

Health and Reproductive Performance of Lactating Dairy Cows with Mild Fatty Liver Receiving Exogenous Glucagon

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Summary and Implications

The data demonstrate that mild fatty liver is associated with decreased general health and reproductive performance as evidenced by more days to first estrus and service, more days open, decreased conception, increased number of days with elevated body temperature at days 9 - 22 postpartum, and increased incidence of mastitis at days 32 - 150 postpartum. The latter two were decreased in dairy cows with mild fatty liver injected with 15 mg/d of glucagon during weeks 2 and 3 postpartum. Therefore, treatment of mild fatty liver with exogenous glucagon or better prevention of fatty liver might improve health and reproductive performance of lactating dairy cows, thereby preventing loss of income of dairy farmers.

Introduction

Fatty liver is a metabolic disease that affects up to 50% of dairy cows in early lactation. Fatty liver is associated with an increased incidence and duration and decreased treatment success of infectious and metabolic diseases, such as mastitis, metritis, and ketosis, as well as decreased reproductive performance in affected cows. A major reason for this association is that hepatic lipid accumulation can impair liver function, such as synthesis and biotransformation of metabolites and proteins and the detoxification and excretion of toxic waste products and xenobiotics, which, in turn, can compromise immune competence and ovarian function.

A previous study from our group demonstrated that severe fatty liver and ketosis can be treated by continuous, intravenous infusions of 10 mg/d of glucagon for 14 days. In that study, cows were induced artificially into fatty liver and ketosis, however, by feed restriction and administration

of 1,3-butanediol, and the effects on health and reproduction were not determined. The objectives of the current study was to determine whether a) mild fatty liver is associated with decreased health status (mastitis, elevated body temperature, and ketosis) and reproductive performance (days to first observed estrus, first service, days open, number of services, conception rate after first service, and overall conception rate) and b) whether glucagon injected subcutaneously at 7.5 or 15 mg/d for 14 days starting at day 8 postpartum could decrease some of the detrimental associations of mild fatty liver on health and reproductive performance.

Materials and Methods

For the experiment, 32 Holstein dairy cows were used that were 32 - 74 months of age, weighed 1190 - 1725 kg, had a body condition score of 3.00 - 4.25 on a 1 - 5 scale, and had completed 1-4 lactations. For the last 4 weeks prepartum, each cow was offered individually, in addition to hay and the total mixed ration that was formulated to meet NRC requirements, 6 kg of cracked corn daily to potentially increase the incidence of mild fatty liver. All cows were visually healthy before parturition. At day 8 postpartum, cows were divided into two groups depending on their liver triacylglycerol concentrations (cutoff value of 1% liver wet weight) at day 8 postpartum. Cows with normal liver ($n = 8$; Saline Normal) and triacylglycerol concentrations <1% of liver wet weight were injected subcutaneously with 60 ml of 0.15 M NaCl every 8 hours (6:00, 14:00, and 22:00 for 14 days starting at 14:00 of day 8 postpartum. Cows with mild fatty liver ($n = 24$; 8 cows randomly assigned to each treatment group) and triacylglycerol concentrations of 1 - 10% of liver wet weight were injected with 0 (Saline Susceptible), 2.5 (7.5 mg/d), or 5 (15 mg/d) mg of lyophilized glucagon. All cows were managed and treated in accordance with guidelines established by the Iowa State University Committee on Animal Care.

Cows were monitored twice daily for flakes in the milk and once daily at 9:00 for body temperature and urinary acetoacetate concentrations. As an indicator of ketosis, Ketostix[®] strips (Bayer Corporation, Tarrytown, NY) were used. Mastitis was defined by flakes in the milk or a somatic cell count in milk of >1,000,000 per ml. Milk samples were analyzed for somatic cell count at two consecutive milking at days 3, 8 (before first injection of glucagon), 11, 15, 22 (before last injection), 25, 28, 36, and 43 postpartum by mid-infrared spectrophotometry (Milk-O-Scan 203; Foss Food Technology, Eden Prairie, MN).

Estrus behavior was monitored for 1 hour twice daily. The reproductive tract was examined by rectal palpation and

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ultrasound by a veterinarian every 2 weeks beginning at 20 days postpartum. Cows that were at least 42 days postpartum, were confirmed open, and had at least one corpus luteum were injected subcutaneously with 5 ml of Lutalyse® (50 mg/ml PGF₂; Pharmacia & Upjohn, Kalamazoo, MI) every 14 days until they showed signs of estrus and then were inseminated. Cows that were at least 45 days postpartum were inseminated 12 hours after they allowed mounting. Cows whose previous insemination passed at least 35 days without further estrus were examined for pregnancy by palpation and ultrasound.

Chi-square tests were used to compare treatment group means of incidence rates for health status (mastitis, body temperatures >39.5 °C, and urinary concentrations >40 mg/dl) and reproductive performance parameter (conception rate after the first service, and overall conception rate). A t-test was used to compare average lengths of time with body temperatures >39.5 °C of treatment groups. Days to first heat, days to first service, and days open were assumed Weibull distributed, and treatment means were compared by using a chi-square test.

Results and Discussion

The data demonstrate that non-treated mild fatty liver was associated with increased incidence of mastitis from days 23 – 150 postpartum (Table 1). Treatment of mild fatty liver with subcutaneous injections of glucagon, in particular at 15 mg/d, decreased the incidence of mastitis (Table 1). The association of mild fatty liver with increased incidence of mastitis from days 23 – 150 postpartum seems surprising because, at days 23 – 42 postpartum, cows had normal liver triacylglycerol concentrations. A possible reason for the higher incidence of mastitis in cows with non-treated mild fatty liver is that mild fatty liver might compromise the immune response to mammary infections, which originated from the periparturient period (Table 1). This possibility is supported by the decreased number of days with elevated body temperatures at 9 – 22 days postpartum of cows with normal liver and cows with mild fatty liver receiving 15 mg/d of glucagon.

Cows with mild fatty liver had a decreased reproductive performance (Table 2). The decreased reproductive performance was associated with a higher incidence of mastitis and a longer period of time in negative energy balance. Five of the 6 cows that did not conceive had mastitis at days 43 – 150 postpartum, whereas only 8 of the 24 cows that conceived had mastitis ($P \leq 0.03$). Fifteen of the 22 cows with mild fatty liver that were inseminated had a lower body weight at days 23 – 42 postpartum than at days 9 – 22, whereas the same was true for only 2 of 8 cows with normal liver ($P \leq 0.03$). Mastitis and negative energy balance both are associated with decreased signs of estrus, conception rate, and embryo survival rates. Subcutaneous injections of glucagon did not improve reproductive performance of cows with mild fatty liver (Table 2). We attribute the lack of positive effects of exogenous glucagon on reproductive parameters to the fact that glucagon did not decrease the length of time during which cows were in negative energy balance. In another study from our group, injections of 15 mg/d of glucagon for 14 days beginning at day 2 postpartum prevented mild fatty liver. Therefore, exogenous glucagon, given directly after calving for 14 days, might improve reproductive performance. Overall, the results indicate that subcutaneous injections of 15 mg/d of glucagon could alleviate some of the detrimental impacts of mild fatty liver on health and reproduction and, therefore, could improve overall performance and profitability of lactating dairy cows.

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Table 1. Health status of cows with normal liver (Saline Normal) or mild fatty liver (Saline Susceptible, 7.5 mg/d Glucagon, 15 mg/d Glucagon) receiving either saline or glucagon at 7.5 or 15 mg/d or saline for 14 days beginning at day 8 postpartum

	Treatment				Type III SS $P > F^a$		
	Saline Normal (n = 8)	Saline Susceptible (n = 8)	7.5 mg/d Glucagon (n = 8)	15 mg/d Glucagon (n = 8)	C1	C2	C3
Incidences:	Number of cows						
Before injection period (days 0-8 postpartum):							
Mastitis	4	5	4	6	NS	NS	NS
Body temp. >39.5°C	5	6	6	5	NS	NS	NS
Acetoac. >40 mg/dl ^b	0	0	2	4	NS	NS	0.02
During injection period (days 9-22 postpartum):							
Mastitis	0	2	1	0	NS	NS	NS
Body temp. >39.5°C	8	6	6	6	NS	NS	NS
Acetoac. >40 mg/dl ^b	1	1	3	3	NS	NS	NS
After injection period (days 23-42 postpartum):							
Mastitis	0	4	1	0	0.02	NS	0.02
Body temp. >39.5°C	2	2	4	3	NS	NS	NS
Acetoac. >40 mg/dl	1	0	1	1	NS	NS	NS
During insemination and conception period (days 43-150 postpartum):							
Mastitis	3	7	2	3	0.04	0.01	0.04
Time length:	Days per week (mean ± SEM)						
During injection period (days 9-22 postpartum):							
Body temp. >39.5°C	2.0 ± 0.5	3.9 ± 0.6	3.0 ± 0.6	1.9 ± 0.5	0.02	NS	0.02
After injection period (days 23-42 postpartum):							
Body temp. >39.5°C	0.4 ± 0.7	0.5 ± 0.6	1.2 ± 0.5	0.9 ± 0.5	NS	NS	NS

^aC1: contrast between Saline Normal and Saline Susceptible (association with Susceptibility); C2: contrast between 7.5 mg/d Glucagon and Saline Susceptible (effect of 7.5 mg/d glucagon); C3: contrast between 15 mg/d Glucagon and Saline Susceptible (effect of 15 mg/d glucagon); NS = not significant at $P > 0.05$; ^burinary acetoacetate concentrations >40 mg/dl.

Table 2. Reproductive health and performance of cows with normal liver (Saline Normal) or mild fatty liver (Saline Susceptible, 7.5 mg/d Glucagon, 15 mg/d Glucagon) receiving either saline or glucagon at 7.5 or 15 mg/d or saline for 14 days beginning at day 8 postpartum

	Treatments				Type III SS $P > F^a$		
	Saline Normal (n = 8)	Saline Susceptible (n = 8)	7.5 mg/d Glucagon (n = 7)	15 mg/d Glucagon (n = 7)	C1	C2	C3
	Days postpartum (mean ± SEM)						
Reproduction:							
First estrus	44 ± 5	54 ± 8	56 ± 6	51 ± 4	0.04	NS	NS
First service	53 ± 2	81 ± 12	74 ± 11	73 ± 10	0.003	NS	NS
Days open	82 ± 24	105 ± 24	137 ± 17	104 ± 26	0.01	NS	NS
Services (n/cow)	1.5 ± 0.4	2.6 ± 0.6	3.3 ± 0.7	2.7 ± 0.8	NS	NS	NS
Conception rate:	Number of cows						
First service	5	4	3	3	NS	NS	NS
Overall	8	5	6	5	0.05	NS	NS

^aC1: contrast between Saline Normal and Saline Susceptible (association with Susceptibility); C2: contrast between 7.5 mg/d Glucagon and Saline Susceptible (effect of 7.5 mg/d glucagon); C3: contrast between 15 mg/d Glucagon and Saline Susceptible (effect of 15 mg/d glucagon); NS = not significant at $P > 0.05$.