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# Acute Effects of Postpartal Subcutaneous Injection of Glucagon and/or Oral Administration of Glycerol on Blood Metabolites and Hormones and Liver Lipids and Glycogen of Holstein Dairy Cows Induced with Fatty Liver Disease

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## A.S. Leaflet R2200

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### Summary

The effects of subcutaneous injection of glucagon and/or oral administration of glycerol on blood metabolites and hormones of Holstein dairy cows induced with fatty liver disease was studied. Twenty multiparous cows were fed a dry cow ration supplemented with 6 kg of cracked corn during the dry period to increase the likelihood of fatty liver disease development. Cows with a body condition score of  $\geq 3.5$  points (1-5 scale) were assigned randomly to one of four treatment groups--saline, glucagon, glucagon plus glycerol, and glycerol. Following treatment, serial blood samples were collected to determine the effect of glucagon and/or glycerol on blood composition. Glucagon injection alone increased postpartal plasma glucose, insulin, glucagon, and plasma urea nitrogen and decreased plasma nonesterified fatty acids (NEFA). Glucagon plus glycerol treatment increased and sustained postpartal plasma glucose, insulin and decreased postpartal plasma NEFA. Administration of glycerol alone increased plasma triacylglycerol (TAG) and decreased plasma NEFA during the postpartal period. Early postpartal treatment of cows with glucagon and/or glycerol increased plasma glucose and decreased plasma NEFA. This response would suggest that these treatments with glucagon and/or glycerol would decrease the likelihood of fatty liver disease in dairy cows.

### Introduction

Dairy cows that are obese or over-conditioned during the periparturient period are susceptible to a complex of metabolic and infectious diseases known as fat cow syndrome. Fat cow syndrome is well known to commence as fatty liver disease before it exacerbates to other interrelated metabolic disorders such as ketosis and milk fever (decreased plasma calcium). Infectious sequelae to fatty liver disease include mastitis and metritis often associated with retained placenta. Other complications commonly seen with disease include left displaced abomasum and laminitis.

Fatty liver disease affects cows in the late prepartal (1 wk before calving) and early postpartal periods (up to 2 wk after calving). The severity of fatty liver disease is classified on the basis of the percentage of accumulated TAG in the liver to assess the potential for deleterious effects to the health, productivity (kg of milk), and reproductivity of dairy cows.

Extensive studies have been undertaken to unravel the etiology of fatty liver disease as the underlying cause of fat cow syndrome. During the final weeks that immediately precede parturition, feed intake progressively decreases to about 30% of normal on the day of parturition. This sharp decrease in feed intake is accompanied with an increased periparturient energy requirement to facilitate colostrum and milk production. This results in a negative energy balance. To accommodate this energy deficit, it is essential for the cow to mobilize body fat reserves. Lipolysis is upregulated and NEFA from adipose tissue overwhelm the liver. Part of NEFA is esterified to be converted into TAG that accumulates in the liver, causing fatty liver disease symptoms. It has been hypothesized that finding a preventative for fatty liver will likely prevent other interrelated metabolic disorders and infectious diseases during the peripartal (around calving) period.

Cows suffering fatty liver are treated similarly to cows affected with ketosis. In mild cases, they are treated with oral glucose precursors such as propylene glycol. In extreme cases, they are treated with intravenous dextrose infusion accompanied by intramuscular glucocorticoid injections. As a preventative against fatty liver disease and ketosis, glycerol mixed with feed has been evaluated. Glycerol drenches also have been assessed to alleviate ketosis.

Therapy of fatty liver disease is frustrating and rarely effective. Our research group recently demonstrated that continuous intravenous infusion of glucagon is efficacious in alleviating the fatty liver-ketosis complex in Holstein dairy cows. Nonetheless, intravenous injection is considered impractical in a dairy farm setting. Subsequently, it was demonstrated that subcutaneous injections of glucagon starting at d 8 postpartum alleviated fatty liver-ketosis complex symptoms from experimentally affected cows. More recently, we have shown that subcutaneous glucagon injections on d 1 prevent development of ketosis. The biochemical basis by which glucagon and/or glycerol exert their preventative action, however, is not fully understood. The objective of this study was to understand the

biochemical basis by which glucagon and/or glycerol prevent fatty liver disease.

The ultimate goal of this study is to provide the U.S. farmers with a slow-release subcutaneous implant of glucagon and/or a glycerol feeding protocol as an effective tool for treating fatty liver disease in dairy cows.

### Materials and Methods

Twenty multiparous Holstein dairy cows with body condition score of 3.5 or better were selected during the dry period. Cows were assigned randomly to one of four treatment groups that received the following treatments:

- 1. Control group:** Cows (n=4), starting at 6:00 h on d 1 postpartum (after calving), received 60 mL of saline (pH 10.25) subcutaneously at 8 h intervals for 14 days.
- 2. Glucagon group:** Cows (n=4), starting at 6:00 h on d 1 postpartum, received 5 mg of glucagon dissolved in 60 mL of saline (pH = 10.25) subcutaneously at 8 h interval for 14 days and once on d 15 postpartum.
- 3. Glycerol group:** Cows (n = 6), starting at 6:00 h on d 1 postpartum, received 400 mL of pure glycerol diluted with 100 mL of water orally once daily for 14 days.
- 4. Glucagon-glycerol group:** Cows (n=6), starting at 6:00 h on d 1 postpartum, received 5 mg of glucagon dissolved in 60 mL of saline (pH=10.25) subcutaneously at 8 h intervals for 14 days and once on d 15 plus 400 mL of pure glycerol diluted with 100 mL of water once daily for 14 days

Blood samples were collected on d 1, d 7, and d 13 postpartum (after calving) using a jugular catheter. Twenty milliliters of blood were collected into two tubes containing Na<sub>2</sub>-EDTA at 15, 10, 5 min before administering each treatment and then at 15, 30, 45, 60, 80, 100, 120, 150, 180, 210, 240, 300, 360, 420, and 480 min after treatment. Tubes were placed on ice until plasma was separated by centrifugation at 500 x g. Plasma was stored at -20<sup>0</sup> for subsequent analyses.

### Results and discussion

We hypothesized that glucagon, glycerol, or glucagon plus glycerol could be administered to early postpartal cows to alleviate or prevent fatty liver disease. This clinical action is likely achieved by improving the energy status of the affected dairy cow. Our results have corroborated this hypothesis. Glucagon and glucagon plus glycerol treatments increased plasma glucose concentration (figures 1, 2, and 3). Glucagon plus glycerol treatment dramatically increased plasma glucose concentrations on d 1, d 7, and d 13 postpartum greater than that of the control group and maintained it at an elevated concentration for longer time than did other treatments (figures 1, 2, and 3). Glucagon treatment decreased plasma NEFA concentrations on d 7 and d 13 (figures 4 and 5). Glucagon plus glycerol decreased plasma NEFA concentrations and maintained it lower for longer time than other treatments on d 1 postpartum (figure 4). Plasma NEFA concentration, however, was decreased by glucagon plus glycerol and

glycerol treatments on all three sampling days. Glycerol treatment maintained plasma NEFA low for longer time than did glucagon plus glycerol treatment on d 7 and 13 postpartum (figures 5 and 6).

Glucagon is a potent glycogenolytic hormone that activates glycogen phosphorylase that causes glycogen to release glucose. Glucagon also increases gluconeogenesis by increasing liver uptake of amino acids that are used as a glucose precursor (data not shown). Furthermore, glucagon increases plasma glucose and by increasing the concentration of rate-limiting gluconeogenesis enzymes.

Glycerol is an efficient glucogenic substrate (can be converted to glucose) because it enters the gluconeogenesis pathway at the triose phosphate level; glycerol conversion to glucose, therefore, is not affected by the rate-limiting enzymes phosphoenolpyruvate carboxykinase and pyruvate carboxylase. Glycerol can increase plasma glucose in the absence of propionate coming from rumen fermentation at time of decreased feed intake.

Glucagon treatment increased plasma glucagon concentration (figures 7, 8, and 9). Also, glucagon treatment increased plasma insulin on d 7 and d 13 postpartum but only slightly on d 1 (figures 10, 11, and 12).

Glucagon, glycerol, and glucagon plus glycerol increased plasma glucose. This response necessitates plasma insulin to increase. The increase in plasma insulin is an essential signal that indicates acute energy abundance. Insulin activates glucose uptake and storage by muscles and stimulate lipogenesis in adipose tissues. Insulin also stimulates ion uptake such as K<sup>+</sup> and PO<sub>4</sub><sup>-3</sup> and amino acid uptake and protein synthesis. Insulin also is needed to restrain metabolic processes that release stored body energy such as lipolysis, ketogenesis, and glycogenolysis. All of these responses suggest that glucagon and glucagon plus glycerol stimulate insulin release that could, in part, cause the metabolic responses that prevent the development of fatty liver by glucagon as shown in earlier research.

### Conclusion

Glucagon and glucagon plus glycerol decreased plasma NEFA concentration and increased plasma glucose and insulin concentration. These metabolic responses may explain why glucagon treatment in previous research decreased fatty liver development. Furthermore, glucagon and glycerol might act additively to improve dairy cow postpartal energy balance.

### Acknowledgement

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Plasma glucose d 1

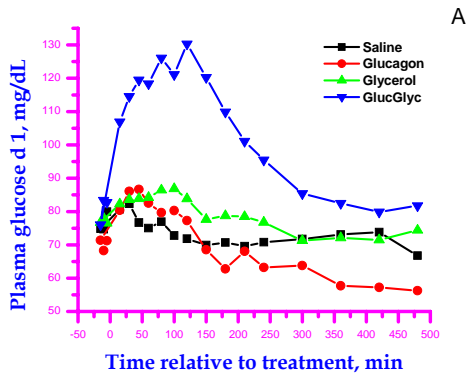


Figure 1. Effect of treatments on plasma glucose concentrations on d 1 postpartum. Glucagon plus glycerol treatments increased plasma glucose.

Plasma glucose d 13

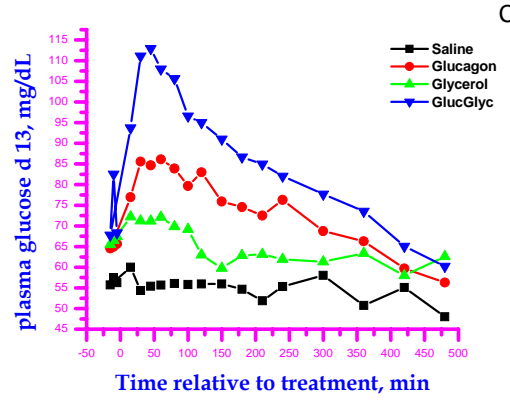


Figure 3. Effect of treatments on plasma glucose concentration on d 13 postpartum. Glucagon plus glycerol treatment increased plasma glucose concentration.

Plasma glucose d 7

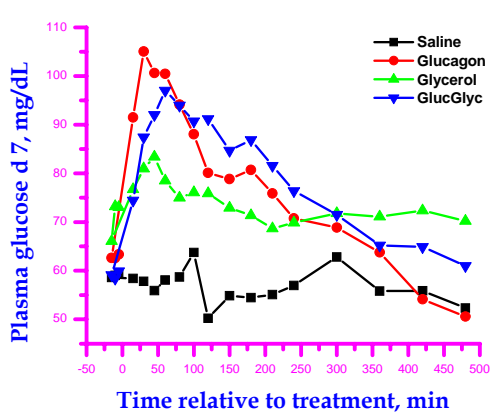


Figure 2. Effect of treatments on plasma glucose concentration on d 7 postpartum. Glucagon and glucagon plus glycerol treatments increased plasma glucose.

Plasma NEFA d 1

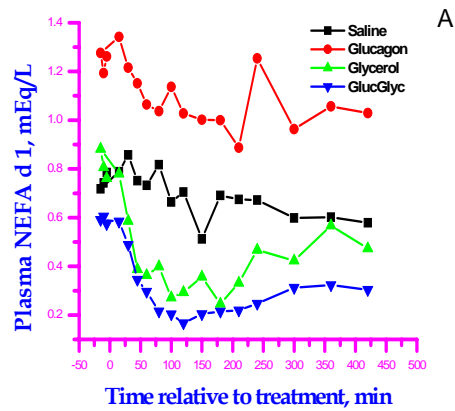


Figure 4. Effect of treatments on plasma NEFA concentration on d 1 postpartum. Glycerol and glucagon plus glycerol treatments decreased plasma NEFA concentration.

Plasma NEFA d 7

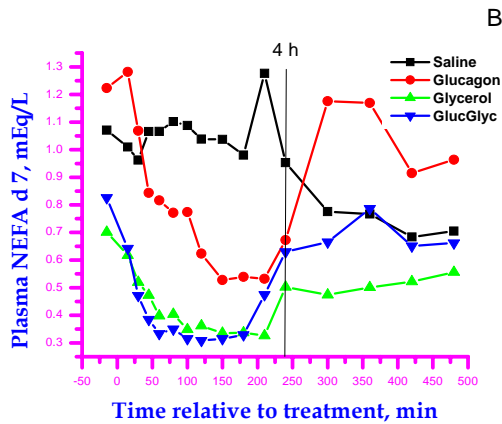


Figure 5. Effect of treatments on plasma NEFA concentration on d 7 postpartum. Glycerol, glucagon, and glucagon plus glycerol treatments decreased plasma NEFA concentration.

Plasma NEFA d 13

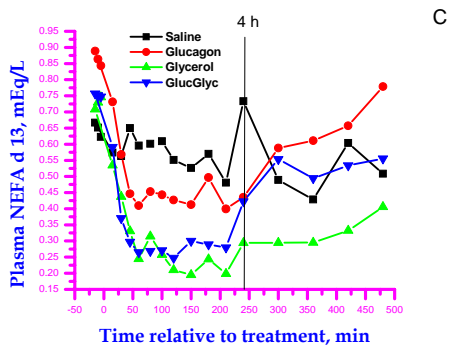


Figure 6. Effect of treatments on plasma NEFA concentration on d 13 postpartum. Glycerol, glucagon, and glucagon plus glycerol treatments decreased plasma NEFA concentration.

Plasma glucagon d 1

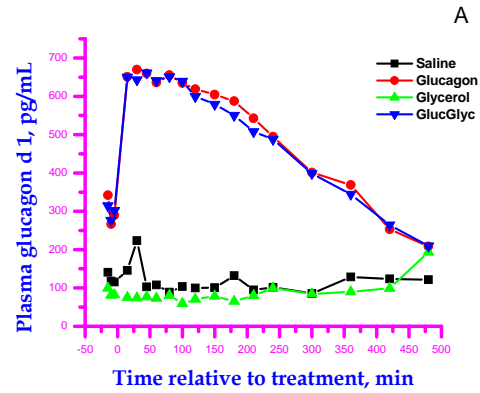


Figure 7. Effect of treatments on plasma glucagon concentration on d 1 postpartum. Glucagon and glucagon plus glycerol treatments increased plasma glucagon concentration.

Plasma glucagon d 7

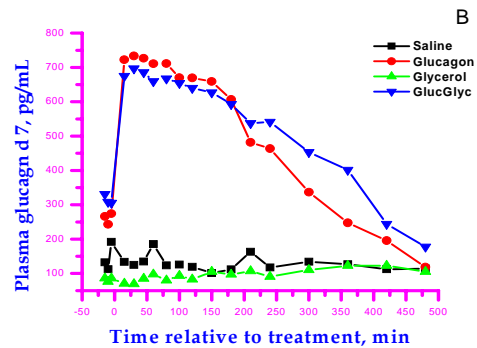


Figure 8. Effect of treatments on plasma glucagon concentration on d 7 postpartum. Glucagon and glucagon plus glycerol treatments increased plasma glucagon concentration.

Plasma glucagon d 13

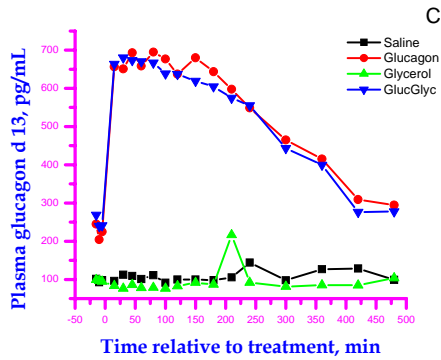


Figure 9. Effect of treatments on plasma glucagon concentration on d 13 postpartum. Glucagon and glucagon plus glycerol treatments increased plasma glucagon concentration.

Plasma insulin d 1

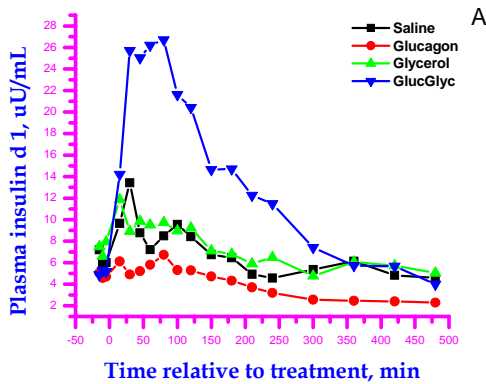


Figure 10. Effect of treatments on plasma insulin concentration on d 1 postpartum. Glucagon plus glycerol treatment increased plasma insulin concentration.

Plasma insulin d 7

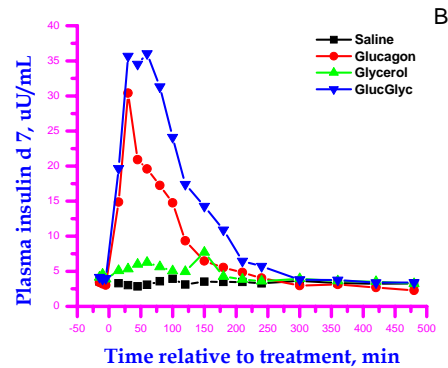


Figure 11. Effect of treatments on plasma insulin concentration on d 7 postpartum. Glucagon and glucagon plus glycerol treatments increased plasma insulin concentration.

Plasma insulin d 13

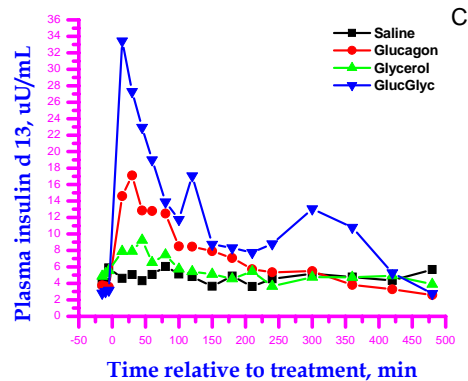


Figure 12. Effect of treatments on plasma insulin concentration on d 13 postpartum. Glucagon plus glycerol treatment increased plasma insulin concentration.



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