < 0.05$).

FIGURE 2. AVERAGE DAILY GAIN TRENDS CALCULATED FROM DATA IN TABLE 2; SOLID LINES INDICATE AVERAGE DAILY GAIN BY TREATMENT AND DASHED LINES ARE TREATMENT TREND LINES.

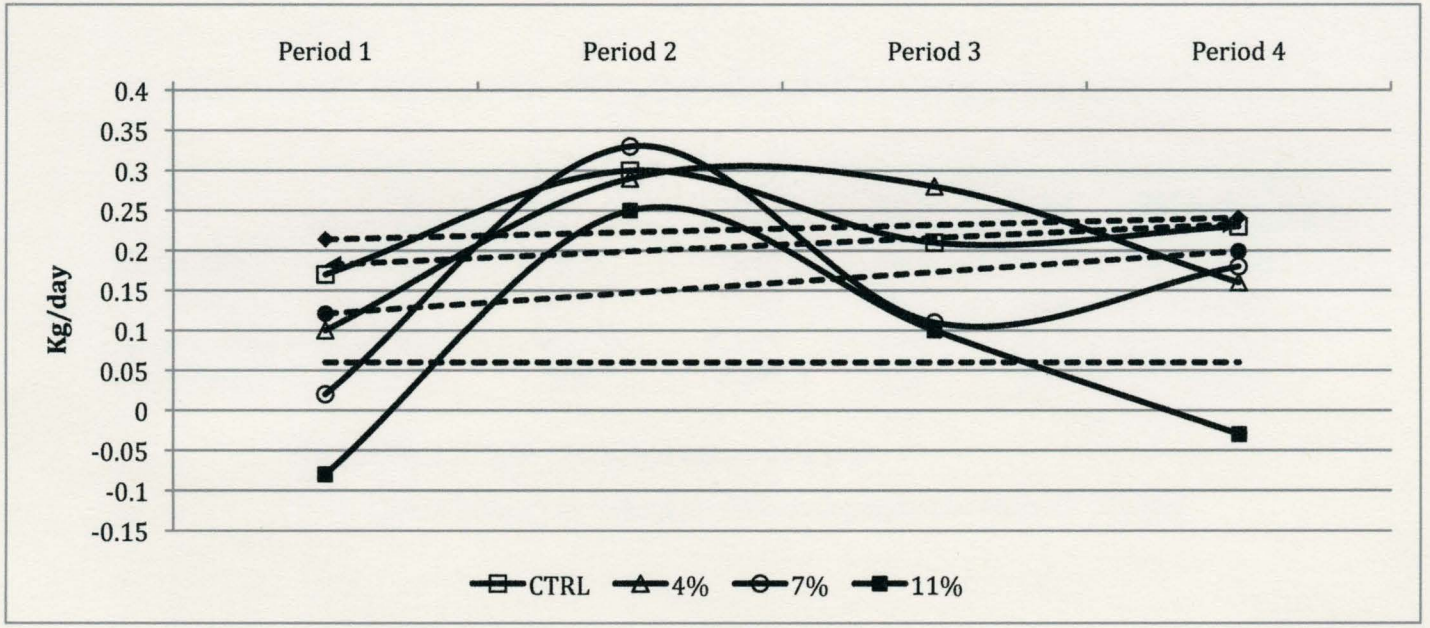


TABLE 3. MAIN EFFECTS OF TREATMENTS ON CARCASS CHARACTERISTICS.

	Treatment					
	CTRL	**4%**	**7%**	**11%**	**SEM**	**TRT (P-value)**
HCW^ (kg)	28.7[a]	28.3[a]	28.0[a]	23.9[b]	0.53	<0.001
LMA* (cm^2)	18.1[a]	17.0[a]	17.3[a]	15.1[b]	0.46	0.002
Back fat (cm)	0.66[a]	0.54[a,b]	0.54[a,b]	0.48[b]	0.07	0.115
Yield Grade	2.4[a]	2.0[a,b]	2.0[a,b]	1.6[b]	0.17	0.038

^Hot carcass weight, *longissimus muscle area.

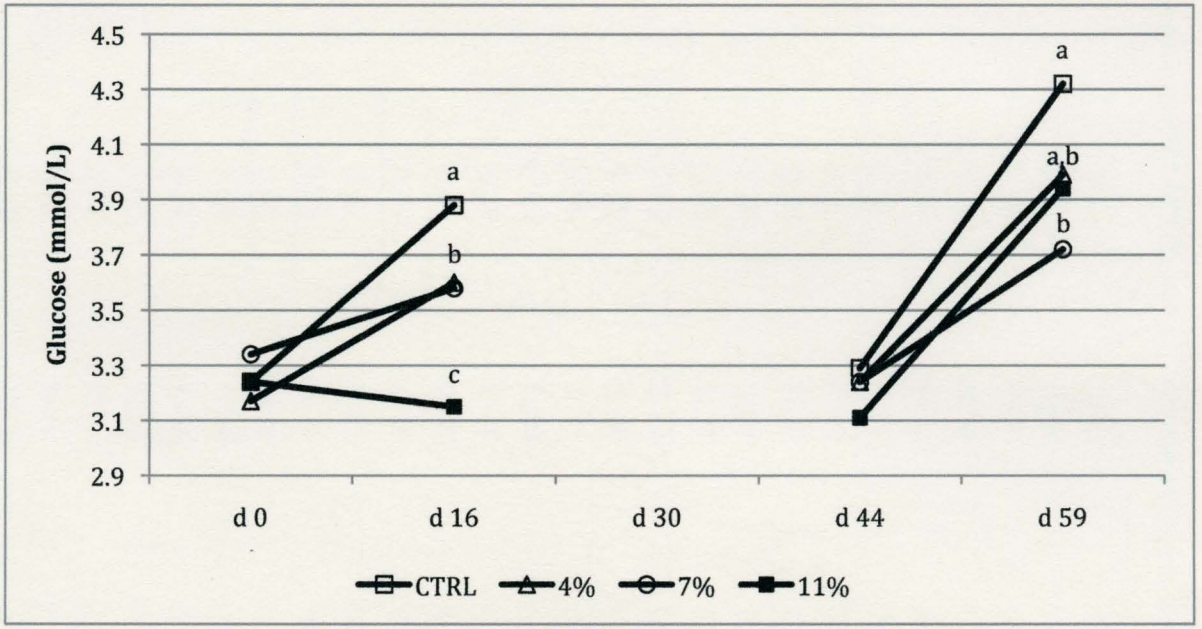
[a, b, c] Means within rows without common superscripts differ ($P < 0.05$).

TABLE 4. PLASMA CONCENTRATIONS OF LACTATE (MMOL/L), INSULIN (AU) AND GLUCOSE (MMOL/L).

	Treatment					P-value		
	CTRL	**4%**	**7%**	**11%**	**SEM**	**TRT**	**Time**	**TRT*Time**
Lactate (mmol/L)	4.08[a]	3.96[a]	3.09[a]	3.32[a]	0.36	0.19	<0.0001	0.11
Insulin (AU)	0.25[a]	0.22[a]	0.19[a]	0.19[a]	0.03	0.48	<0.0001	0.36
Glucose (mmol/L)	3.68[a]	3.50[a,b]	3.47[a,b]	3.36[b]	0.09	0.10	<0.0001	0.02
d 0	3.24[a]	3.17[a]	3.34[a]	3.24[a]	0.10			
d 16	3.88[a]	3.60[b]	3.58[b]	3.15[c]	0.12			
d 44	3.29[a]	3.24[a]	3.24[a]	3.11[a]	0.18			
d 59	4.32[a]	3.99[a,b]	3.72[b]	3.94[a,b]	0.28			

[a, b, c] Means within rows without common superscripts differ ($P < 0.05$).

FIGURE 3. BLOOD GLUCOSE CONCENTRATIONS (MMOL/L); CALCULATED FROM DATA IN TABLE 4.



[a, b, c] Data points without common superscripts differ ($P < 0.05$).

TABLE 5. TISSUE FATTY ACID CONCENTRATION P-VALUES DETERMINED FROM GAS CHROMATOGRAPHY DATA ANALYSIS.

	P-value		
Item	**SQ adipose**	**Skeletal mm.**	**Liver**
Fatty acid			
C6:0	0.36	0.27	0.29
C7:0	0.33	0.27	0.29
C8:0	0.36	NA	0.29
C9:0	0.39	0.65	0.47
C10:0	0.36	0.23	0.47
C11:0	0.08	0.87	NA
C12:0	0.65	0.87	0.89
C13:0	0.36	0.87	0.01
C14:0	0.35	0.87	0.23
C14:1 (9c)	0.41	0.71	0.29
C15:0	0.52	0.11	0.45
C16:0	0.55	0.60	0.71
C16:1 (9c)	0.54	0.45	0.78
C17:0	0.17	0.40	0.36
C18:0	0.21	0.45	0.20
C18:1 (9c)	0.31	0.30	0.58
C18:10t12c	0.09	0.46	0.54
C18:10t12c	0.42	0.12	0.47
C18:9c11t	0.93	0.30	NA
C18:9c12c	0.05	0.78	0.27
C18:9t11t	0.56	0.74	0.29
C19:0	0.34	0.54	0.63
C20:0	0.95	0.30	0.47
C20:4 (5,8,11,14 cis)	0.28	0.92	0.30
C20:5 (5,8,11,14,17 cis)	0.28	0.64	0.03
C21:0	0.40	0.46	0.43
C22:0	0.67	0.89	0.50
C22:1 (w-9)	0.44	0.47	0.40
C22:6 (4,7,10,13,16,19)	0.63	0.22	0.37
C23	0.44	0.58	0.23
C24	NA	0.36	0.52
C24:1 (15c)	0.47	0.35	0.95

TABLE 6. TREATMENT MEANS FOR SIGNIFICANT EFFECTS OF FATTY ACID TISSUE COMPOSITION.

		Treatment					P-value
Item		**CTRL**	**4%**	**7%**	**11%**	**SEM**	**TRT**
Tissue	Fatty acid, %						
Subcutaneous adipose	9-cis,12-cis-linoleic acid	$26.19^{a,b}$	30.35^{b}	8.73^{a}	12.25^{a}	6.3	0.05
Liver	Tridecanoic acid	0.00^{a}	0.00^{a}	1.33^{b}	0.36^{a}	0.3	0.01
Liver	(all-cis)-5,8,11,14,17-eicosapentaenoic acid	$1.46^{a,b}$	2.25^{b}	0.33^{a}	1.00^{a}	0.4	0.03

[a, b, c] Means within rows without common superscripts differ ($P < 0.05$).

APPENDIX B: STATISICAL MODELS

BIOCHEMICAL PARAMETERS

Glucose and lactate:

```
proc mixed method = reml data=lacglu covtest cl;
class trt pen day;
model LACf= trt|day/ddfm=kr;
*model GLUf= trt|day/ddfm=kr;
random pen (trt);
repeated day/type=ar(1) subject=pen(trt) rcorr;
lsmeans trt|day/pdiff;
run;

proc glm data=lacglu;
where day eq 0;
class trt;
model LACf =trt;
*model GLUf =trt;
lsmeans trt /pdiff;
run;
```

Insulin:

```
proc mixed method = reml data=insulin covtest cl;
class trt pen day;
model insulinF= trt|day insulinI/ddfm=kr solution;
random pen(trt);
lsmeans trt|day/pdiff;
run;
proc glm data=insulin;
where day eq 0;
class trt;
model insulinI =trt;
lsmeans trt /pdiff;
run;
```

CARCASS CHARACTERISTICS

Hot carcass weight:

```
proc mixed method = reml data=carcassHCW covtest cl;
where pen ne 6;
class trt pen;
model hcw= trt/ddfm=kr;
lsmeans trt/pdiff;
run;
```

Longissimus muscle area:

```
proc mixed method = reml data=carcassREA covtest cl;
where pen ne 6;
class trt pen;
model rea= trt/ddfm=kr;
lsmeans trt/pdiff;
run;
```

Backfat:

```
proc mixed method = reml data=carcassBF covtest cl;
where pen ne 6;
class trt pen;
model bf= trt/ddfm=kr;
lsmeans trt/pdiff;
run;
```

Fatty acid composition:

```
proc mixed method = reml data=ffa1 covtest cl;
class   tissue trt percent;
model percent = trt /ddfm=kr;
lsmeans trt/pdiff;
run;
```

PERFORMANCE

Body weight:

```
proc mixed method = reml data=BW covtest cl;
where pen ne 6 and day ne 0;
class trt pen day;
model bwf = trt|day bwi/ddfm=kr solution;
random pen(trt);
*repeated day/type=ar(1) subject=pen(trt) rcorr;
*repeated day/type=sp(exp) (day1) subject=pen(trt) rcorr;
lsmeans trt|day/pdiff cl;
run;

proc glm data=BW;
where day eq 0 and pen ne 6;
class trt;
model bwf =trt;
lsmeans trt /pdiff;
run;
```

ADG:

```
proc mixed method = reml data=adg covtest cl;
where period eq 4;
class trt pen period;
model adg = trt|period /ddfm=kr;
random pen(trt);
lsmeans trt|period/pdiff;
run;
```

Dressing percent:

```
proc mixed method = reml data=dressingpercent covtest cl;

class  trt pen bwf hcw dp;

model dp = trt /ddfm=kr;

random pen(trt);

run;
```

Dry matter intake (DMI):

```
proc mixed method = reml data=dmi covtest cl;

where day eq 59;

class trt pen day;

model dmi = trt|day dmiI/ddfm=kr;

random pen(trt);

lsmeans trt|day/pdiff;

run;
```

Gain to feed efficiency (GF):

```
proc mixed method = reml data=GF covtest cl;

where period eq 5;

class trt pen ;

model gf = trt /ddfm=kr;

random pen(trt);

lsmeans trt/pdiff;

run;
```

Ultrasound:

```
proc glm data=ultrasound;
where day eq 1;
class trt;
model rea =trt;
lsmeans trt /pdiff;
run;
```