PEER REVIEWED

Improving Health and Productivity in Holstein Dairy Cows Diagnosed with Twins: Evaluation of Feeding Strategies

Helen Aceto[1], *BSc, PhD, VMD;* **Robert Gallop**[2,3], *PhD;* **Randall H. Rieger**[2,3], *BA, MS, PhD;*
Dina H. Appleby[3,4], *BS, MS;* **Billy I. Smith**[1], *DVM, MS, DABVP*
[1]*Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, New Bolton Center, Kennett Square, PA 19348*
[2]*Department of Mathematics, West Chester University, 15 University Avenue, West Chester, PA 19383*
[3]*West Chester Statistics Institute, 408 Mitchell Hall, West Chester, PA 19383*
[4]*Center for Clinical Epidemiology and Biostatistics, School of Medicine, University of Pennsylvania, Philadelphia, PA 19102*
Corresponding author: Billy I. Smith, Tel.: 1-610-925-6310; Fax: 1-610-925-6803;
E-mail address: bis@vet.upenn.edu

Abstract

Holstein cattle diagnosed with twins pose difficult managerial decisions for dairy producers. Since there is no effective method to reduce the incidence of twinning, it is important to better understand how these metabolically challenged animals should be handled to maximize health and productivity. The objectives of this study were first, to develop a metabolic profile of Holstein cows carrying twins and second, to investigate how implementation of a simple on-farm management intervention affected cows with twins by comparing metabolic status, disease events, and milk production in animals subject to typical dry-cow management (control: low-energy diet from dry-off for five weeks, followed by a nutrient-dense ration for three weeks prior to calving) and those exposed to atypical feeding management (treatment: nutrient-dense ration for the entire eight weeks of the dry period). A total of 36 cows confirmed with twins by transrectal ultrasonography were randomly allocated to control (19) or treatment (17) diets. Cows in the treated group had a steady decline in body weight over the dry period, while cows in the control group maintained weight and then declined precipitously after the change in diet. There was no beneficial effect on disease events, and there was an unexplained negative impact on mastitis incidence. Cows in the treated group had significantly higher postpartum serum β-hydroxybutyrate ($P < 0.0001$) and mastitis ($P = 0.04$) levels. Compared to other cows in the study herd, delivery of twins did not appear to affect milk production; within cows with twins there was no significant effect of diet on milk yields.

Key words: bovine, serum metabolites, twinning, ration, periparturient disease

Résumé

Les bovins Holstein diagnostiqués avec des jumeaux soulèvent des problèmes de régie épineux pour les producteurs laitiers. Parce qu'il n'existe pas de méthode efficace pour réduire l'incidence de gémellité, il est donc important de mieux comprendre comment ces animaux stressés métaboliquement doivent être gérés pour maximiser leur santé et leur productivité. Les objectifs de cette étude étaient d'abord de développer un profil métabolique de vaches Holstein portant des jumeaux et ensuite d'examiner comment l'implantation d'une simple intervention de régie à la ferme affecterait les vaches avec des jumeaux en comparant le statut métabolique, les évènements de maladie et la production de lait d'animaux sujets à la régie traditionnelle du tarissement (témoin : un régime faible en énergie pendant cinq semaines suivant le tarissement suivi d'une ration riche en nutriments pendant trois semaines avant le vêlage) et d'autres animaux exposés à une régie alimentaire riche en nutriments pendant l'entière période de huit semaines du tarissement (groupe traité). Un total de 36 vaches gestantes de jumeaux diagnostiqués par échographie transrectale ont été allouées au hasard dans le groupe traité (n=17) ou le groupe témoin (n=19). Les vaches dans le groupe traité perdaient du poids de façon soutenue durant le tarissement alors que les vaches témoins ont maintenu leur poids avant une chute prononcée suivant le changement de ration. Il n'y avait

pas de bénéfice au niveau des évènements de maladie et il y a eu un impact négatif inexpliqué sur l'incidence de la mammite. La concentration sérique postpartum de β-hydroxybutyrate ($P < 0.0001$) et l'incidence de la mammite ($P = 0.04$) étaient significativement plus élevées chez les vaches traitées. En comparaison avec les autres vaches du troupeau d'étude, le vêlage de jumeaux n'a pas semblé influencer la production de lait; et chez les vaches avec des jumeaux, il n'y avait pas d'effet significatif de la ration sur la production de lait.

Introduction

Holstein cattle diagnosed with twins pose difficult managerial decisions for dairy producers. Twinning rates of 3 to 6% have been reported in dairy cattle.[29,44] The incidence appears to be increasing over time. In 1974, Scanlon *et al*[39] reported a twinning rate of 2.8% in over 2,000 pregnancies. Between 1978 and 1994, the range was reported to be 1.4 to 4.8%.[5,12,28,32,37] More recently, twinning rates of 6.8% in over 4,400 pregnancies and 5.3% in over 2,000 pregnancies were reported in Holstein dairy cattle in the United States.[9,41] The occurrence of twins in cows has been associated with genetics,[3,41] breed,[5,47] parity,[15,26,42,46] ovulation rate,[38,47] and milk production.[22]

If the number of cows carrying twin pregnancies increases, dairy producers may be forced to rethink how they manage these animals. Producers and veterinarians have limited options for managing cows giving birth to twins. One option is to abort all cows diagnosed with twins at the time of pregnancy determination. Because of the serious negative side effects resulting from cows carrying twins, a decision to abort and rebreed may be a reasonable choice, certainly a decision that veterinarians and producers make every day. However, this does not eliminate the possibility of a second twin pregnancy occurring in these cows. Cows calving with twins are at greater risk of subsequent twinning,[3,41] so the effort of aborting and rebreeding may only result in another twin pregnancy.

A second, equally unsatisfactory choice is to allow the cow to carry the calves to term, which could result in serious negative consequences. Complications associated with carrying twins make it a highly undesirable pregnancy outcome for most cows. There are numerous problems related to cows delivering twins. These problems occur before, at the time of, and after parturition. The principle problem occurring prior to parturition is abortion[7,13,14,27] and subsequent effects on fertility. At the time of parturition, cows calving with twins have higher risks for stillbirth, retained placenta, and uterine infections.[5,7,11,16] Cattle delivering twins are nearly four times more likely to have a retained placenta, and 10 times more likely to have a dystocia, which in itself leads to an increased incidence of retained placenta.[7] Retention of fetal membranes is the major factor predisposing cattle to uterine infections.[1,7] Postpartum uterine infection can lead to a wide spectrum of related problems, ranging from systemic illness that could result in death of the animal to poor fertility.[30,35,36] Research has shown that cattle delivering twins have an increased number of days open (33 days) when compared to cows having a single birth.[30] Every additional day open beyond the targeted breeding date could cost the producer between $2 and $5.40,[10,33] for a potential total cost of $166-$178, demonstrating a negative economic effect of twinning, particularly the performance of Holstein cows.

Identifying strategies to optimize management of cows carrying twins, thereby minimizing the negative effects of twinning, is highly desirable. Since there is currently no effective method for reducing twinning rates, it is important to better understand how these animals should be managed to maximize health and productivity. Before we can develop strategies to help deal with cows carrying twins, it is necessary to evaluate how a twin pregnancy influences the metabolic status of the dam. The objectives of this study were 1) to develop a metabolic profile of Holstein cows carrying twins and 2) to determine the effects of feeding a treatment group a higher nutrient-dense ration for the entire eight-week dry period prior to calving when compared to a control group fed a traditional low nutrient-dense ration for the first five weeks of the dry period, followed by three weeks on a high nutrient-dense ration.

Materials and Methods

Experimental Design

The study was a randomized clinical trial. A high-producing Holstein dairy herd using the consultative services of the University of Pennsylvania Field Service Section was chosen for the study due to excellent management practices, including animal health management and meticulous record keeping, and the owner's willingness to participate.

Animals and Management

At the time of the study, the herd comprised approximately 625 Holstein cows milked twice daily with a mature equivalent (ME) 305-day milk yield of 23,400 lb (10,612 kg), and bulk-tank fat and protein contents of 3.5 and 3.0%, respectively. Cows were housed in a conventional six-row, free-stall barn and fed a total mixed ration (TMR) during the entire lactation. Cattle received recombinant bovine somatotropin (rbST)[a] starting on or near day 85 postpartum, and then every 14 days through the remainder of lactation.

The farm's routine reproductive program and the methods used to identify cows with twins have been pre-

viously described.[42] Thirty-six Holstein cows identified with twin conceptions were selected for participation in the current study. Examinations using transrectal ultrasonography[b] to identify animals carrying twins were performed at various times over a 28-month period beginning in August of 2001, through December of 2003. At the time of scheduled dry-off, cows diagnosed with twins and selected for the study were randomly assigned, by coin toss, to one of two dry-cow groups. The control group of 19 cows experienced a traditional dry-cow period where they were first placed in a far-off dry-cow group for five weeks and then moved to a close-up dry-cow group for the remaining three weeks of their dry period. The 17 cows in the treatment group were immediately placed into a close-up dry-cow group, and remained there for the entire eight weeks of their dry period.

Rations

The dry-cow rations were based on TMR composed primarily of corn-silage, hay, and a grain-based mineral mix. The primary difference between the far-off and close-up mixes was that the latter contained less hay and higher levels of concentrate, with the aim of increasing nutrient density as cows approach parturition in accordance with standard transition cow management. A once monthly sample of the TMR supplied to the control and treatment groups was collected for every month in which there were cows in the study. A composite sample of each ration was obtained by combining random grab samples across the entire feed bunk. Samples were thoroughly mixed prior to submission for analysis. A total of 16 such samples of each diet underwent standard analysis by wet chemistry[c] for dry matter (DM), crude protein (CP), adjusted protein (AP), soluble protein (SOLPRO), acid detergent fiber (ADF), neutral detergent fiber (NDF), ash, calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), and pH. To assess differences in the nutrient density and Ca/P levels of the rations, only the DM, CP, SOLPRO, ADF, NDF, Ca, and P were included in subsequent statistical analyses.

Health and Production Data Collection

Body Condition and Weight. Body weight (BW) of all study cows was estimated by heart girth taping, and a body condition score (BCS) was determined using a standard five-point scale in 0.25 increments at the time of drying off, at close-up, and at three weeks after calving.

Milk Production. Daily milk production of the study participants was recorded by means of electronic milk meters[d] for 30 days after calving.

Post-Calving Diseases. For 30 days post-calving, occurrence of the following diseases were monitored and recorded in a herd management system:[e] retained placenta (RP), acetonemia (ketosis), periparturient hypocalcemia (milk fever), mastitis, and uterine infection (metritis).

Serum Metabolites

Blood samples were collected weekly from all study animals by coccygeal venipuncture starting eight weeks prior to parturition (-8) and for six weeks post-parturition (+6). Blood was allowed to coagulate, was then refrigerated at 4.4°C (40°F) and cleaned from clots within 24 hours of collection. Serum was recovered by centrifugation at 4,500 *g* and stored frozen at -20°C (-4°F). Samples were analyzed for β-hydroxybutyrate (BHB; mg/dL),[f] non-esterified fatty acids (NEFA; mEq/L),[g] and cholesterol (CHOL; mg/dL),[f] using commercially available testing kits following manufacturers instructions.[f,g] Total protein (TP; g/dL) was also measured by standard refractometry.[h]

Statistical Analysis

Statistical analyses were performed using SAS statistical software.[i] Primary objectives of the analyses were to: analyze differences in feed composition between the two diets (far-off and close-up); compare changes in estimated BW and BCS at three time points (dry-off, close-up, and 21 days post-parturition); compare milk production between the two groups (treated and control); investigate any differences in post-calving disease between the two groups; and analyze any differences between control and treated groups in blood levels of NEFA, BHB, CHOL, and TP. For ration data, paired two-sample t-tests were conducted on each of the nutrients to compare the mean value of measurements from far-off ration samples with the mean for the same nutrient from close-up samples. A hierarchical linear model (HLM) was used to evaluate rates of change of milk production over 30 days post-calving and determine if these rates differed between the two groups. Estimated BW and BCS were also compared by HLM for examining the rate of change between groups per assessment. In addition, to examine the change in weight parameters from dry-off to close-up and close-up to 21 days post-calving, we used a linear piece-wise mixed-effects model that modeled separate rates of change for the two periods.[40] In the case of post-calving diseases, because the number of disease events was low for most diseases considered, Fisher's exact test was the most appropriate test of statistical significance. For each condition, logistic regression was used to model how the independent variables for treatment group (treatment vs control), prior twins (Yes/No), and parturition date (date of parturition before or after due date) was related to disease occurrence. Preliminary inspection of serum metabolite data included calculation of means at each pre- and post-parturition time-point, followed by plotting of mean profile plots us-

ing cubic splines. The graphical profiles did not indicate a linear, piece-wise, or easily identifiable polynomial curve; therefore, general mixed-model analysis of variance (MMANOVA) was implemented. There is a need to inspect the validity of assumptions for the statistical model used.[24] A key assumption for the use of the General Linear Model (GLM) framework, and its extensions including the MMANOVA, is normality of the outcome measure. Transformations for non-normality were applied when appropriate for subsequent analyses of all blood measurement levels. The MMANOVA does not assume a linear relationship between the outcome variable and time. This framework models the means per group over the respective time period and the covariance between the repeated measures over the assessments, which allows for on-average contrasts between groups, while accommodating all available data, as well as the within correlation of the repeated observations.

Results

Ration Analysis

The ration constituents DM, CP, SOLPRO, ADF, NDF, Ca, and P were compared for both diets. There was much variation between months of measure. Nevertheless, for most months there were differences in the analytes between the two diets. Overall means for the 16 samples of each diet that were analyzed and the results of paired two-sample t-tests are shown in Table 1. With the exception of P, where there was no significant average difference ($P = 0.3093$), all of the other ration constituents were significantly different across the two diets (Table 1).

Health and Production

Body Condition and Weight. On average, the mean BCS profiles showed a decrease across assessment points for the treated group. In controls, the BCS remained relatively steady from dry-off to close-up and then exhibited an accelerated rate of decline relative to treated cows from the close-up point to the 21-day assessment (Figure 1A). Examination of linear change over the three measurement points indicated no statistical difference between groups (F (1,31) = 0.34, $P = 0.56$). Rates of change per measurement point were -0.41 BCS units (SE = 0.075) for control and -0.34 BCS units (SE = 0.089) for the treated group.

Examination of the change in two parts, dry-off to close-up and close-up to 21 days, indicated no statistical difference between groups on either leg of time (F (1,29) = 0.11, $P = 0.74$ for dry-off to close-up; F (1,29) = 0.83, $P = 0.37$ close-up to 21 days). For estimated BW, the linear change over time (Figure 1B) indicated no statistical difference between groups (F (1,30) = 1.00, $P = 0.33$). Rates of change per measurement point were -62.57 lb (-28.38 kg) (SE = 17.88 lb (8.11 kg)) for controls and -91.27 lb (-41.39 kg) (SE = 22.48 lb (10.20 kg)) for the treated group. There was a marginal difference between groups over the dry-off to close-up leg of time (F (1,28) = 4.16, $P = 0.051$). Over dry-off to close-up, controls on average increased BW by 19.31 lb (8.76 kg) (SE = 35.01 lb (15.91 kg)), whereas in treated animals on average BW decreased by 92.44 lb (41.92 kg) (SE = 42.18 lb (19.13 kg)). There was no difference between groups from close-up to 21 days (F (1,28) = 1.17, $P = 0.29$). On average during this period, control BW decreased by 152.4 lb (69.12 kg) (SE = 37.6 lb (17.05 kg)) while treated animals decreased by 90.79 lb (41.17 kg) (SE = 42.72 lb (19.37 kg)). Interestingly, there was a consistent weight loss over the two measurement periods for the treated group.

Milk Production. Although there was large variability in these data, the average profiles indicate consistent increases with a near linear trajectory for both groups over time (Figure 2). There was no statistically significant difference in the rate of change in milk pro-

Table 1. Overall means (%) of ration constituents in far-off and close-up dry cow diets.

Ration constituent*	Overall mean far-off	Overall mean close-up	Difference	*P*-value**
DM	38.64	46.04	-7.088	0.0008
CP	15.85	17.22	-1.408	0.0319
SOLPRO	7.24	6.29	0.858	0.0141
ADF	27.06	24.74	2.579	0.0006
NDF	41.61	38.50	3.540	0.0029
Ca	0.64	0.48	0.154	< 0.0001
P	0.33	0.35	-0.014	0.3093

*DM = dry matter; CP = crude protein; SOLPRO = soluble protein; ADF = acid detergent fiber; NDF = neutral detergent fiber; Ca = calcium; P = phosphorus

**Means were compared by a two-sample paired t-test

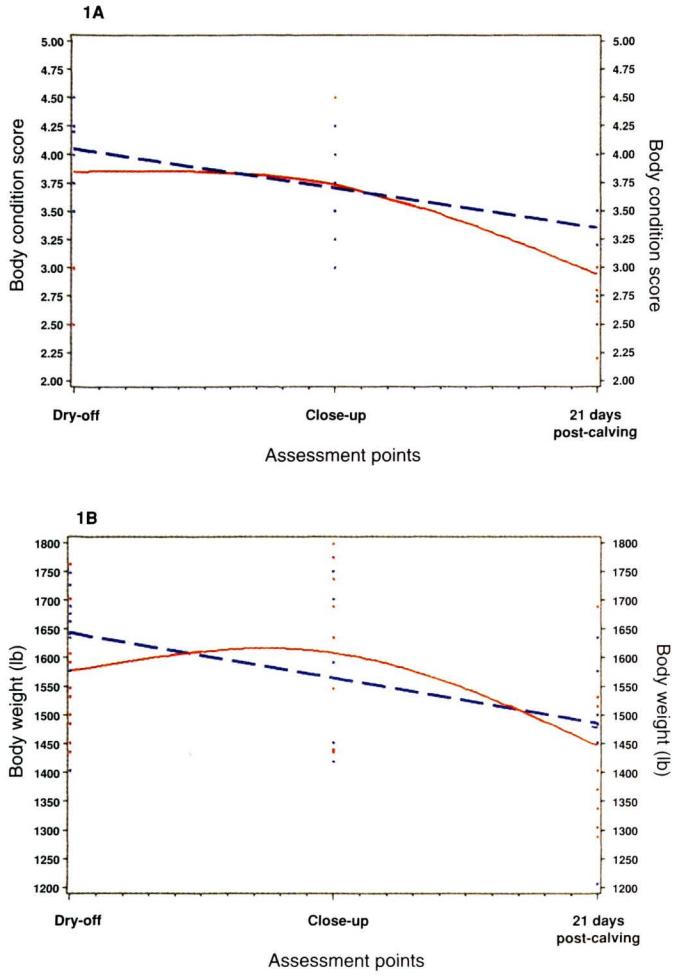


Figure 1A and 1B. Individual profiles with the average trend line for (A) body condition scores and (B) body weight (lb) of control (standard dry-cow management; solid red) and treated (nutrient-dense ration; dashed blue) cows with twins over the dry and periparturient periods.

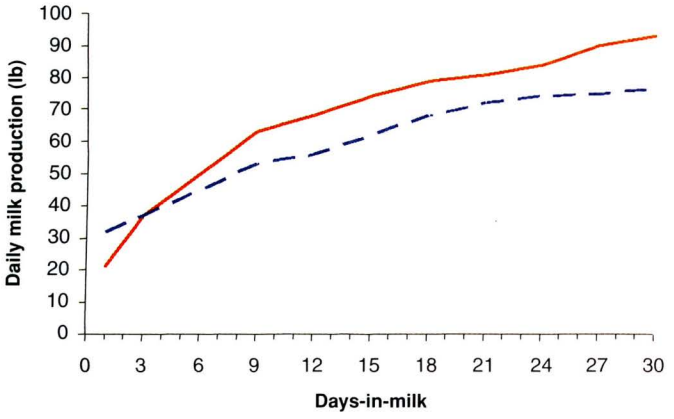


Figure 2. Daily milk production (lb) during the immediate post-calving period in control (standard dry-cow management; solid red) and treated (nutrient-dense ration; dashed blue) cows with twins.

duction over the 30 days between groups ($F(1,26) = 0.72$, $P = 0.40$). On average, the rate of change for the control group was 1.70 lb (0.77 kg) (SE = 0.21 lb (0.10 kg)) per day, whereas the rate of change for the treated group was 1.42 lb (0.64 kg) (SE = 0.25 lb (0.11 kg)) per day, corresponding to an on-average difference in rates of 0.28 lb (0.13 kg) (SE = 0.33 lb (0.15 kg)) per day.

Post-Calving Diseases. No cows in this study developed periparturient hypocalcemia. The overall number of events was low for most diseases considered. Disease data are summarized in Table 2. Only mastitis was significant at the 0.05 level, and was more common within the treatment group than the control group. Having prior twins was significant only for ketosis ($P = 0.0344$), with an odds ratio for disease having had prior twins equal to 13.33 (95% confidence interval 1.25-142.8). The logistic model supported the Fisher's exact test results for all tests of overall disease events (having now controlled for group and parturition date). The only significant result was mastitis ($P = 0.0365$).

Serum Metabolites

β-hydroxybutyrate (BHB). On average, prior to calving there was no difference in serum levels of BHB between the control and treated groups ($P = 0.97$), but post-calving, there was separation between the two groups ($P < 0.0001$, Figure 3). On average there was a 4.81 (SE = 1.08) unit (mg/dL) difference between the treated and control groups, with higher BHB in treated animals than in controls (Figure 4).

Non-Esterified Fatty Acids (NEFA). There was a trend towards NEFA being higher pre-calving in controls than in the treated group ($P = 0.096$), with on average a 0.076 (SE = 0.044) unit (mEq/L) difference between treated and control animals (Table 3). Post-calving, there was no statistically significant difference between groups ($P = 0.51$).

Cholesterol (CHOL). There was no significant difference in CHOL level between groups either pre- ($P = 0.49$) or post-calving ($P = 0.95$, Table 3).

Total Protein (TP). As with cholesterol, there was no significant difference between groups either pre- ($P = 0.33$) or post-calving ($P = 0.42$, Table 3).

Discussion

In all cows, carriage of twins can be problematic. This is particularly true in high-producing dairy cattle if twinning should result in abortion.[7,13,14,27] When twins are carried to term, metabolic problems with concurrent loss of body condition and increased levels of periparturient disease have been observed.[5,7,11,16] This, in turn, may subsequently result in decreased production and/or reduced fertility.[30] As there are currently no effective methods of reducing twinning rates or ending twin preg-

Table 2. Post-parturition disease events in control and treated cows calving with twins.

Disease	Disease events control group N (%)§	Disease events treatment group N (%)§	Two-sided *P*-value*
Ketoacidosis	5 (26)	5 (29)	1.000
Retained placenta	7 (37)	9 (53)	0.5027
Displaced abomasum	3 (16)	5 (29)	0.4338
Mastitis	1 (5)	6 (35)	0.0365[a]
Uterine infection	14 (74)	12 (71)	1.000

§Control group = 19 cows: control cows were fed a low-energy diet from dry-off for five weeks, followed by a nutrient-dense ration for three weeks prior to calving. Treatment group = 17 cows: cows were fed a nutrient-dense ration for the entire eight weeks of the dry period.
*Fisher's exact test
[a]Significant difference in mastitis between groups

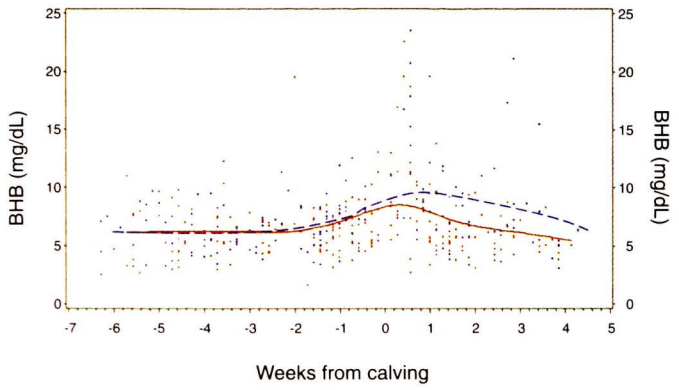


Figure 3. Individual profiles with the average trend line for serum β-hydroxybutyrate levels (BHB; mg/dL) during the periparturient period in control (standard dry-cow management; solid red) and treated (nutrient-dense ration; dashed blue) cows with twins.

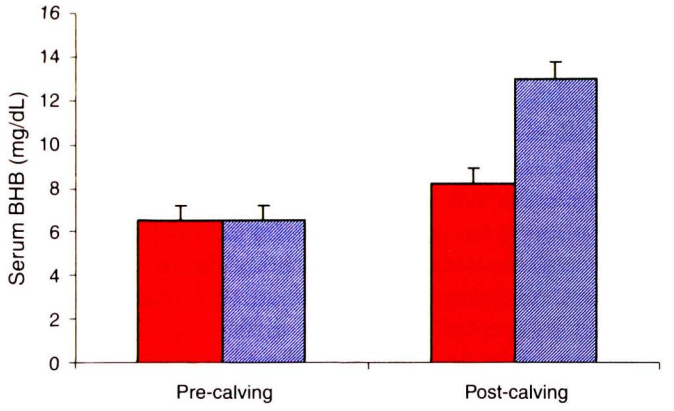


Figure 4. On-average serum β-hydroxybutyrate levels (mean + SEM, BHB; mg/dL) during the pre- and post-calving phases in control (standard dry-cow management; solid red) and treated (nutrient-dense ration; hatched blue) cows with twins.

nancies, understanding the metabolically challenged animal is critical to designing management strategies to alleviate problems associated with carrying twins. Currently, little information exists concerning the metabolic profile of cows carrying twins and there are no studies documenting the effect of specific management strategies on outcomes in these animals. This study traced the metabolic status of cows carrying twins from dry-off to 28 days (30 days for milk production and disease events) post-calving in control animals subject to typical dry-cow management (low-energy diet from dry-off for five weeks, followed by a nutrient-dense ration for three weeks prior to calving) and in treated cows that were exposed to atypical feeding management (nutrient-dense ration for the entire eight weeks of the dry period).

Feed samples from the far-off low-energy diet and the close-up high nutrient-dense ration were significantly different, with the exception of P. The close-up diet supplied a more nutrient-dense ration throughout the dry period in the treated group. Target dry matter intakes were 28-30 lb (12.70-13.61 kg) and 23-24 lb (10.43-10.88 kg) per day for the far-off and close-up diets, respectively. For both rations, Ca intake was designed to be less than 50 g per day. Target levels of NDF were less than 50% for the far-off ration and less than 45%, but with a minimum of 40%, for the close-up ration. Because the overall means of NDF were 41.6% and 38.5% in the far-off and close-up diets, respectively, other ration constituents, especially starch and protein, would increase correspondingly.[j] As a result, both rations were more energy-dense than intended.

Parameters used to assess metabolic status were comparable with previously published studies and comprised BW, BCS, BHB, NEFA, CHOL, and TP.[2,8,18,31,34] Treated cows fed the more nutrient-dense ration throughout the dry period exhibited a steady, almost linear decline in both BW and BCS throughout

Table 3. On-average pre- and post-calving levels of serum metabolites in control and treated groups of cows with twins.

Serum metabolite*	Pre-calving average			Post-calving average		
	Control (SE)	Treatment (SE)	*P*-value**	Control (SE)	Treatment (SE)	*P*-value**
BHB (mg/dL)	6.5 (0.7)	6.5 (0.7)	0.97	8.2 (0.7)	13.0 (0.8)	< 0.0001
NEFA (mEq/L)	0.49 (0.03)	0.41 (0.03)	0.096	0.73 (0.03)	0.77 (0.04)	0.51
CHOL (mg/dL)	140.2 (4.8)	145.0 (5.1)	0.49	130.6 (5.2)	131.2 (5.8)	0.95
TP (g/dL)	6.87 (0.13)	6.69 (0.13)	0.33	6.57 (0.14)	6.41 (0.15)	0.42

*BHB – β hydroxybutyrate; NEFA = non-esterified fatty acids; CHOL = cholesterol; TP = total protein
**General mixed model analysis of variance (MMANOVA)

the study period. During the far-off period, control cows maintained BCS and there was a marginal increase in the average BW. After diet change both BCS and BW of cows in the control group declined. During the post-diet change period, close-up to parturition, the rate of decline in both measures was greater in control than in treated cows.

Over the years, the dairy industry has utilized heart-girth taping and BCS as measures of BW, but due to their rather subjective nature the reliability of these measures has been questioned. The finding that control animals tended to maintain weight, as measured by tape, during the far-off period while treated cows showed a steady decline was somewhat unexpected. Nevertheless, when the entire study period is considered, cows in the treatment group receiving a single ration had a more predictable decrease in body weight than did the controls, where there was a precipitous decline when animals were switched from the far-off to the close-up ration. The differing profiles of BW change are likely the result of receiving a single fixed ration versus transitioning between two diets of different nutrient density. While the results of this study did not demonstrate an effect on the subsequent health of the cows, the effect of diet and BW change may be worthy of further evaluation since only a small number of animals were used in this study.

Results of this study provide the first report of baseline data for levels of serum metabolites during the periparturient period in cows carrying twins. Compared to previously reported values for BHB, NEFA, CHOL, and TP over the same period in cows carrying a single calf, the levels observed here in cows with twins were not markedly different.[2,8,18,34] When moving from the pre- to post-parturient period, the directionality of phase changes in each metabolite were similar to other reports in the literature.[2,8,18] For example, levels of both BHB and NEFA increased post-calving compared to pre-calving, while CHOL and TP showed an immediate post-calving decline followed by a rebound increase. Only one metabolite in this study was significantly different between groups: BHB pre-calving was the same in both control and treatment groups, but diverged post-calving and was significantly higher in cows receiving a nutrient-dense ration throughout the dry period. Although not fully understood, prepartum nutrition could play a role in postpartum energy balance and disease resistance. Results similar to those observed here have been reported in previous studies[2,8] in cows with a single calf (i.e. lower postpartum BHB levels in cows fed a low-energy, far-off diet compared to animals fed a high-energy ration), and were also associated with high levels of uterine infection.[8]

The effect of the two feeding management strategies on disease occurrence and milk production was also assessed up to 30 days following parturition. With the exception of periparturient hypocalcemia (0%) and mastitis in the control group (5%), the incidence of all conditions measured were markedly higher (2.5-6 times) than commonly accepted benchmarks for dairy herds:[17,31] uterine infection (> 70%; target < 10%), retained placenta (> 35%; target < 10%), acetonemia (> 25%; target < 5%), and displaced abomasum, often considered a sequel to the aforementioned conditions (> 15%; target < 3%). All of the cattle in this study exhibited the same trend in disease outcomes reported in the literature for cows delivering twins.[5,7,11,16] For the most part, altered feeding management during the dry period had no effect on the occurrence of disease. Although levels of mastitis in the control group were significantly lower than that observed in treated cows, it is difficult to ascribe the difference to feeding management. However, serum levels of post-calving BHB were significantly higher in treated versus control animals, and it is possible that this exacerbated periparturient immunosuppression, thereby increasing the likelihood of mammary gland infection. It has been reported that cattle with postpartum ketonemia and elevated BHB have a depressed

immune response that may be related to poor neutrophil function,[4,25,45] and neutrophils in particular are crucial for protecting the mammary gland.[23,43] While levels of uterine infection were elevated in both groups, this outcome may have been influenced more by the presence of retained placenta[1,7,19,20] rather than by overt immunosuppression.

Cows enrolled in the current study would be considered excellent producers with average milk yields of about 80-90 lb (36.28-40.82 kg) by 30 days-in-milk, which was in line with the herd's average. Contrary to some reports and clinical impressions,[6,21] our data suggest that cows delivering twins do not produce less milk than cows delivering a single calf. Average daily milk output in control animals was initially slightly lower than that recorded in treated cows. By day 3, however, production in both groups was the same, and after that controls consistently had higher average daily milk yields than did treated cows. Although the on-average difference in rate of change per day over the 30-day observation period was 0.28 lb (0.13 kg) per day higher in controls, the difference between groups was not statistically significant. The slightly lower milk production in treated cows may have been related to significantly higher levels of mastitis seen in that treatment group.

Conclusion

The objective of this research was to show that implementation of a simple on-farm management intervention could provide dairy producers with a practical method of meeting the greater nutritional demands of cows carrying twins. The study provided valuable data regarding baseline levels of serum metabolites commonly used to assess metabolic status, and demonstrated differences in BW profiles of cows subjected to standard dry-cow management compared to treated cows receiving high nutrient-dense rations throughout the dry period. However, it failed to show any sparing effect on disease events, and with limited exceptions did not appear to influence serum metabolites or milk production. Cows in the treated group had significantly higher postpartum serum BHB and mastitis levels. This study utilized a relatively small number of animals on a single farm. Use of a larger number of animals across several farms, and the inclusion of equivalent groups of cows carrying a single calf, may result in more conclusive findings.

Footnotes

[a]POSILAC®, Elanco Animal Health, Greenfield, IN
[b]Shimadzu-350 XL, Shimadzu Corporation, Columbia, MD
[c]Wet chemistry standard analysis, Cumberland Valley Analytical Services Inc., Hagerstown, MD
[d]BouMatic, Perfection 3000 Milk Meter, Madison, WI
[e]DairyCOMP 305, Valley Agricultural Software, Tulare, CA
[f]Sigma Diagnostics, St. Louis, MO
[g]WACO Pure Chemical Industries, Ltd., Richmond, VA
[h]J351 clinical refractometer, Jorgensen Laboratories, Loveland, CO
[i]SAS™ 9.1 software, SAS Institute Inc., Cary, NC
[j]Personal communication Dr. Robert Munson, University of Pennsylvania and Pennsylvania Animal Diagnostic Laboratory System Field Investigation, Kennett Square, PA

Acknowledgments

This research was supported by The University of Pennsylvania Research Foundation. The authors wish to thank WalMoore Dairy, Inc., Chatham, PA, especially Charlene Mowrey, for all their help, expertise, and enthusiasm when handling these dairy cattle, and Bonnie Vecchiarelli, University of Pennsylvania, New Bolton Center, for laboratory analysis of serum metabolites.

References

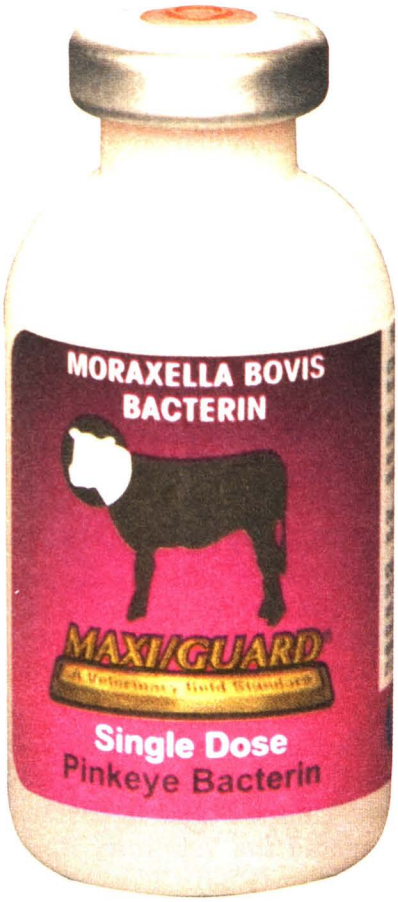
1. Bartlett PC, Kirk JH, Wilke MA, Kaneene JB, Mather EC: Metritis complex in Michigan Holstein-Friesian cattle: incidence, descriptive epidemiology and estimated economic impact. *Prev Vet Med* 4:235-248, 1986.
2. Beever DE: The impact of controlled nutrition during the dry period on dairy cow health, fertility and performance. *Anim Reprod Sci* 96:212-226, 2006.
3. Bendixen PH, Ostenacu PA, Andersson L: Case-referent study of cystic ovaries as a risk indicator for twin calvings in dairy cows. *Therio* 31:1059-1066, 1989.
4. Burvenich C, Paape MJ, Hill AW, Guidry AJ, Miller RH, Heyneman R, Kremer WD, Brand A: Role of the neutrophil leucocyte in the local and systemic reactions during experimentally induced *E. coli* mastitis in cows immediately after calving. *Vet Q* 1:45-50, 1994.
5. Cady RA, Van Vleck LD: Factors affecting twinning and effects of twinning in Holstein dairy cattle. *J Anim Sci* 46:950-956, 1978.
6. Chapin CA, Van Vleck LD: Effects of twinning on lactation and days open in Holsteins. *J Dairy Sci* 63:1881-1886, 1980.
7. Correa MT, Erb H, Scarlett J: Path analysis for seven postpartum disorders of Holstein cows. *J Dairy Sci* 76:1305-1312, 1993.
8. Dann HM, Litherland NB, Underwood JP, Bionaz M, D'Angelo A, McFadden JW, Drackley JK: Diets during far-off and close-up dry periods affect periparturient metabolism and lactation in multiparous cows. *J Dairy Sci* 89:3563-3577, 2006.
9. Day JD, Leon DW, Franti CE: Twin pregnancy diagnosis in Holstein cows: discriminatory powers and accuracy of diagnosis by transrectal palpation and outcome of twin pregnancies. *Can Vet J* 36:93-97, 1995.
10. De Vries A: Determinants of the cost of days open in dairy cattle. *Proc 11th Symp Int'l Soc Vet Epidem and Econ*, Cairns, Australia, August 2006. Available at http://www.animal.ufl.edu/devries/publications/2006/isv_11_1114.pdf. Accessed March 22, 2010.
11. Echternkamp SE, Gregory KE: Effects of twinning on gestation length, retained placenta, and dystocia. *J Anim Sci* 77:39-47, 1999.
12. Eddy RG, Davies O, David C: An economic assessment of twin births in British dairy herds. *Vet Rec* 129:526-529, 1991.

13. Erb RW, Morrison RA: Effects of twinning on reproductive efficiency in a Holstein Friesian herd. *J Dairy Sci* 42:512-518, 1959.
14. Foote RH: Factors affecting gestation length in dairy cattle. *Therio* 15:553-559, 1981.
15. Fricke PM, Wiltbank MC: Effect of milk production on the incidence of double ovulation in dairy cows. *Therio* 52:1133-1143, 1999.
16. Gregory KE, Echternkamp SE, Cundiff LV: Effects of twinning on dystocia, calf survival, calf growth, carcass traits, and cow productivity. *J Anim Sci* 74:1223-1233, 1996.
17. Gröhn YT, Erb HN, McCulloch CE, Saloniemi HS: Epidemiology of metabolic disorders in dairy cattle: association among host characteristics, disease, and production. *J Dairy Sci* 7:1876-1885, 1989.
18. Guo J, Peters RR, Kohn RA: Effect of a transition diet on production, performance and metabolism in periparturient dairy cows. *J Dairy Sci* 90:5247-5258, 2007.
19. Gunnink JW: Pre-partum leukocytic activity and retained placenta. *Vet Q* 6:52-54, 1984.
20. Gunnink JW: Retained placenta and leukocytic activity. *Vet Q* 6:49-51, 1984.
21. Kay RM: Changes in milk production, fertility and calf mortality associated with retained placentae or the birth of twins. *Vet Rec* 102:477-479, 1978.
22. Kinsel ML, Marsh WE, Ruegg PL, Etherington WG: Risk factors for twinning in dairy cows. *J Dairy Sci* 81:989-993, 1998.
23. Kehrli ME Jr, Shuster DE: Factors affecting milk somatic cells and their role in health of the bovine mammary gland. *J Dairy Sci* 77:619-627, 1994.
24. Keselman HJ, Algina J, Kowalchuk RK, Wolfinger RD: A comparison of two approaches for selecting covariance structures in the analysis of repeated measurements. *Comm Statist Simulation Comput* 27:591-604,1998.
25. Kremer WD, Noordhuizen-Stassen EN, Grommers FJ, Schukken YH, Heeringa R, Brand A, Burvenich C: Severity of experimental *Escherichia coli* mastitis in ketonemic and nonketonemic dairy cows. *J Dairy Sci* 76:3428-3436, 1993.
26. Labhsetwar AP, Tyler WJ, Casida LE: Analysis of variation in some factors affecting multiple ovulations in Holstein cattle. *J Dairy Sci* 46:840-842, 1963.
27. Lopez-Gatius F, Hunter RHF: Spontaneous reduction of advanced twin embryos: its occurrence and clinical relevance in dairy cattle. *Therio* 63:118-125, 2005.
28. Markusfeld O: Risk of recurrence of eight periparturient and reproductive traits of dairy cows. *Prev Vet Med* 9:279-286, 1990.
29. Markusfeld O: Periparturient traits in seven high dairy herds. Incidence rates, association with parity, and interrelationships among traits. *J Dairy Sci* 70:158-166, 1987.
30. Marsh WE, Dijkhuizen AA, Morris RS: An economic comparison of four culling decision rules for reproductive failure in United States dairy herds using dairyORACLE. *J Dairy Sci* 70:1274-1280, 1987.
31. Mulligan FJ, O'Grady L, Rice DA, Doherty ML: A herd health approach to dairy cow nutrition and production diseases of the transition cow. *Anim Reprod Sci* 96:331-353, 2006.
32. Nielen M, Schukken YH, Scholl DT, Wilbrink HJ, Brand A: Twinning in dairy cattle: a study of risk factors and effects. *Therio* 32:845-862, 1989.
33. O'Connor ML: *Systematic Breeding Program for Dairy Cows*. The Pennsylvania State University, College of Agricultural Sciences, Cooperative Extension. Available at http://www.das.psu.edu/research-extension/dairy/pdf/systematicbreeding.pdf. Accessed March 22, 2010.
34. Oetzel GR: Monitoring and testing dairy herds for metabolic disease. *Vet Clin North Am Food Anim Pract* 3:651-674, 2004.
35. Olson JD, Bretzlaff K, Mortimer RG, Ball L: The metritis-pyometra complex, in Morrow DA (ed): *Current Therapy in Theriogenology*, ed 2. Philadelphia, WB Saunders Co., 1986, pp 227-236.
36. Paisley LG, Mickelsen WD, Anderson PB: Mechanisms and therapy for retained fetal membranes and uterine infections of cows: a review. *Therio* 25:353-381, 1986.
37. Peeler EJ, Otte MJ, Esslemont RJ: Interrelationship of periparturient diseases in dairy cows. *Vet Rec* 134:129-132, 1994
38. Rivera H, Lopez H, Fricke PM: Fertility of Holstein dairy heifers and synchronization of ovulation and timed AI or AI after removed tail chalk. *J Dairy Sci* 87:2051-2061, 2004.
39. Scanlon PF, Gordon I, Sreenan JM: Multiple ovulations, multiple pregnancies and multiple births in Irish cattle. *J Ir Dept Agric Fish* 70:2-18, 1974.
40. Schwarz CJ: The Mixed-Model ANOVA: The truth, the computer packages, the books. *Amer Statistician* 47:48-59, 1993.
41. Silva del Rio N, Stewart S, Rapnicki P, Chang M, Fricke PM: An observational analysis of twin births, calf sex ratio, and calf mortality in Holstein dairy cattle. *J Dairy Sci* 90:1255-1264, 2007.
42. Smith BI: Case Report – diagnosing twin pregnancies in Holstein cows: accurate diagnosis by transrectal ultrasonography and outcome of twin pregnancies. *Bov Pract* 42:76-84, 2008.
43. Sordillo LM, Shafer-Weaver K, DeRosa D: Immunobiology of the mammary gland. *J Dairy Sci* 80:1851-1865, 1997.
44. Stevenson JS, Call EP: Reproductive disorders in the periparturient dairy cow. *J Dairy Sci* 71:2572-2583, 1988.
45. Suriyasathaporn W, Daemen AJ, Noordhuizen-Stassen EN, Dieleman SJ, Nielen M, Schukken YH: Beta-hydroxybutyrate levels in peripheral blood and ketone bodies supplemented in culture media affect the in vitro chemotaxis of bovine leukocytes. *Vet Immunol Immunopathol* 68:177-186, 1999.
46. Von Vandeplassche M, Butaye R, Bouters R: The twin capacity of the uterus in heifers and cows. *Dtsch Tieriirzgl Wschr* 86:470-473, 1979.
47. Wiltbank MC, Fricke PM, Sangsritavong S, Sartori R, Ginther OJ: Mechanisms that prevent and produce double ovulations in dairy cattle. *J Dairy Sci* 75:2998-3007, 2000.

Summer Pinkeye Dread?

Stop the CONTAGIOUS SPREAD!

It's Not Too Late To Vaccinate!



Too many cattlemen have experienced pinkeye's contagious disease spread following antibiotic treatment of infected calves. Outbreaks can occur multiple times in summer months. Pinkeye is simply not yet under control.

There's an easier way…**MAXI/GUARD®** Pinkeye Single Dose can be administered to the herd simultaneously with your 1st antibiotic treatment to ensure that you *treat only once*. **Immunize with MAXI/GUARD® when administering antibiotics and stop the contagious spread and need for further treatments.**

A 2ml subcutaneous dose of **MAXI/GUARD®** provides the highest protection and broadest disease strain coverage available. It shortens the outbreak duration and reduces eye damage. Decreased scarification means reduced blindness and higher profits from market cattle.

A strong safety record, minimal injection site reactions and ease of syringeability make it a smart choice to control outbreaks.

New Single Dose Protection increases your convenience and lowers disease control costs.

It's the Smart Way to Control Summer Pinkeye Outbreaks.

MAXI/GUARD®
A Veterinary Gold Standard

ADDISON BIOLOGICAL LABORATORY

Addison Biological Laboratory, Inc.
507 North Cleveland Avenue
Fayette, Missouri 65248 USA
800-331-2530
www.addisonlabs.com
info@addisonlabs.com