Effect of Isoflupredone Acetate With or Without Insulin on Energy Metabolism, Reproduction, Milk Production, and Health in Dairy Cows in Early Lactation

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ABSTRACT

Glucocorticoids are commonly used to treat cows with clinical ketosis and fatty liver disease, but their use is controversial. The objectives of the present study were to investigate the effects of isoflupredone acetate alone or with insulin on the energy metabolism of dairy cows in early lactation in a large double-blind, randomized clinical trial. A total of 1,162 Holstein cows and first-lactation heifers were randomly assigned to receive 1 of 3 treatments between the day of parturition and 8 DIM: group A, 20-mg i.m. injection of isoflupredone and 100 units of insulin; group B, 20-mg i.m. injection of isoflupredone; group C (control group), 10-mL i.m. injection of sterile water. Treatments were randomized across 24 dairy farms located near Guelph, Ontario, Canada. Serum samples obtained at the time of treatment and at wk 1 and 2 following treatment were analyzed for β -hydroxybutyrate, nonesterified fatty acids, glucose, calcium, potassium, sodium, and chloride. Cows were assigned a body condition score at the time of enrollment. Data were analyzed using a repeated-measures mixed model that accounted for the effects of parity and body condition score, and the random effects of cow and farm. Cows that received isoflupredone with insulin and isoflupredone alone had higher β -hydroxybutyrate and nonesterified fatty acid concentrations 1 wk after treatment compared with control cows. Cows that received isoflupredone acetate plus insulin had lower glucose concentrations at 1 wk after treatment. Calcium concentrations 1 wk after treatment were lower for cows that received isoflupredone and insulin or isoflupredone only compared with control cows. Serum sodium, potassium, and chloride concentrations were not influenced by treatment. The effect of treatment on the proportion of cows with subclinical ketosis was evaluated with a logistic regression model. Over

the 2 wk following treatment, a significant increase in the prevalence of subclinical ketosis was observed in the isoflupredone plus insulin group relative to the control group. Among 972 cows that were not ketotic at enrollment, cows that received isoflupredone acetate plus insulin or isoflupredone acetate only were, respectively, 1.72 and 1.59 times more likely than control cows to develop subclinical ketosis 1 wk after treatment. There were no treatment effects on test-day milk production, milk fat and protein percentages, or the intervals from calving to first insemination or pregnancy.

Key words: energy, peripartum, glucocorticoid, insulin

INTRODUCTION

The transition period, from 3 wk before to 3 wk after parturition, is critically important to the health, production, and profitability of dairy cows (Drackley, 1999). During the transition period, feed intake is at the lowest point of the lactation-gestation cycle, and the demand for nutrients, particularly energy, is increasing at the greatest rate a cow will ever experience (Grummer et al., 2004). Most health disorders occur during this time. One of the major predisposing causes of these periparturient diseases is negative energy balance. Although virtually all cows go through some degree of negative energy balance postcalving (Herd, 2000), it is the degree of negative energy balance that most likely contributes to disease. A recent study of 25 Holstein dairy herds indicated that more than 40% of dairy cows suffered from subclinical ketosis in early lactation (Duffield, 2000). Clinical ketosis has been shown to significantly reduce milk yield in dairy cows, with an average loss of production of 25%, or 353.4 kg per lactation (Lucey et al., 1986; Rajala-Schultz et al., 1999). Even with subclinical ketosis, there is a loss of 1.0 to 1.5 kg/d of milk production (Dohoo and Martin, 1984). Cows with subclinical ketosis were 8 times more likely to develop a displaced abomasum (LeBlanc et al., 2005).

When treating cows for negative energy balance, it is essential that the need for glucose be met, and that

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the ketogenic process in the liver be reduced (Herdt, 2000; Hungerford, 1990). Typically, an intravenous bolus of 50% dextrose solution is used to this end. However, the effects of this therapy are short-lived and must be repeated for 2 to 4 d following initial treatment (Hungerford, 1990). Therefore, glucocorticoids, propylene glycol, or sodium propionate are often administered (Radostits et al., 2000; Pickett et al., 2003). Drenching with propylene glycol has a slightly beneficial effect on reducing NEFA and BHBA levels (Pickett et al., 2003). Insulin has been recommended as a treatment for ketosis, although its use has not been widespread, probably as a result of concern for aggravating hypoglycemia in ketotic cows (Foster, 1988). Slow-release insulin has also been used for the therapy of ketosis, and has resulted in increased DMI and milk yield. In a study with 37 cows, insulin reduced liver triglyceride and NEFA levels over a few days (Hayirli et al., 2002).

Glucocorticoids are commonly used to treat cows with clinical ketosis and fatty liver disease (Foster, 1988; Andrews et al., 1991; Shpigel et al., 1996; Bruss, 1997; Radostits et al., 2000). When it was first observed that glucocorticoids appeared to be an effective treatment for spontaneous ketosis, the hypothesis was that the disease was due to adrenal cortical insufficiency. This theory has fallen into disfavor because ketotic cows have been shown to have higher plasma levels of glucocorticoids than healthy cows (Bruss, 1997). Glucocorticoids probably have their effect by stimulating proteolysis and inhibiting glucose use in muscle, thereby providing gluconeogenic precursors and stimulating the rate of gluconeogenesis (Foster, 1988; Bruss, 1997). However, the use of glucocorticoids is controversial because they might increase NEFA release from adipose tissue and thereby aggravate ketosis (Baird and Heitzman, 1971; Slavin et al., 1994; Hippen et al., 1999; Bobe et al., 2004). Two studies (Furll et al., 1993; Furll and Furll, 1998) reported significant increases in plasma glucose concentrations and decreases in concentrations of NEFA and BHBA in plasma, but the dosing regimen of 5 daily injections of prednisolone was not typical of clinical practice. The objective of this study was to evaluate the effect of isoflupredone acetate with or without insulin, when administered in early lactation, on energy metabolism, blood electrolytes, reproductive performance, and milk production.

MATERIALS AND METHODS

Study Design

A total of 1,162 animals from 20 farms near Guelph, Ontario, Canada, and 4 farms near Kemptville, Ontario, Canada, were enrolled in a double-blind, randomized clinical trial. Dairy herds were selected based on

the willingness of the herd operators to participate in the study as well as the enrollment of the herd in DHIA milk recording. Cows were enrolled between the day of calving and 8 DIM. At enrollment, animals were randomly assigned to receive 1 of 3 treatments: 1) group A, 20 mg of isoflupredone acetate i.m. (10 mL of Predef two times, Pfizer, Kirkland, Quebec, Canada) in the left hind leg plus a 100-unit subcutaneous (s.c.) injection of insulin (1 mL of Humulin Ultralente, Eli Lilly and Co., Indianapolis, IN) in the right leg; 2) group B, 20-mg i.m. injection of isoflupredone acetate in the left hind leg plus a 1-mL s.c. injection of sterile water in the right leg; 3) group C (control group), 10-mL i.m. injection of sterile water in the left hind leg plus a 1-mL s.c. injection of sterile water in the right leg.

Herd size ranged from 35 to 450 lactating Holstein cows, and the rolling herd average for milk production was between 7,625 and 11,895 kg. All herds were fed a TMR. The predominant forages used on these farms were hay, alfalfa haylage, and corn silage, and the main concentrates were corn, mixed grain, soybean meal, roasted soybeans, and commercial protein supplements. Six herds had tie-stall barns, and the others used free-stall facilities. All farm personnel, veterinarians, and researchers were masked to the treatment. Lists of the cows that were due to calve were generated for each farm, and the randomization scheme was followed according to the order of calving. Cows were enrolled between April 12 and September 1, 2005. Every farm was visited weekly in the morning on the same day of the week and at approximately the same time of the day.

Blood was collected from the coccygeal vein into 10-mL vacuum tubes (Becton Dickinson, Franklin Lakes, NJ) at the time of enrollment, and again at both 7 d (wk +1) and 14 d (wk +2) after enrollment. Cows were scored for body condition on a scale of 1 to 5, in increments of 0.25 (Edmonson et al., 1989), at the time of enrollment.

Sample Handling and Laboratory Procedures

Blood samples were chilled on ice packs immediately after collection and within 3 h were centrifuged at $733 \times g$ for 10 min. Harvested sera were frozen and later submitted to the Animal Health Laboratory at the University of Guelph for determination of BHBA, NEFA, glucose, calcium, inorganic phosphorus, potassium, sodium, and chloride concentrations. All biochemical tests were conducted on an automated analyzer (Hitachi 911 Analyzer, Roche Diagnostics, Laval, Quebec, Canada). Reagents for all tests were supplied by Roche Diagnostics (Laval).

Data Management and Statistical Analysis

The data were checked for errors and compared with written reports; outliers were rechecked to ensure that values were accurate. Because serum metabolites were measured over time, a repeated-measures approach using ANOVA with mixed linear models was used in SAS (fixed effects of treatment and covariates, random effects of cow and herd; SAS Inst. Inc., Cary, NC). All outcome variables were screened for normality by visual assessment of the distributions and calculation of kurtosis and skewness. The distributions of calcium and potassium were normal, whereas the distributions of BHBA, NEFA, phosphorus, chloride, and sodium were skewed to the right and were transformed with the natural logarithm to achieve a normal distribution. The distribution of glucose was transformed to square root to get a normal distribution. Several covariance structures were evaluated for each analyzed metabolite: compound symmetry, unstructured, autoregressive order 1, autoregressive heterogeneous order 1, Toeplitz, and Toeplitz heterogeneous. The covariance structure for each model that resulted in Akaike's information criterion closest to zero was used (Wang and Goonewardene, 2004). Cow variables included treatment, parity, BCS at enrollment, time (i.e., sample), and the occurrence of retained placenta (RP), milk fever, and subclinical ketosis (except for the models in which ketosis was the outcome). Subclinical ketosis was defined as a serum BHBA concentration of $\geq 1,400 \mu\text{mol/L}$ (Duffield, 2000). Cows that had a BCS of ≤ 3.0 were classified as thin, a BCS of 3.25 or 3.5 as fair, and a BCS of ≥ 3.75 as fat. Parity was classified into 3 groups: 1, 2, and ≥ 3 . Cow and herd were considered as random effects to account for the correlation between observations of the same cow and correlations between cows within the same herd. All variables were offered to each model and then removed in a backward stepwise elimination approach. Interactions between treatment and the significant covariates were tested and included in the final model if significant. The interaction between time (enrollment, wk +1, and wk +2) and treatment was tested. If there was a significant interaction, data were reanalyzed after stratification by sample time. Because there were 3 samples for each cow, a Bonferroni correction of the probability value was used ($P < 0.016 = 0.05$ divided by 3) when there was stratification on time. For each of the models for which a significant treatment effect was found, least squares means and standard errors by treatment were plotted for each outcome.

The treatment effect on the proportion of cows with subclinical ketosis was evaluated with multivariable logistic regression models with PROC GENMOD of SAS

(SAS Inst. Inc.). The models included treatment, sample time, parity, and BCS class. The pretreatment sample served as the referent for the incidence or cure of subclinical ketosis. The impact of treatment on both prevention (cows with BHBA $< 1,400 \mu\text{mol/L}$ that remained nonketotic) and resolution (those with subclinical ketosis that became normal) were modeled separately by logistic regression as described above.

Definitions of periparturient health events were based on Duffield et al. (1999). The association of treatment with disease outcomes, including death and culling, by 61 DIM was the chi-squared statistic. Variables that were significant at $P < 0.20$ were included in the multivariable logistic regression analyses.

Individual test-day data for milk production were collected for all cows from DHIA (Canwest DHI, Guelph, Ontario, Canada) records. Data from the first 3 DHI tests after calving were used to assess the effect of treatment on test-day milk production and components. The 305-d milk production projection at the second test day was also analyzed as an outcome. Milk production data were analyzed using repeated-measures ANOVA (PROC MIXED in SAS, SAS Inst. Inc.). Separate models were built for each of test-day milk weight, test-day milk fat percentage, and test-day milk protein percentage. Variables considered in each model included treatment, BCS at enrollment, parity group, test-day SCC linear score, subclinical ketosis before treatment, and disease (cows that had one or more illnesses after parturition were considered diseased; otherwise, cows were considered healthy).

Reproductive performance was measured by the intervals from calving to first breeding and from calving to pregnancy by using survival analysis. Cows were followed for 9 mo until pregnant or culled. Cows in the herd that remained nonpregnant after 9 mo were retained in the analysis and were censored at that time. The effects of treatment on time to first breeding and pregnancy were analyzed with multivariable survival analysis using Cox's proportional hazards regression (PROC PHREG in SAS, SAS Inst. Inc.). The models included treatment, parity, BCS at enrollment, disease (cows having one or more illnesses postcalving), and a random herd effect.

RESULTS

Metabolic and Health Data

A total of 376 cows were given isoflupredone plus insulin (group A), 397 cows received isoflupredone only (group B), and 389 cows received the placebo (group C). There was no difference in the distribution of parity or BCS between treatment groups. Approximately 31% were in first lactation, 30% were in second lactation,

Table 1. Parity and BCS of cows that received isoflupredone acetate plus insulin (group A), isoflupredone acetate only (group B), or a placebo (group C) once in the first week of lactation

Item	Total	Group A (n = 376)	Group B (n = 397)	Group C (n = 389)
Parity				
1	363	115	126	122
2	352	109	122	121
≥3	447	152	149	146
BCS at enrollment				
<3.0	346	112	136	98
3.25 to 3.5	674	217	215	242
≥3.75	142	47	46	49

and the remainder (39%) were in third parity or greater (Table 1).

The concentrations of NEFA, BHBA, glucose, and calcium were significantly influenced by treatment. Treatment $\times$ time interactions were significant for each of these outcomes. No significant treatment effects were found for phosphorus, potassium, sodium, or chloride. There were no other significant interactions between treatment and the other model variables for any of the analyses. The random effects of farm and cow were significant ($P < 0.05$) in all models, but there were no interactions of farm with treatment of any metabolite.

Seventy-six cows had RP and 14 cows had milk fever. In addition, 190 cows had subclinical ketosis at enrollment. All the models controlled for the effects of RP, milk fever, and preexisting subclinical ketosis, but there were no significant interactions between treatment and these 3 diseases.

Accounting for the effects of parity, BCS, and the random effects of cow and farm, BHBA concentrations were significantly higher for cows that had been treated with isoflupredone plus insulin ($P = 0.0003$) or isoflupredone only ($P = 0.0147$) than control cows 1 wk after treatment, and tended to be higher for cows treated with isoflupredone plus insulin than control cows ($P = 0.0185$) 2 wk after treatment (Figure 1). Accounting for the effects of parity, BCS, and the random effects of cow and farm, NEFA concentrations were significantly higher for cows treated with isoflupredone plus insulin ($P = 0.0009$) or isoflupredone only ($P = 0.0127$) than control cows 1 wk after treatment (Figure 2). Accounting for the effects of parity, BCS, and the random effects of cow and farm, glucose concentrations were significantly lower for cows treated with isoflupredone plus insulin both 1 wk ($P = 0.0045$ and $P = 0.0041$) and 2 wk after treatment ($P = 0.0046$ and $P = 0.0051$) compared with cows treated with isoflupredone only or the placebo (Figure 3). Accounting for the effects of parity, BCS, and the random effects of cow and farm, calcium concentrations were significantly lower for cows that had been treated with isoflupredone plus insulin ($P <$

0.0001) or isoflupredone only ($P = 0.0043$) at wk +1 in comparison with control cows (Figure 4).

The logistic regression model for the prevalence of subclinical ketosis included treatment, time, parity, and BCS class. When all cows were considered together, over the 2 wk following treatment a significant increase in the prevalence of subclinical ketosis was observed in the isoflupredone plus insulin group relative to the control group (odds ratio = 1.47, 95% confidence interval = 1.2 to 1.79, $P = 0.0002$). Among 972 cows that were not ketotic at enrollment, cows that received isoflupredone plus insulin or isoflupredone only were, respectively, 1.72 and 1.59 times more likely than control cows to develop subclinical ketosis 1 wk after treatment (Table 2). Cows that received isoflupredone plus insulin were at a risk 1.46 times greater than control cows of having subclinical ketosis 2 wk after treatment. Among 190 cows that were ketotic before treatment, neither treatment improved the resolution of ketosis, relative to controls, and cows that received isoflupredone plus insulin tended (OR = 2.0, $P = 0.06$) to be more likely than control cows to remain ketotic. Complete health data were available for 905 cows from 16 out of 24 farms. There were no significant effects of treatment on clinical disease risks (Table 3).

Production and Reproduction

Data from 1,102 cows were available for evaluation of the treatment effect on test-day milk production and milk components. The final models for test-day milk production and for fat and protein percentages included parity, DHI test number, SCC linear score, BCS at treatment administration, and disease, and the random effects of cow and herd. There were no treatment effects on test-day milk yield ($P = 0.19$), fat percentage ($P = 0.72$), or protein percentage ($P = 0.14$; Table 4). There were no treatment $\times$ test-day interactions. Similarly, there was no treatment effect on projected 305-d milk yield. Table 5 presents a summary of reproductive indices by treatment. The intervals from calving to first breeding and to pregnancy were not influenced by treatment.

DISCUSSION

To our knowledge, this is the largest field study on the effects of a corticosteroid with or without insulin on the energy metabolites and performance of dairy cows. The main findings of the present study were that isoflupredone alone or with insulin had no preventive effect on subclinical ketosis 1 to 2 wk after treatment. Unexpectedly, BHBA and NEFA concentrations were significantly higher for cows treated with isoflupredone

Figure 1. Least squares means (accounting for parity, BCS, and random effects of cow and farm) and standard errors for serum BHBA concentrations at enrollment, and 1 and 2 wk after treatment in dairy cows that received isoflupredone acetate plus insulin (●), isoflupredone acetate only (○), or placebo (▼) once in the first week of lactation. There was an interaction of treatment with time ($P = 0.058$). Treatments marked with different letters were significantly different ($P < 0.016$) at that sample time.

with or without insulin 1 wk after treatment, and cows that received isoflupredone plus insulin had lower glucose concentrations 1 and 2 wk after treatment.

Little information is available about the effects of glucocorticoids, alone or with insulin, on lipolysis in dairy cows. Kronfeld and colleagues (Kronfeld, 1966; Robertson, 1966) evaluated a single treatment with a glucocorticoid (dexamethasone or flumethasone) plus insulin, a glucocorticoid alone, or a placebo in 40 cows with clinical ketosis per treatment and reported clinical recovery rates of 68, 56, and 23%, respectively. However, the small numbers of animals and limitations of statistical methods at the time made it impossible to evaluate possible confounding effects on the efficacy of treatments. Clinical success was measured within 5 d, and the ketosis cases occurred later in lactation than in the current study. In a clinical trial, Shpigel et al. (1996) compared the relative efficacy of dexamethasone or flumethasone alone or in combination with rapid i.v. infusion of glucose for treatment of ketosis. They treated 127 cows in 4 treatment groups and measured serum glucose and BHBA 2 d after treatment. They concluded that treatment of ketosis with a corticosteroid alone was less efficacious than with glucose and a

corticosteroid, based on a greater relapse risk in those that received corticosteroid alone.

Holtenius and Holtenius (1996) reported that classical clinical ketosis generally occurred 3 to 6 wk after calving in cows whose glucose demand for milk production exceeded the capacity for glucose production. However, the present study addressed the effects of a glucocorticoid alone or with insulin in clinically healthy cows in the first week after calving. Among this population of cows, we found that glucocorticoids with or without insulin might have a detrimental effect on energy balance in early lactation. Whether similar effects could be expected in cows with clinical ketosis or fatty liver cannot be determined from the present results.

Glucocorticoids increase gluconeogenesis (Foster, 1988; Bruss, 1997; Rijnberk and Mol, 1997). Gluconeogenesis and ketogenesis are, under most circumstances, related directly; that is, as one increases, so does the other (Herdt, 1988). Increased ketogenesis is associated with an increased rate of gluconeogenesis in association with an increased activity of a key gluconeogenic enzyme (phosphoenolpyruvate carboxykinase). It was shown in rats that dexamethasone increased hormone-sensitive lipase mRNA levels in vitro by approximately

Figure 2. Least squares means (accounting for parity, BCS, and random effects of cow and farm) and standard errors for serum NEFA concentrations at enrollment, and 1 and 2 wk after treatment in dairy cows that received isoflupredone acetate plus insulin (●), isoflupredone acetate only (○), or placebo (▼) once in the first week of lactation. There was a significant interaction of treatment with time ($P = 0.03$). Treatments marked with different letters were significantly different ($P < 0.016$) at that sample time.

4-fold (Slavin et al., 1994). Hormone-sensitive lipase is the enzyme responsible for hydrolysis of triacylglycerol from adipocytes into glycerol and NEFA. Nonesterified fatty acids are released into the blood stream and are taken up by the liver. In the liver, NEFA can be reesterified to triacylglycerol or be oxidized to acetyl-CoA. In early lactation, the supply of glucose and glucose precursors is limited and little glucose flows into the Krebs cycle, resulting in little or no citrate leaving the mitochondria for malonyl-CoA production. Low malonyl-CoA concentrations result in activation of carnitine palmitoyltransferase-I, inducing rapid transfer of NEFA into mitochondria. Mitochondrial metabolism of NEFA stimulates the production of both glucose and ketone bodies (Herdt, 2000). There is an increase in the NADH:NAD ratio, which would promote the conversion of oxaloacetate to malate, thereby depleting oxaloacetate. With the depletion of oxaloacetate and oxaloacetate deficiency, there is an insufficient condensing partner for acetyl-CoA for the Krebs cycle. The acetyl-CoA is then readily diverted to ketone bodies (Kaneko, 1997). Whole-cell oxaloacetate concentrations are decreased during ketosis (Baird et al., 1968), but whether this decrease is a reflection of mitochondrial oxaloacetate is not known (Grummer, 1993). Regardless of how low

mitochondrial oxaloacetate levels might be in the liver, ketogenesis will not occur at a significant rate without sufficient precursor in the form of NEFA (Bruss, 1997). In addition, glucocorticoids promote the release of AA and their subsequent conversion to glucose in the liver. Glucogenic AA can be degraded to pyruvate or an intermediate in the Krebs cycle. Leucine (a six-carbon, branched-chain AA) cannot be converted to the glucogenic AA; thus, as protein is broken down, Leu and other branched-chain AA (Ile and Val) are converted to ketone bodies (Rooyackers and Nair, 1997). The net effect of glucocorticoids on protein degradation includes production of both glucose and ketones.

However, in the short term, glucocorticoids may cause a transitory hyperglycemia (because of the reduced milk production and glucose yield from gluconeogenesis), but after several days, ketogenesis is increased. Additionally, glucocorticoids may increase lipolysis, thereby increasing the supply of NEFA and contributing to the increased serum NEFA concentrations and increased ketonemia observed 7 d after treatment in the present study. Dutch researchers showed that a single dose of dexamethasone-21-isonicotinate significantly increased the plasma glucose concentra-

Figure 3. Least squares means (accounting for parity, BCS, and random effects of cow and farm) and standard errors for serum glucose concentrations at enrollment, and 1 and 2 wk after treatment in that dairy cows received isoflupredone acetate plus insulin (●), isoflupredone acetate only (○), or placebo (▼) once in the first week of lactation. There was a significant interaction of treatment with time ($P = 0.003$). Treatments marked with different letters were significantly different ($P < 0.016$) at that sample time.

tion on d 2, but not on d 4, 7, and 9, in heifers in early lactation (Jorritsma et al., 2004).

On the other hand, around parturition there is increased lipolysis in adipose tissues and very little stimulus for reesterification of fatty acids in adipose tissue (McNamara and Hillers, 1986; Smith and Walsh, 1988). In the first weeks after parturition, neither glucose nor insulin were observed to stimulate reesterification of fatty acids (Metz and Van Den Bergh, 1977). Fatty acids and triglycerides in the liver inhibit the removal of circulating insulin by the liver, which results in hyperinsulinemia (Oehler et al., 1982; Arner et al., 1983). Because of decreased insulin binding in the liver, the pathway for fatty acid metabolism might be switched from lipogenesis to oxidation and ketogenesis (Holtenius and Holtenius, 1996). The key issue regarding ketogenesis is the competition for substrate between esterification and oxidation (McGarry and Foster, 1972).

The effects of insulin on energy balance may vary depending on the stage of lactation and the physiological status of lactating dairy cows. However, there is evidence that insulin resistance exists in peripheral muscle, adipose, and hepatic tissues in early lactation. Several researchers who have documented insulin resistance have found that the addition of more insulin

to the system did not help to suppress fatty acid mobilization, increase adipose tissue uptake, or stimulate hepatic glycolysis (McCann and Reimers, 1995; Opsomer et al., 1999; Bell et al., 2000).

It is common for glucose concentrations to decrease during the first weeks of lactation in dairy cows, but the decline in glucose concentrations 1 and 2 wk after treatment in cows that received isoflupredone plus insulin might be due to a hypoglycemic effect of insulin. Hayirli et al. (2002) showed that insulin had more dramatic effects on plasma glucose concentrations than on plasma NEFA concentrations. They concluded that immediately after parturition, lipolysis in adipose tissue is more resistant to the effects of insulin than is glucose metabolism in tissues such as the liver. The present results showed that insulin had a significant effect on plasma glucose, but not a suppressive effect on lipolysis in adipose tissue, as measured by circulating NEFA concentration. However, the activity of insulin is expected to last no more than 24 h (Hayirli et al., 2002). It is not clear why a treatment effect on glucose was detected 7 d after therapy.

Our results showed that potassium and chloride concentrations were not influenced by treatments. In some

Figure 4. Least squares means (accounting for parity, BCS, and random effects of cow and farm) and standard errors for serum calcium concentrations at enrollment, and 1 and 2 wk after treatment in that dairy cows received isoflupredone acetate plus insulin (●), isoflupredone acetate only (○), or placebo (▼) once in the first week of lactation. There was a significant interaction of treatment with time ($P = 0.017$). Treatments marked with different letters were significantly different ($P < 0.016$) at that sample time.

studies, it was found that repeated treatments with isoflupredone acetate and, when used in combination with other treatment modalities for ketosis, such as insulin and glucose, isoflupredone acetate might result in profound hypokalemia and hypochloremia (Sielman et al., 1997; Peek et al., 2002; Coffey et al., 2006). Our results did not detect hypokalemic and hypochloremic effects of a single dose of isoflupredone acetate.

Although short-term changes in electrolytes would not have been detected in this experiment, no cases of weakness or recumbency were observed. The lower concentrations of calcium for cows that had been treated with isoflupredone with or without insulin may be due to decreased DMI. Cows that received isoflupredone with or without insulin had higher concentrations of BHBA and NEFA, which may decrease DMI

Table 2. Logistic regression models of the preventive and curative effects of isoflupredone acetate plus insulin (group A) or isoflupredone acetate only (group B) on subclinical ketosis (serum BHBA $\geq 1,400$ $\mu\text{mol/L}$)¹

Time after treatment	Treatment	β estimate	Robust SE	Odds ratio	95% confidence interval	P-value
Preventive effect of treatment ²						
Week 1	A	0.5416	0.2008	1.72	1.16–2.55	0.007
	B	0.4618	0.1989	1.59	1.07–2.34	0.02
Week 2	A	0.3773	0.1912	1.46	1.003–2.12	0.048
	B	0.0721	0.1947	1.07	0.67–1.57	0.71
Curative effect of treatment ³						
Week 1	A	0.6854	0.3677	2.00	0.97–4.12	0.06
	B	0.3164	0.3666	1.37	0.67–2.81	0.39
Week 2	A	0.3631	0.3534	1.44	0.72–2.87	0.30
	B	–0.1018	0.3612	0.90	0.44–1.83	0.77

¹Group C (cows that received placebo) was the referent group. The models account for the significant effects of parity and BCS at treatment.

²Among cows with BHBA $< 1,400$ $\mu\text{mol/L}$ at enrollment ($n = 972$).

³Among cows with BHBA $\geq 1,400$ $\mu\text{mol/L}$ at enrollment ($n = 190$).

Table 3. Risk of disease in Holstein cows treated with isoflupredone acetate plus insulin, isoflupredone acetate only, or placebo once within 8 d after calving

Health variable	Isoflupredone acetate plus insulin (n = 301)		Isoflupredone acetate only (n = 303)		Placebo (n = 301)		Chi-squared <i>P</i> -value
	Risk of disease, %	Number of affected cows	Risk of disease, %	Number of affected cows	Risk of disease, %	Number of affected cows	
Milk fever ¹	1.33	4	1.98	6	0.33	1	0.13
Retained placenta ¹	4.32	13	6.26	19	4.98	15	0.55
Abomasal displacement	3.3	10	3.6	11	2	6	0.45
Clinical ketosis	4.3	13	3.6	11	4.6	14	0.82
Metritis	3.6	11	6.6	20	6	18	0.24
Clinical mastitis	8	24	7.6	23	6	18	0.60
Lameness	1.3	4	0.7	2	1.6	5	0.52
Sold <61 DIM	7.3	22	5	15	4.3	13	0.24
Died <61 DIM	4	12	4.6	14	3	9	0.58

¹These diseases occurred prior to treatment.

(Vazquez-Anon et al., 1994; Grummer et al., 2004). In addition, Hiresch et al. (1998) showed that under appropriate conditions, glucocorticoids act in a fashion similar to calcitonin in restricting hypercalcemia and in lowering blood calcium.

In the present study we found no association of treatment with the incidence of clinical disease. However, with <400 cows per group, the power of this study to detect clinical disease effects was limited.

Despite the treatment effects on metabolites, there were no effects on milk production or milk components. The administration of glucocorticoids to healthy lactat-

ing cows has been reported to result in a significant depression in milk yield lasting for 3 to 4 d (Braun et al., 1970; Hartmann and Kronfeld, 1973; Wierda et al., 1987), which has been attributed to a reduction in glucose uptake by the mammary gland. It is likely that glucocorticoids antagonize the actions of insulin within the mammary gland and decrease the amount of glucose available for lactose synthesis (Hartmann and Kronfeld, 1973). These appear to be short-term effects of glucocorticoids, because in the present study isoflupredone was associated with a lower, not higher, serum glucose concentration 1 wk following administration.

Table 4. The effects of DHI test day, treatment (group A, isoflupredone acetate plus insulin; group B, isoflupredone acetate only; group C, placebo), BCS, parity, disease, and subclinical ketosis on milk production and milk components by repeated-measures ANOVA¹

Variable	Outcome											
	Test-day yield, kg			Test-day fat, %			Test-day protein, %			305-d projection		
	Mean	SD	<i>P</i>	Mean	SD	<i>P</i>	Mean	SD	<i>P</i>	Mean	SD	<i>P</i>
Treatment			0.19			0.30			0.39			0.12
A	36.78	9.4		3.53	1.01		2.94	0.65		9,967	1,866	
B	36.06	9.6		3.53	0.99		2.97	0.72		9,740	2,054	
C	36.28	8.8		3.55	0.99		2.98	0.63		9,762	1,826	
Test day			<0.0001			<0.0001			<0.0001			
1	35.66	9.9		3.58	1.11		2.94	0.76				
2	38.13	9.1		3.35	0.91		2.88	0.60				
3	35.30	8.3		3.44	0.94		3.06	0.65				
BCS pretreatment			0.42			<0.0001			0.13			0.48
<3.0	36.36	9.2		3.44	0.99		2.97	0.69		9,727	1,943	
3.25 to 3.5	36.38	9.1		3.56	0.99		2.97	0.66		9,866	1,889	
≥3.75	36.34	9.9		3.68	1.07		2.96	0.69		9,822	2,015	
Parity			<0.0001			<0.0001			<0.0001			<0.0001
1	30.76	6.6		3.51	0.81		3.06	0.62		9,082	1,655	
2	38.00	8.4		3.46	0.90		2.95	0.58		10,015	1,793	
3	39.78	9.6		3.60	0.97		2.92	0.63		10,279	2,043	
Clinical disease			0.82			0.006			0.14			0.33
Yes	37.73	9.7		3.41	0.95		2.88	0.66		10,037	2,045	
No	36.13	9.2		3.56	1.02		2.99	0.66		9,782	1,896	
Subclinical ketosis pretreatment			0.19			0.70			0.018			0.72
Yes	35.64	9.4		3.61	0.98		2.9	0.64		9,717	1,695	
No	36.49	8.7		3.53	0.98		3.0	0.68		9,838	1,957	

¹There was no treatment × time interaction.

Table 5. Summary of reproductive performance in dairy cows that received isoflupredone acetate plus insulin, isoflupredone acetate only, or placebo once in the first week of lactation¹

Treatment	n	Outcome					
		Time to first insemination			Time to pregnancy		
		Median, d	Hazard ratio	P	Median, d	Hazard ratio	P
Isoflupredone with insulin	371	72	0.87	0.13	100	0.94	0.48
Isoflupredone only	360	70	0.95	0.57	100	0.95	0.59
Control	382	68		Referent	102		

¹Cows were followed for 9 mo until pregnancy or culling and the data were analyzed using Kaplan-Meier and Cox proportional hazards survival analyses, accounting for the effects of parity, BCS at enrollment, clinical disease, and the random effect of herd.

Our results showed that administration of isoflupredone alone or with insulin in the first 8 d of calving had no impact on reproductive performance. Glucocorticoids may prolong the estrous cycles of cattle (Stoebel and Moberg, 1982) by inhibiting LH secretion (Li and Wagner, 1983) and by inhibiting follicular function (Spicer and Chamberlain, 1998). The majority of previous research used dexamethasone as a glucocorticoid drug, and none of the cited studies evaluated the effect of isoflupredone on reproduction. Most of these reports refer to the need for high doses of glucocorticoids to alter gonadotropin secretion. The glucocorticoid dose or duration of treatment used in this study was not associated with reproductive performance.

CONCLUSIONS

This study demonstrated that preventive administration to early-lactation cows of a single dose of isoflupredone acetate, alone or in combination with long-acting insulin, offered no metabolic, production, or reproductive benefits in lactating dairy cattle. Treatment unexpectedly increased NEFA and BHBA and decreased glucose concentrations 1 to 2 wk later. Treatment of clinically healthy early-lactation dairy cows with isoflupredone with or without long-acting insulin is not recommended, based on this study. The lack of a treatment benefit in cows with preexisting subclinical ketosis cannot necessarily be generalized to cows with clinical ketosis or fatty liver disease, but does highlight the need for a continued search for effective therapies for ketosis.

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