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Evaluation of Calcium Propionate as a  
Nutrient to Prevent Dark Cutting Beef

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# Evaluation of Calcium Propionate as a Nutrient to Prevent Dark Cutting Beef

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Dietary feeding of calcium propionate (NutroCAL™) as a nutritional supplement was unsuccessful in the prevention of dark cutting beef.

## Summary

*The objective of the study was to evaluate the ability of calcium propionate to prevent the dark cutting condition in beef. Angus crossbred steers (n=14) were assigned to two treatments: control and 0.25 lb of calcium propionate (NutroCAL™) per day for 14 days prior to (and 12 days after) the application of artificial stress with epinephrine at day 0. Biopsies of the longissimus muscle and blood samples were obtained at days -2, 0, 1, 3, 6 and 9. Calcium propionate had no effect on muscle glycogen content, extent of glycogen depletion, or glycogen repletion rates.*

## Introduction

Dark cutting beef is caused by total or partial depletion of muscle glycogen prior to slaughter. The depletion primarily occurs when the animal suffers stress. Dark cutting beef has an undesirable color to consumers, a different flavor profile and a shorter shelf life (more rapid microbial spoilage). These characteristics decrease the value of the beef, causing an economic loss for the producer. In the carcass of an unstressed animal, muscle glycogen will be converted to lactic acid, and cause a muscle pH decline from 7.0 to about 5.6 – the

normal pH for beef. Muscle pH does not decline in meat from stressed animals because of low glycogen levels, causing a dark color and a high pH.

When NutroCAL™ was fed to dairy cattle, serum insulin and blood glucose levels increased. Both of these conditions are favorable for muscle glycogenesis and may increase the concentration of glycogen inside the muscle. Our study was conducted to evaluate feeding of calcium propionate (NutroCAL™) to prevent the dark cutting condition in beef.

## Procedure

Fistulated, Angus crossbred steers (n=14) were randomly assigned to one of two treatments, the control or 0.25 lb of NutroCAL™ (calcium propionate) mixed with a diet that consisted of dry rolled corn, wet corn gluten, alfalfa and limestone (77.5% DM, 13.3% CP). Steers were fed 80% of *ad libitum* to ensure complete intake of the NutroCAL™. The steers were under this treatment 14 days prior to the first biopsy and during the 12 days that biopsies were performed. The animals were kept in individual pens with water *ad libitum*.

Biopsies were performed along the *longissimus* muscle using a biopsy needle on days -2, 0, 1, 3, 6 and 9; with day 0 being the day of stress. The skin in the incision area was anesthetized with a subcutaneous injection of lidocaine. Following surgery, incisions were treated with penicillin to minimize the risk of infection and one or two sutures were made. Blood samples for insulin and glucose measurements were obtained from the jugular vein immediately prior to biopsy. During the course of the study, six sites (3/side) along the *longissimus* muscle in the region of the loin were randomized for day of sampling.

Muscle glycogen, glucose, and glu-

cose-6-phosphate levels were determined using an amyloglucosidase assay, and lactate levels were determined using a lactate dehydrogenase assay.

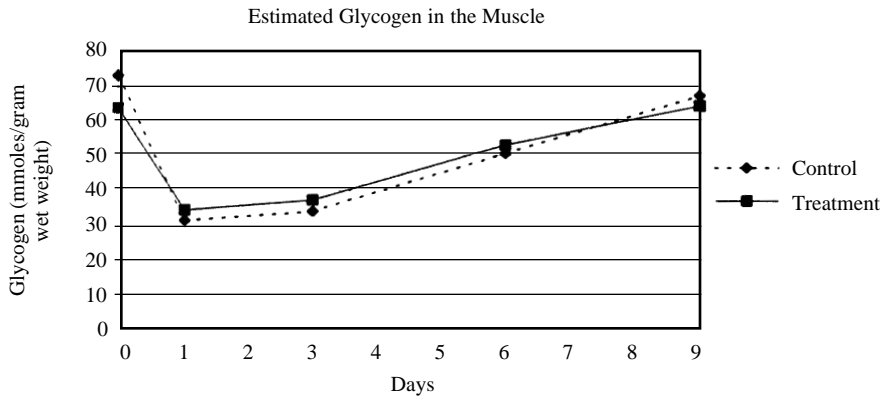
Glucose was determined using the Sigma Glucose Trinder Kit and insulin was determined using the DSL-1600 Insulin Radio-immunoassay Kit.

After biopsies and blood were obtained on day 0, the animals were artificially stressed by subcutaneous injection of 6 mg of epinephrine per 100 lb of live weight. The dose was equally divided in two parts and applied five hours apart. After the last injection, 1.0 lb of NutroCAL™ (calcium propionate) was placed into the rumen through the fistula in an effort to provide calcium propionate to the animal immediately after stress. Biopsies were conducted after training by a veterinarian and under the approval of the Institute Animal Care and Use Committee.

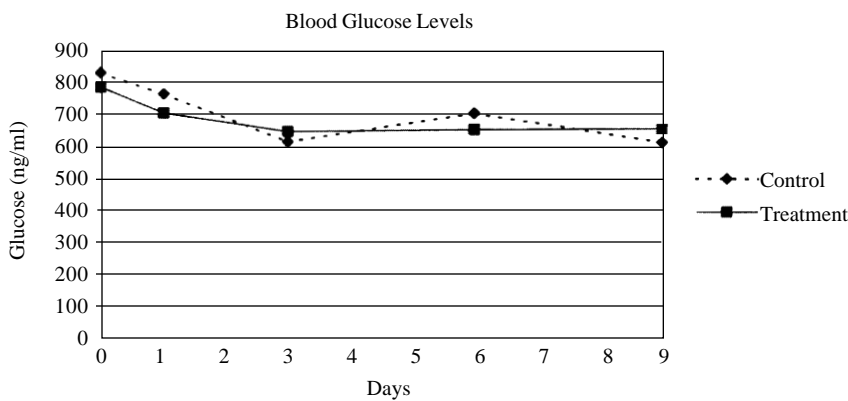
## Results

Day -2 biopsies were taken to indicate initial muscle glycogen levels. Unfortunately, the sampling methods were not performed consistently, resulting in excessive blood and fatty tissue in the samples. As a result, the glycogen levels were exceptionally low and were not credible. These results were excluded from the analysis. Subsequent sampling periods yielded biopsies of much higher quality; essentially all muscle with little, if any, fatty tissue and (or) excess blood.

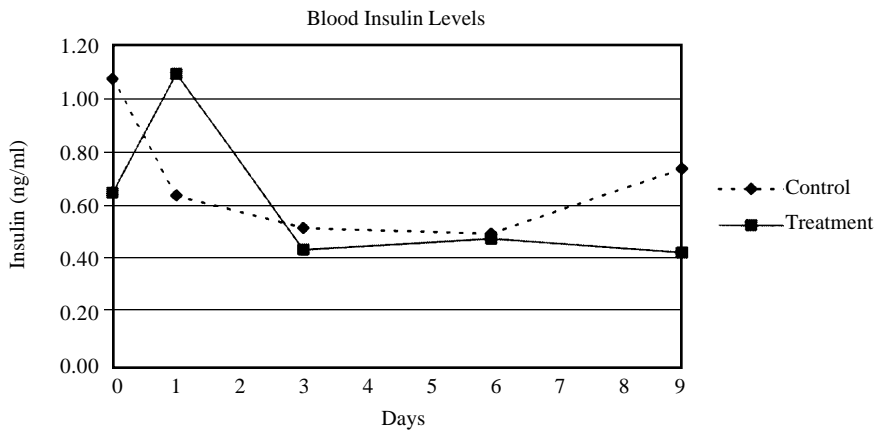
Figure 1 presents the estimated amount of glycogen in muscle during the course of the study. No significant differences were found in muscle glycogen content between the control and the NutroCAL™-fed steers at any sampling period, indicating a failure of calcium propionate to enhance muscle glycogen content, minimize glycogen



**Figure 1.** Estimated glycogen levels in the muscle pre and post stress. The first injection of epinephrine for artificial stress was applied after the day 0 biopsies were obtained.



**Figure 2.** Glucose levels in the blood pre and post stress. The first injection of epinephrine for artificial stress was applied after the day 0 biopsies were obtained.



**Figure 3.** Insulin levels in the blood pre and post stress. The first injection of epinephrine for artificial stress was applied after the day 0 biopsies were obtained.

loss as a consequence of stress, and/or hasten the repletion of glycogen stores in muscle. The figure also shows that the use of epinephrine to produce artificial stress in steers was an appropriate method because the glycogen levels decreased significantly in all 14 animals.

The readings on day 0 were taken prior to the application of epinephrine and so indicate baseline levels of muscle glycogen. These values are in line with a muscle glycogen survey conducted by our laboratory the previous year (2001 *Beef Report*, p. 109-110),

although they were slightly lower than expected. This could be related to previous stress of the animal in the holding pens or to the stress generated by the initial biopsy procedure on day -2. Reports in the literature indicate stress created during the biopsy procedure is fairly low. The fact that the animals did replenish muscle glycogen stores during the biopsy sequence is evidence that minimal stress was experienced.

Previous research indicated glycogen values above 60-65 mmole/kg were needed to prevent dark cutting. The repletion rates for redeposition of muscle glycogen were not different (Figure 1) between the two treatments ( $P > 0.05$ ). These data suggest that to avoid being considered a dark cutter, animals require 8 days or more to replace sufficient muscle glycogen lost as a consequence of significant stress. Unfortunately, the extent of glycogen depletion caused by common stresses, rather than injection of epinephrine, are not well characterized, so it is impossible to know how much recovery time is needed.

Similar patterns of blood glucose changes were observed for the two treatments (Figure 2). There was a gradual decline up to three days following stress, followed by a leveling off period. Feed intake (which was not monitored) would likely have followed the same pattern.

Insulin levels fluctuate hourly within the blood. It is not clear if the higher level of insulin noted in the control cattle on day 0 is a consequence of sampling time or a true treatment effect (Figure 3). The fluctuations of insulin levels are comparable in magnitude to the normal changes in cattle under normal conditions. The increase in blood insulin that occurred after the 1.0 lb of NutroCAL™ was placed directly into the rumen through the fistula was apparently not big enough to produce a change in the muscle glycogen or blood glucose levels and it may be due to other factors like feeding time.

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