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Production Responses of Holstein Dairy Cows to a Sodium Propionate Supplement Fed Postpartum to Prevent Hyperketonemia

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Simple Summary: Hyperketonemia can be associated with decreased milk yield in early lactation and an increase in periparturient diseases. The objective of this study was to determine if feeding an exogenous source of propionate increased milk and milk component yield and reduced the incidence of hyperketonemia and other health events in Holstein dairy cows. In this study, the prevalence of hyperketonemia was low in multiparous cows and high in primiparous cows along with a high incidence of metritis. Multiparous cows supplemented with sodium propionate had lower prevalence of health events, produced more milk fat and tended to produce more milk during the postpartum period than control cows. This indicates that feeding sodium propionate is beneficial for multiparous cows during the postpartum period.

Abstract: Hyperketonemia is common in cows postpartum and is associated with a decrease in milk production, reproductive efficiency, and increased risk of periparturient diseases and early culling from the herd. The objective of this research was to determine if feeding an exogenous source of propionate increased milk and milk component yield and reduced the incidence of hyperketonemia and other health events in Holstein dairy cows. Cows were systematically enrolled in the control group (C) or sodium propionate treatment group (SP) in a randomized block design. A subset of cows was sampled for blood glucose and betahydroxybutyrate (BHB) concentrations in milk at 3, 7, and 14 days using a NovaMax® Plus™ meter (Nova Diabetes Care, Inc., Billerica, MA, USA). Data were analyzed using a mixed model. Average blood BHB and glucose concentrations during the postpartum period did not differ between treatments for multiparous or primiparous cows (C = 0.53 ± 0.02, SP: 0.55 ± 0.02 mmol BHB/L, p = 0.5; C = 44.0 ± 0.77, SP = 43.0 ± 0.78 glucose mg/dL, p = 0.6). However, the prevalence of hyperketonemia and metritis was high in primiparous cows (C = 35.6% and 19.8%, respectively; SP = 35.8% and 18.9%, respectively). Blood glucose was inversely related to BHB concentration for cows below 40 mg/dL blood glucose. Feeding sodium propionate during the postpartum period increased milk fat yield (C = 1.71; SP = 1.86 kg/day, p = 0.01), tended to increase milk yield in multiparous cows (C = 39.3; SP = 40.5 kg/day, p = 0.06) and increased milk fat yield in primiparous cows (C = 1.18; SP = 1.27 kg/day, p = 0.02). Including sodium propionate in the total mixed ration is beneficial to reduce health events and increase milk fat production in multiparous cows but may only increase milk fat production in primiparous cows.

Keywords: blood glucose; betahydroxybutyrate; sodium propionate

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Citation: Wukadinovich, M.; Rossow, H.A. Production Responses of Holstein Dairy Cows to a Sodium Propionate Supplement Fed Postpartum to Prevent Hyperketonemia. *Dairy* **2023**, *4*, 527–540. https://doi.org/10.3390/dairy4040036

Academic Editor: Anna Bassols

Received: 17 August 2023
Revised: 21 September 2023
Accepted: 27 September 2023
Published: 29 September 2023

1. Introduction

Hyperketonemia (blood betahydroxybutyrate, BHB, >1.0 mmol/L) [1] has an estimated prevalence of 45% to 69% and is associated with decreased dry matter intake, negative energy balance, low milk production, impaired nervous function, substantial weight loss and decreased reproductive efficiency that can lead to other periparturient diseases [2–4]. On commercial dairies, BHB concentration is commonly measured because it is the most stable and abundant ketone body and can be easily measured with handheld meters [5]. Glucose is not commonly used to evaluate hyperketonemia because it is under tight homeostatic control, but it can be indicative of energy status as ketone accumulation is often accompanied by hypoglycemia [6]. However, a minimum level of blood glucose to prevent hyperketonemia has not been established for dairy cows.

Inclusion of glucose precursors into the total mixed ration (TMR) provides a non-invasive method of delivering gluconeogenic supplements and can increase blood glucose and decrease ketone levels by providing a more constant supply of gluconeogenic precursors compared to oral drenching [7]. Propionate has been supplemented to postpartum cows to increase blood glucose because it is a glucose precursor [8,9]. However, improvement in blood glucose and BHB levels and prevention of hyperketonemia and other transitions diseases has been inconsistent when feeding propionate [10–12]. The objective of this research was to determine if feeding an exogenous source of propionate increases milk and milk component yield and reduces the incidence of hyperketonemia and health events in Holstein dairy cows.

2. Materials and Methods

All procedures involving animals were approved by the Animal Care and Use Committee protocol # 20598 approval date 13 June 2018, PHS Animal Assurance number A3433-01 at the University of California, Davis.

2.1. Experimental Design and Treatments

Holstein multiparous and primiparous cows were assigned according to even or odd ear tag numbers to the control (C) or sodium propionate (SP) treatment pen just before calving in a randomized block design with 2 pens, 2 treatments (1 C and 1 SP) and 2 periods. The SP total mixed ration (TMR) included 0.49 kg/cow/day of a molasses-based liquid sodium propionate supplement (Innovative Liquids LLC, El Dorado Hills, CA, USA) and the C TMR was balanced to have similar sugar levels by including 0.25 kg/cow/day of molasses. Each TMR (Table 1) was mixed and fed in a separate load.

In the first period, cows were continuously enrolled for 5 weeks and followed from 1 day in milk (DIM) to 21 DIM in the postpartum pens and then moved to a high lactating pen. Then SP and C TMR were switched between postpartum pens and cows were continuously enrolled for 5 weeks and followed from 1 DIM to 21 DIM in the postpartum pens for the second period. A sample size of 108 cows per pen period was adequate to determine if milk production was different between the two treatment groups assuming a 5% increase in fat-corrected milk (FCM) in multiparous cows in response to supplementation with a glucose precursor, a standard deviation of 7.5 kg/day FCM during the transition period which was typical for transition cows at this dairy, an alpha of 0.05 and power of 0.90.

Multiparous cows (226) were enrolled in the C pen for period 1 and 157 for period 2, and 211 SP cows were enrolled for period 1 and 140 for period 2. Primiparous cows (107) were enrolled in the C pen for period 1 and 63 for period 2, and 101 SP cows were enrolled for period 1 and 74 for period 2. Random subsets of 73 multiparous C cows, 39 primiparous C cows, 81 multiparous SP cows and 36 primiparous SP cows were bled for BHB and glucose analyses at 3 timepoints: 3, 7, and 14 DIM.

After spending 3 weeks in the postpartum pen, each cow was randomly assigned and moved to 1 of 5 milking pens according to parity. Multiparous cows were moved into 1 of 3 pens and primiparous cows were moved to 1 of 2 pens. Multiparous and primiparous cows received different TMR during the follow-up period (Table 1; FUP).

2.2. Animals, Housing and Care

The study was conducted on a 6000-cow commercial dairy in the California San Joaquin Valley from July 2017 to February 2018. Prior to freshening, multiparous and primiparous cows were housed separately in dry open lot corrals at 90% capacity of the pens. During the postpartum period, multiparous and primiparous cows were housed together in 2 free stall pens at 75% capacity of the pens. All free stall pens were equipped with fans that turned on at an ambient temperature of 27 °C and sprinklers at the feed bunk turned on every 10 min. Postpartum cows were fed once daily 0600 h) and feed was pushed up every 2 h. After the postpartum period, pens were fed twice daily for multiparous (700 and 1200 h) and primiparous cows (800 and 1200 h). Cows were milked 3 times daily (200 h, 900 h, 1700 h) in an 80-cow rotary milking parlor equipped with fans and sprinklers.

Table 1. Average nutrient analysis and ingredients for control (C) and sodium propionate treatment (SP) TMR during the postpartum and follow-up period.

	Postpartum [1] TMR			**Follow-Up [2] TMR**	
Item, % DM	**C [3]**	**SP [4]**	**SD**	**Multiparous [5]**	**Primiparous**
Nutrient					
DM	54.5	54.2	0.64	60.6	62.5
CP	18.0	17.9	0.23	20.2	20.1
ADF	20.9	21.2	0.34	19.6	19.7
NDF	33.7	34.1	0.44	28.9	29.4
Lignin	4.0	4.0	0.06	5.1	4.9
Starch	20.3	19.8	0.56	28.5	28.6
Ash	7.6	7.6	0.13	5.1	5.1
Calculated energy, MJ/kg [6]					
NE_l	7.8	7.7	0.4	13.8	13.8
NE_m	8.3	8.3	0.4	14.7	14.7
NE_g	5.2	5.1	0.4	9.6	9.6
Ingredients					
Wheat straw	2.6	2.6			
Almond hulls				6.1	4.5
Alfalfa hay	20.7	20.7		12.4	12.7
Wet distillers grain	3.4	3.3		5.3	5.9
Corn silage	22.3	21.6		18.4	21.1
Rolled corn	21.5	21.0		26.3	25.2
Cottonseed	6.1	6.1		6.3	6.1
Canola	13.3	13.3		13.9	13.3
Corn gluten	4.5	4.5		5.5	5.3
Milk cow mineral [7]	2.8	2.8		2.9	2.8
Salt	0.3	0.3		0.4	0.4
Megalac-R [8]	0.6	0.6			
Megalac [9]	0.6	0.6		1.3	1.5
Molasses [10]	1.3	2.6		1.2	1.2

[1] Postpartum period defined as 1–21 DIM during which multiparous and primiparous cows were housed together. [2] Follow-up period defined as 22–147 DIM during which multiparous and primiparous cows were housed separately. TMR nutrient contents were estimated using [13]. [3] Control (C) multiparous and primiparous cows. [4] Sodium propionate treated (SP) multiparous and primiparous cows. [5] Multiparous cows from lactations 2–7. [6] Estimated energy using [13]. [7] SQ 810 (Arm & Hammer Animal Nutrition Princeton, NJ, USA), calcium carbonate, MagOx54, almond shell, urea, dicalcium phosphate, Avalia 4 (Zinpro Eden Prairie, MN, USA), zinc sulfate, manganese sulfate, Celmanax (Arm & Hammer Animal Nutrition Princeton, NJ, USA), biotin 1%, Sel-Plex 2000 (Alltech, Inc., Nicholasville, KY, USA), vitamin A, vitamin D3, vitamin E, copper sulfate, EDDI, selenium. [8] Megalac-R (Arm & Hammer Animal Nutrition Princeton, NJ, USA). [9] Megalac (Arm & Hammer Animal Nutrition Princeton, NJ, USA). [10] Molasses included in C postpartum and follow-up diets and SP follow-up diets was from Penny Newman Grain Co., Inc. (Stockton, CA, USA). Molasses supplement fed to SP postpartum cows was a liquid molasses-based sodium propionate supplement added to SP TMR daily at 0.91 kg/cow/d.

2.3. TMR Sample Analyses

Amounts of TMR offered and refused were recorded daily using the software EZFeed (DHI-Provo, Provo, UT, USA) and daily pen head counts were recorded using Dairy Comp (Valley Ag. Software, Tulare, CA, USA). Average daily dry matter intake (DMI) in the postpartum period were estimated by subtracting refusals from delivered TMR and dividing the total by the number of cows in the pen. Feed samples were collected daily immediately after feed delivery via the grab sampling method [14] and pooled weekly for TMR nutrient content analysis for both treatment pens. The TMR samples were stored at −20 °C until sent for nutrient analyses to Analab (Fulton, IL, USA). Samples were analyzed for dry matter, acid detergent fiber, neutral detergent fiber, crude protein, fat, ash, and lignin using wet chemistry analysis [15], starch using near-infrared reflectance spectroscopy (based on predictive equations developed at Analab), and mineral analysis using inductively coupled plasma-mass spectrophotometry [15] for Ca, P, Mg, K, Na, Fe, Cu, Mn, S, Cl and Zn. Feed samples were not collected during the follow-up period, but nutrient content was estimated using [13] and energy calculations were estimated using [16].

2.4. Blood Measurements and Health Event Recording

All health events $\leq$ 150 DIM were collected from DairyComp (Valley Ag Software, Tulare, CA, USA). Events included metritis, retained placenta (RP), mastitis, lameness, displaced abomasum (DA), pneumonia, ill (fever), and culled or died. Hyperketonemia was defined by blood BHB > 1.0 mmol/L measured by research personnel. Blood samples were collected from multiparous and primiparous cows at 3, 7, and 14 DIM from the same cows. Cows were bled at 600 h at the beginning of feeding. Blood samples were obtained by coccygeal venipuncture using Vacutainer tubes (Becton-Dickenson, Franklin Lakes, NJ, USA) containing sodium fluoride and potassium oxalate to inhibit glycolysis and blood clotting, respectively. Whole blood samples were analyzed cow-side within 2 h of collection using NovaMax® Plus™ meters (NM; Nova Diabetes Care, Inc., Billerica, MA, USA). Ketone NM test strips were used to determine the concentration of BHB (mmol/L) and glucose NM strips were used to determine glucose levels (mg/dL).

The NM meter has been used in previous research monitoring glucose and BHB levels [17–19]. Performance of the meter was tested against other meters for glucose monitoring [20]. They found that none of the meters estimated blood glucose levels accurately. We used the NM meter as an indicator of low blood glucose only and, therefore, a risk factor for hyperketonemia. The NM meter also was evaluated for diagnosing hyperketonemia by measuring BHB level [18]. They determined the Nova Vet meter was acceptable, but the NM meter was not. The Nova Vet meter uses the same method to measure BHB but included a calibration step to calibrate the meter to different concentrations of BHB in cow blood which involves a 1.25 slope adjustment. The NM meter does not include this step, since it is for human use. Since they did not use the slope correction for the NM meter, it did not perform well in their analyses. If the slope adjustment is used, BHB $\geq$ 1.0 mmol/L plasma, the BHB definition of hyperketonemia becomes 1.25 mmol/L which agrees with other definitions of hyperketonemia [21,22]. Dairy producers are unlikely to go the extra step of using the correction factor, therefore, the BHB numbers presented in this paper are uncorrected.

2.5. Milk Data

Daily milk yields were collected using Dairy Comp 305 (Valley Ag. Software, Tulare, CA, USA) from 2 to 147 DIM and averaged by week in milk (WIM; 1 to 21). Milk components, milk fat, milk protein, lactose, somatic cell counts (SCC), solids not fat (SNF), and milk urea nitrogen (MUN), were analyzed weekly during the postpartum period using Tulare County Dairy Herd Improvement Association milk testers (Bentley Instruments ChemSpec 150, Chaska, MN, USA). Energy corrected milk and FCM were calculated using weekly milk yield averages and component yields [23].

2.6. Statistical Analyses

Pen period was the experimental unit of interest for all analyses. To be included in statistical analyses, cows must have remained on treatment in the postpartum pen for a minimum of 14 days but no more than 21 days. All cows that remained in the hospital for 2 days or more during the postpartum period were omitted from all analyses. Cows with missing milk yields for 4 days or more during the postpartum period (2–21 DIM) and cows missing 7 days consecutive or more from the FUP period (22–147 DIM) were also omitted from milk analyses.

Data collected during the postpartum period were analyzed using a mixed model (Mixed Procedure; v. 9.4, SAS Institute 2022) with weekly repeated measures for milk yield (including energy corrected milk and 3.5% FCM), milk component yields and percentages and blood glucose and BHB. Fixed effects were treatment, period or block, week, interaction of sequence and treatment, interaction of week and treatment, and cow previous 305-day mature equivalent milk yield. Random effects were pen nested within sequence, pen nested within treatment, interaction of week and pen nested within treatment and cow nested within interaction of treatment and pen. Variables that did not contribute to the model were removed by backwards elimination. Data from primiparous and multiparous cows in the postpartum period were analyzed separately.

To quantify at what blood glucose concentrations BHB levels began to rise, glucose ranges were separated into 10 mg/dL increments for C primiparous cows only. There were no differences in C multiparous cows between blood glucose and BHB concentrations. Cow blood glucose levels were divided in categories as follows: $\leq$ 30, 31–40, 41–50, 51–60, and 61–70 mg/dL glucose. Relationship of each cow BHB to blood glucose category was analyzed using the Mixed procedure of SAS with category of blood glucose vs. blood BHB as a repeated measure at 3, 7 and 14 DIM and cow as a random effect.

3. Results and Discussion

3.1. Nutrient, TMR and DMI Analysis

There were no differences in TMR nutrient composition between treatments during the study (Table 1). Nutrient delivery was consistent with less than 1% variation across TMR samples.

There were no differences in DMI for postpartum pens between treatments (Table 2). However, pens were mixes of multiparous and primiparous cows so differences in intake by parity were not able to be quantified. Past studies which have infused sodium propionate intraruminally have observed a decrease in DMI [24,25]. However, this was not observed in this study probably due to slower absorption of fed sodium propionate. Hypophagic effects have also not been observed when administering oral drenches with glucose precursors [26] which is a method used to treat hyperketonemia on commercial herds. Dry matter intake was not impacted by feeding propionate at 0.11 kg/day during the transition period. Feeding transition cows a supplement of 0.41 kg/day of calcium propionate, propylene glycol and calcium salts of fatty acids increased DMI 1 week before calving and maintained DMI postpartum compared to control cows [27]. Feeding glucose precursors may be more beneficial than providing glucose precursors through delivery methods such as infusions since there is less stress involved.

3.2. Impact of SP Supplementation on Milk and Milk Components in Multiparous and Primiparous Cows

Multiparous and primiparous cows yielded 0.15 and 0.09 kg/day more fat, respectively during the postpartum period (Table 2). This led to increased FCM, energy-corrected milk and fat yield and % in multiparous cows and only an increase in fat yield and % in primiparous cows. Increases in milk fat have been reported in other studies when propionate was infused or fed, potentially because increasing glucose supply can increase fat synthesis and milk yield [10,12,28]. However, there were no differences between milk yields during the follow-up period to 150 DIM for any treatment.

Table 2. Least square mean blood and milk production parameters for multiparous and primiparous cows in the control and sodium propionate treatment groups.

	Postpartum [1]				Follow-Up		
	C [2]	SP	SEM	*p* Value	C	SP	SEM
DMI, kg/d [3]	19.3	18.9	0.9	0.5			
Multiparous cows [4]	383	351					
Blood glucose, mg/dL	41.7	42.4	1.0	0.6			
Blood BHB, mmol/L	0.46	0.50	0.04	0.4			
Milk, kg/d	39.3	40.5	0.6	0.06	44.8	44.8	0.40
FCM, kg/d [5]	43.8	46.9	0.8	<0.01			
ECM, kg/d [6]	42.9	45.5	0.8	<0.01			
Fat, kg/d	1.71	1.86	0.04	<0.01			
Fat, %	4.72	5.00	0.08	<0.01			
Protein, kg/d	1.18	2.20	0.02	0.4			
Protein, %	3.27	3.23	0.03	0.3			
Lactose, kg/d	1.70	1.75	0.03	0.09			
Lactose, %	4.62	4.64	0.02	0.3			
Previous lactation yield, kg/cow [7]	10,522	10,370	110	0.2			
Primiparous cows	170	175					
Blood glucose, mg/dL	45.6	43.8	2.0	0.3			
Blood BHB, mmol/L	0.59	0.60	0.06	0.9			
Milk, kg/d	30.2	30.6	0.6	0.5	34.6	34.8	0.47
FCM, kg/d	32.9	34.4	0.9	0.1			
ECM, kg/d	32.1	33.2	0.8	0.1			
Fat, kg/d	1.18	1.27	0.04	0.02			
Fat, %	4.25	4.49	0.09	0.02			
Protein, kg/d	0.89	0.90	0.02	0.6			
Protein, %	3.13	3.13	0.04	0.9			
Lactose, kg/d	1.41	1.43	0.03	0.6			
Lactose, %	4.89	4.88	0.03	0.6			

[1] Postpartum period defined as 2 to 21 DIM, follow-up period defined as 22 to 147 DIM. There were no differences between treatments during the follow-up period. [2] C is control treatment, SP is sodium propionate treatment. [3] Multiparous and primiparous cows were not separated in the postpartum pens thus DMI does not account for parity intake differences. [4] All multiparous cows (lactations 2 to 7). [5] Fat corrected milk (3.5% fat): (0.4324 × kg of milk) + (16.216 × kg of fat). [6] Energy corrected milk (3.5% fat, 3.2% protein): (0.3246 × kg of milk) + (12.86 × kg of fat) + (7.04 × kg of protein). [7] Previous lactation 305 d milk yield equivalent for multiparous cows.

3.3. Impact of SP Supplementation on Hyperketonemia and Health Issues

Prevalence of hyperketonemia across farms can be impacted by occurrence of diseases such as metritis, DA or lameness, management strategies, and high milk production. Hyperketonemia can occur spontaneously due to lactation demands or due to complications from other transition issues and can ultimately have long-term downstream negative impacts on cow milk and reproductive productivity [29]. Prevalence has been noted to range from 43% to 54% for BHB > 1.0 mmol/L [30,31] and 2% to 15% for BHB > 1.4 mmol/L [1,30]. However, the overall prevalence of hyperketonemia in the current study was low in multiparous cows compared to the previous numbers reported, but was relatively high in primiparous cows (Table 3). Supplementing with SP did not reduce hyperketonemia, mastitis, displaced abomasum, ill or culled or dead cows for primiparous cows. First lactation cows do not generally experience hyperketonemia [29] and susceptibility to hyperketonemia increases with successive lactations [32]. The increased incidence of hyperketonemia in the primiparous cows in this study was likely a secondary health event due to another transition issue such as metritis. In this study, 80% of hyperketonemic primiparous cows in both treatment groups had concurrent health complications with metritis. Cows with metritis consumed 4 kg/day less than healthy cows during the first 3 weeks postpartum, likely caused by discomfort from metritis [33]. Additionally, health disorders with concomitant reduced appetite are often followed by hyperketonemia [34]. Complications from these transition issues may have reduced the feed intake of primiparous cows and subsequently reduced ruminal propionate supply to the liver for gluconeogenesis and increased NEFA mobilization and ketone production. Thus, primiparous cows with health events during the postpartum period were likely potentiating the impacts of negative energy balance due to reduced DMI (metritis) and so did not appear to benefit from SP inclusion in the TMR.

Table 3. Prevalence of health events for multiparous and primiparous cows in the control (C) and sodium propionate (SP) supplemented groups during the first 60 DIM.

	Multiparous [2]		**Primiparous**	
Health Event [1], %	**C**	**SP**	**C**	**SP**
Total events	38.5	34.5	35.6	35.8
Hyperketonemia [3]	8.2	6.1	28.2	30.6
Metritis	10.6	7.5	19.8	18.9
Retained placenta	2.7	1.5	6.9	0.9
Mastitis	14.2	14.0	5.0	8.5
Lameness	5.8	5.0	2.0	0.9
Displaced abomasum	0	1.0	0	1.9
Ill [4]	4.0	4.0	1.0	4.7
Pneumonia	1.3	1.5	1.0	0
Culled or died [5]	4.9	3.5	4.0	4.7

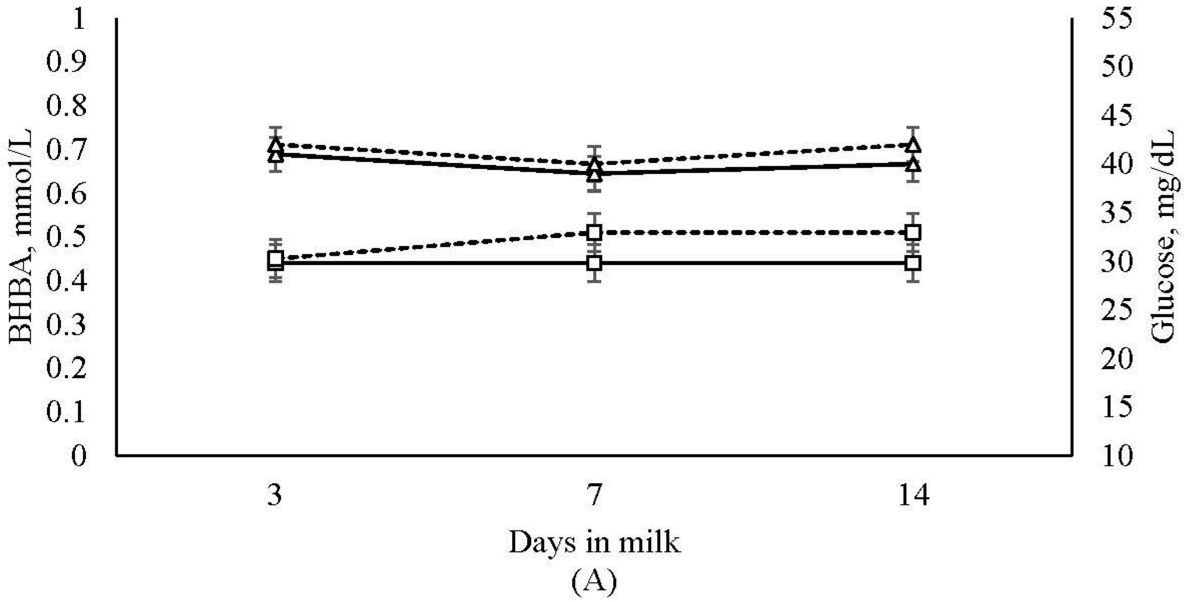
[1] Includes all multiparous and primiparous cows in C (553) and SP treatment (526). Multiparous and primiparous cows with more than one recorded health event were only recorded once. [2] Cows in lactations 2 to 7. [3] Hyperketonemia was defined as BHB > 1.0 mmol/L. [4] Ill multiparous and primiparous cows were reported as down, fever, or treated with antibiotics but were not categorized with any other health event. [5] Multiparous and primiparous cows that were culled or died were also counted in other health events.

3.4. Impact of SP Supplementation on Blood Metabolites by Parity

Reducing the occurrence of hyperketonemia begins with maintaining adequate blood glucose levels to reduce lipolysis and subsequent ketone production. Propionate has been a well-researched option for treatment of hyperketonemia because it has been estimated to account for 50% to 60% of gluconeogenesis during the transition period [9].

Primiparous cows had greater blood glucose levels than multiparous cows during the first two WIM ($p < 0.01$; Figure 1A,B). Additionally, primiparous cows had greater levels of blood BHB than multiparous cows during the first two WIM ($p < 0.01$). There were no overall improvements in blood glucose or BHB levels from SP supplementation (Table 2), but there were interactions with DIM of blood sample for glucose in primiparous cows (Figure 1B). Primiparous SP cows had a tendency for lower blood glucose concentrations during the first week in milk, but supplementation had increased their blood glucose above C cows by two weeks in milk implying that it was taking longer for SP to increase blood concentrations. Blood BHB followed a similar pattern which is unexpected since increasing blood glucose should decrease blood BHB. The increase in BHB may be due to the high prevalence of metritis in both treatments. If DMI has decreased and the source of SP is the

TMR, it may take a longer exposure to SP or a higher dose in the TMR to increase blood glucose and decrease BHBA.



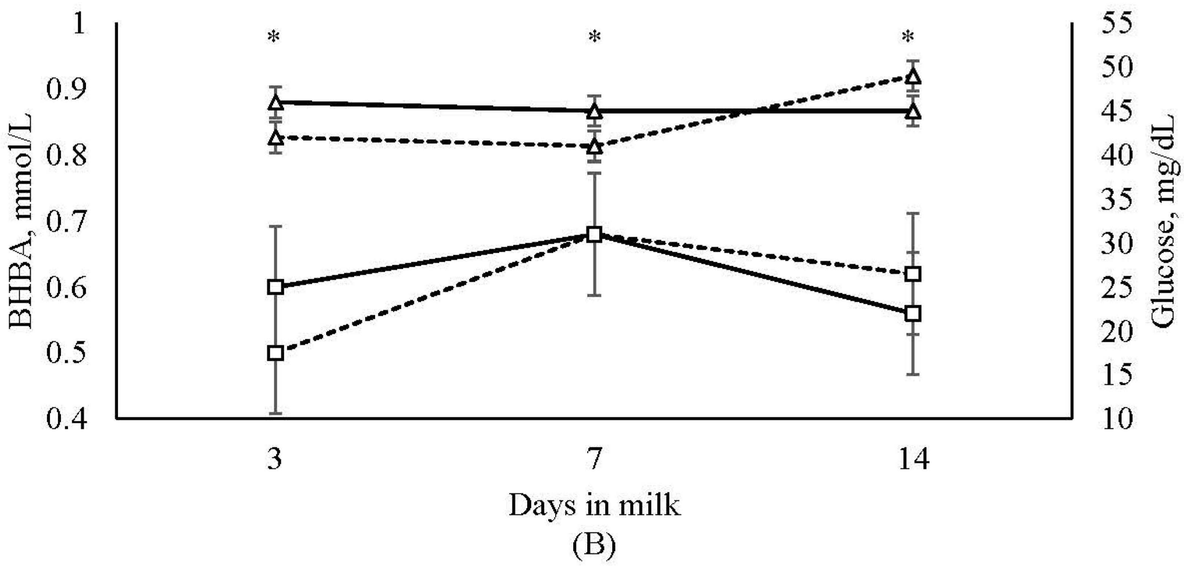


Figure 1. Effect of SP supplementation on blood BHBA and glucose concentrations in multiparous and primiparous cows during the postpartum period. Multiparous (**A**) and primiparous cows (**B**) were fed a control (C, — —) or molasses-based sodium propionate (SP, ———) supplement from approximately 1–21 DIM and separated according to parity during the follow-up period (22–147 DIM). Blood glucose (Δ) and BHBA (☐) concentrations from 3, 7, and 14 DIM are expressed as LSM ± SEM. * indicates $0.05 < p < 0.1$ between blood glucose levels in multiparous cows.

These findings agree with mixed responses observed in other feeding trials. A study by [35] fed peripartum cows 0.11 kg/day of a propionate supplement and reported no improvement of plasma glucose levels, but there was a transient decrease in non-esterified fatty acid levels and urine ketone score during the second week of lactation. Feeding 0.41 kg/day of a calcium propionate, propylene glycol and calcium salts of fatty acids combination during the peripartum period increased blood glucose and insulin and decreased non-esterified fatty acid concentrations during the transition period [26]. Cows fed 0.12 kg/day of propionate during the peripartum period observed no differences in plasma glucose or non-esterified fatty acid levels compared to non-supplemented cows [10]. Overall, supplementing with SP in the current study did not consistently improve blood glucose or BHB levels in multiparous or primiparous cows.

3.5. Association of Blood Glucose and BHB Levels

Although low levels of glucose and increased ketone levels match the clinicopathologic definition of hyperketonemia [36–38] the role of glucose in the etiology of hyperketonemia is controversial because glucose is regulated under tight homeostatic control. In the current study, glucose and BHB tended to be inversely related in control primiparous cows at glucose levels below 40 mg/dL (Figure 2; $p < 0.1$). Because the potential treatment effect of SP on blood metabolites cannot be separated, SP primiparous cows were not included in the analysis. Due to the low incidence of hyperketonemia in multiparous cows, there were no differences between glucose ranges and average blood BHB levels (Figure 1A) and, therefore, they were not included in analyses to quantify thresholds. Primiparous cows in glucose ranges $\leq$ 40 mg/dL had greater blood BHB levels than primiparous cows in glucose ranges > 40 mg/dL during the first two WIM (0.89 $\pm$ 0.07 vs. 0.52 $\pm$ 0.04 BHB mmol/L; $p < 0.001$). These data suggest a glucose threshold of 40 mg/dL below which cows respond with increased ketone formation. Thus, this study considered $\leq$40 mg/dL of glucose to be low and >40 mg/dL of glucose to be adequate. Similarly, ref. [39] noted that blood glucose levels < 30 mg/dL were associated with hyperketonemia. Although glucose is under tight homeostatic control, reduced availability of glucose leads to less oxaloacetate and succinyl CoA availability which are required for complete oxidation of non-esterified fatty acids [40] and use of ketone bodies for fuel in extrahepatic tissues [41]. Thus, depletion of glucose leads to increased ketogenesis.

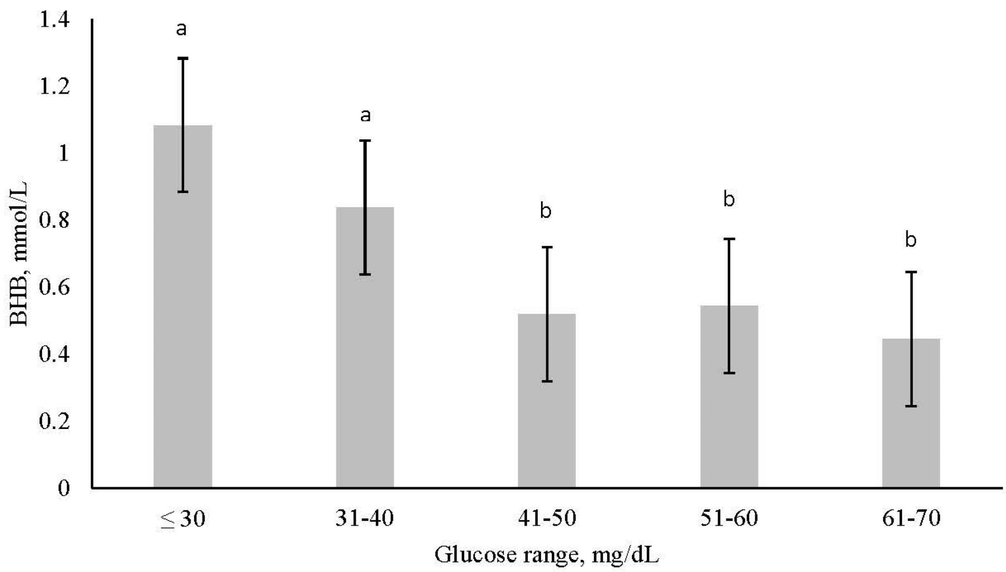
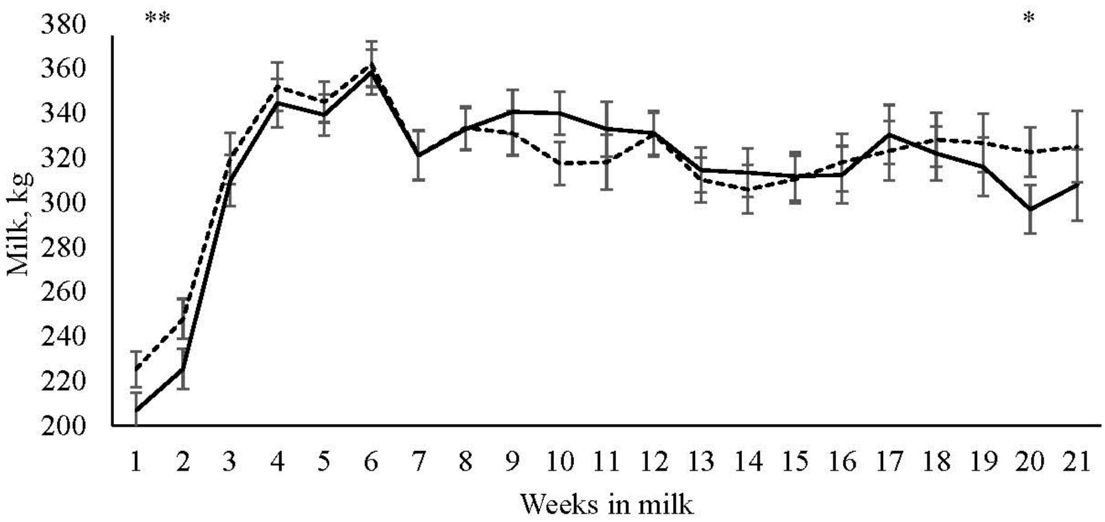


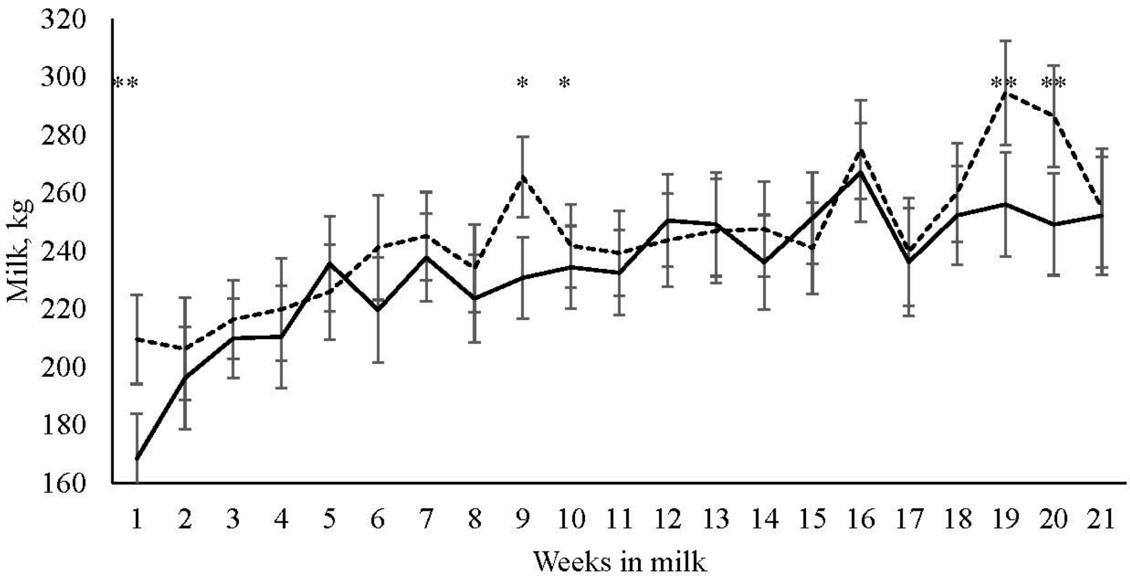
Figure 2. Association of blood glucose and BHB during the postpartum period in control primiparous cows. Values are expressed as LSM $\pm$ SEM. Letters that are different illustrate a tendency of $p \leq 0.1$.

3.6. Response of Low Glucose Multiparous and Primiparous Cows to SP Supplementation

To evaluate how milk yield was impacted by cows experiencing different levels of metabolic stress, glucose status was used to evaluate milk response to SP supplementation (Figure 3). Multiparous and primiparous cows were analyzed separately within treatment group based on low blood glucose ($\leq$40 mg/dL) or adequate (>40 mg/dL) glucose status. Low glucose SP multiparous cows (Figure 3A) tended to produce more milk than low glucose C multiparous cows during the postpartum period (p = 0.09) and also at 20 WIM (p = 0.05). Supplementation with SP tended to increase milk yield in low glucose primiparous cows overall (Figure 3B) during the postpartum period (p = 0.1) and at 9 (p = 0.02), 10 (p = 0.02), 19 (p = 0.07), and 20 WIM (p = 0.08). Multiparous and primiparous cows with adequate glucose levels did not have increased milk yield with SP supplementation from 1 to 21 WIM (Figure 3C,D). Thus, supplementation helped low glucose multiparous and primiparous cows increase milk production by providing more substrate for lactose synthesis which increased milk yield [42] but did not affect milk yield in adequate glucose cows. Studies often fail to show an increase in milk yield when glucose precursors are fed [10,43,44] or orally drenched [27,45]. Since milk yield is closely related to glucose supply to the mammary gland, stressed cows with lower blood glucose levels experience more beneficial effects with glucose supplementation [39,46]. Thus, when cows are stressed by environmental factors such as decreased feed intake, transition diseases and high milk yield, supplementing with propionate may mitigate the decrease in energy balance and help cows recover milk yield.



(A)



(B)

Figure 3. *Cont.*

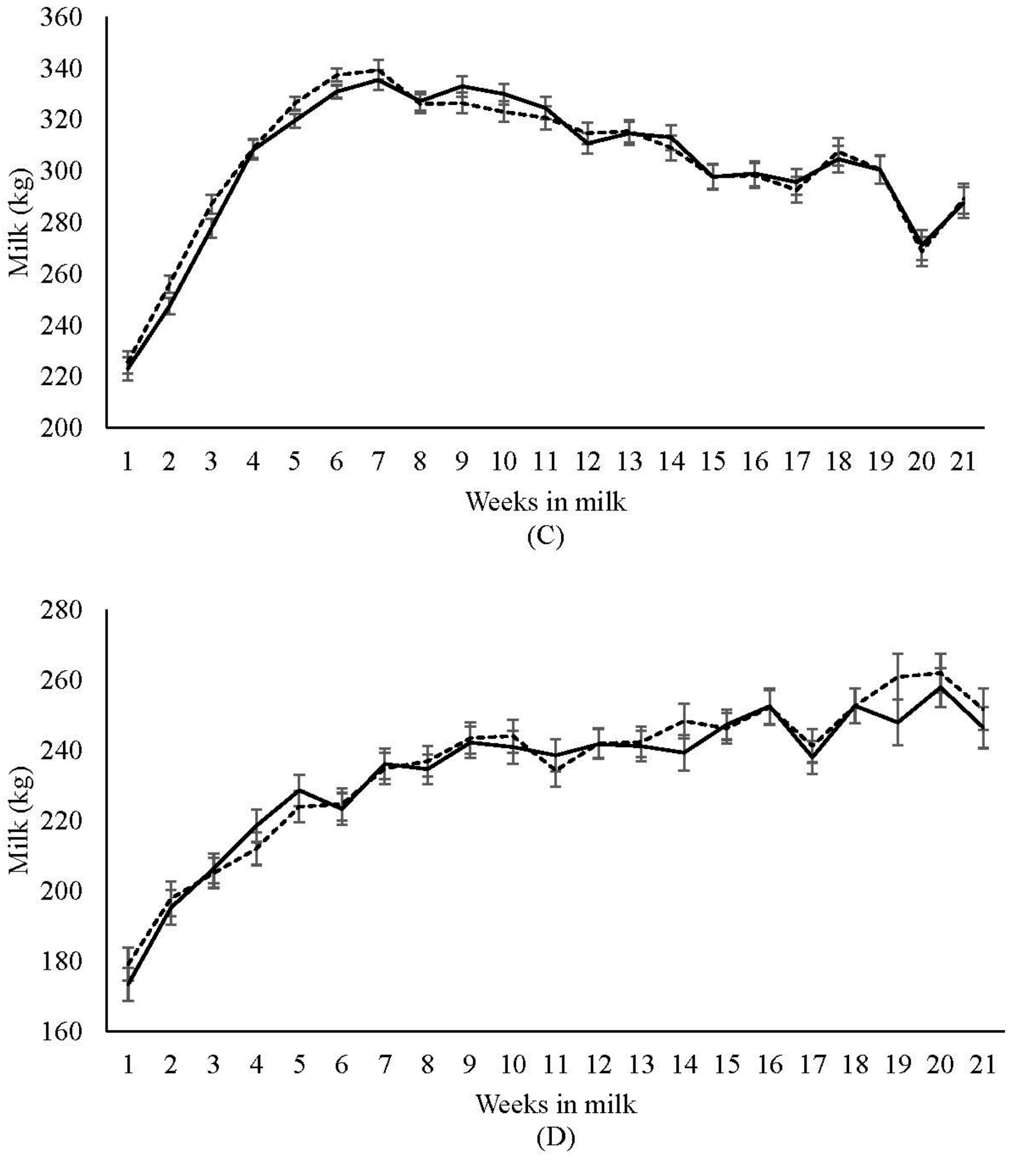


Figure 3. Impact of SP supplementation on milk yield during the first 21 weeks in milk in multiparous and primiparous cows that were considered low blood glucose (≤40 glucose mg/dL) or adequate blood glucose (≥40 glucose mg/dL) during the postpartum period. Multiparous low glucose (**A**), primiparous low glucose (**B**), multiparous adequate glucose (**C**) and primiparous adequate glucose (**D**) cows were housed together in a control pen (C, —) or molasses-based sodium propionate pen (SP, ———) from approximately 1–21 DIM and separated according to parity during the follow-up period (22–147 DIM). Weekly milk is expressed as LSM ± SEM. * indicates $p < 0.1$ and ** indicates $p \leq 0.05$ between C and SP treatment groups.

4. Conclusions

This study showed that primiparous cows with low glucose ≤ 40 mg/dL benefitted from SP supplementation via increases in milk yield during early lactation and intermittently until 21 WIM likely due to propionate providing more substrate for lactose synthesis. Primiparous cows had relatively high BHB probably due to a higher prevalence of metritis. Blood glucose and BHB were inversely related in primiparous control cows at blood glucose levels below 40 mg/dL indicating that this may be a threshold between adequate and low blood glucose. These data suggest there is value in identifying low glucose cows during the postpartum period as they are likely to have greater ketone levels and milk yield could increase with exogenous glucose intervention.

Author Contributions: Conceptualization, H.A.R.; methodology, H.A.R. and M.W.; validation, H.A.R. and M.W.; formal analysis, H.A.R. and M.W.; investigation, H.A.R. and M.W.; resources, H.A.R.; data curation, H.A.R. and M.W.; writing—original draft preparation, M. Wukadionvich; writing—review and editing, H.A.R.; visualization, M.W.; supervision, H.A.R.; project administration, H.A.R.; funding acquisition, H.A.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Innovative Liquids, LLC, El Dorado Hills, CA, USA, grant number 000. Funding for the graduate student was provided by the Feed Industry Fellowship from California Dairy Research Foundation during the time of this study, grant number P-17-004-UCD-HR-SUST.

Institutional Review Board Statement: All procedures involving animals were approved by the Animal Care and Use Committee protocol # 20598 approval date 13 June 2018, PHS Animal Assurance number A3433-01 at the University of California, Davis.

Informed Consent Statement: Not Applicable.

Data Availability Statement: Data is available on request.

Conflicts of Interest: The authors declare no conflict of interest. The funder, Innovative Liquids LLC supplied the sodium propionate product and did not have any role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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