Short communication: Relationships between physical form of oats in starter, rumen pH, and volatile fatty acids on hepatic expression of genes involved in metabolism and inflammation in dairy calves

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ABSTRACT

In early-weaning programs, dietary effects on calf rumen development have been studied extensively, but very little information is available about the effects of a solid diet on hepatic metabolism in preweaned dairy calves. The objective of this experiment was to determine the effect of physical form of oats in calf starter on the expression of key hepatic gluconeogenic, β -oxidation, and acute phase protein genes in preweaned dairy calves. Samples were analyzed from 3 experiments that fed either ground or whole oats in calf starters. Briefly, 7 calves were slaughtered at 5 wk of age in experiment 1, 6 were slaughtered at 6 wk in experiment 2, and 7 were slaughtered at 7 wk in experiment 3, and liver tissue was collected for gene expression analysis. Calves from experiments 1 and 2 were cannulated, and their rumen pH and volatile fatty acids were measured during treatment periods. The mRNA expression of gluconeogenic enzymes pyruvate carboxylase (*PC*), cytosolic and mitochondrial phosphoenolpyruvate carboxykinase (*PCK1* and *PCK2*), fatty acid oxidation enzyme carnitine palmitoyltransferase I (*CPT1*), and positive acute phase protein haptoglobin (*HPT*) was measured by real-time quantitative reverse-transcriptase PCR. Expression of *HPT* was greater in calves fed whole oats than in calves fed ground oats; however, *PC*, *PCK1*, *PCK2*, and *CPT1* were not affected by the physical form of oats. All enzymes (*PCK1*, *PCK2*, *HPT*, and *CPT1*) except *PC* were affected by experiment; *PCK1* and *CPT1* had greater expression in experiment 2 than in experiments 1 and 3. Expression of *PCK2* was similar in experiments 2 and 3 but greater than experiment 1. Expression of *HPT* was similar in experiments 1 and 2 but greater than experiment 3. The mRNA expression of enzymes *PCK1*, *PCK2*, and *CPT1* differed between

experiments 1 and 2 and was negatively correlated with rumen propionate and butyrate but had a positive relationship with rumen acetate. Similarly, rumen pH was different in experiments 1 and 2, averaging 5.69 in experiment 1 and 4.81 in experiment 2, and there was a negative correlation between mRNA expression of rate-limiting gluconeogenic *PCK1*, *PCK2*, and β -oxidation *CPT1* enzymes and rumen pH of calves in experiments 1 and 2. We concluded that the physical form of oats in calf starter did not affect gene expression of gluconeogenic and β -oxidation enzymes in preweaned dairy calves. However, lower rumen pH may be related to the upregulation of these enzymes.

Key words: calf, rumen development, rumen pH, hepatic gene expression

Short Communication

Developmental changes create differences in production and utilization of energy substrates in ruminants. Adult ruminants predominantly use short-chain fatty acids (SCFA) for energy, whereas neonatal calves rely on glucose. Hepatic gluconeogenesis is critical for meeting glucose needs of young calves because milk lactose is not sufficient to meet glucose requirements (Haga et al., 2008; Hammon et al., 2012). In ruminants, propionate is the primary substrate for gluconeogenesis. When SCFA are deficient, the calf liver can use alternative substrates for gluconeogenesis. The calf liver has a 7 to 8 times greater capacity to use lactate for gluconeogenesis than the liver of adult ruminants (Donkin and Armentano, 1995). This increased utilization of lactate for gluconeogenesis in preweaning calves may protect them from negative effects of ruminal lactic acidosis. In preweaning calves, rumen pH often remains in the range of SARA (<5.6 pH), but unlike adult ruminants, this decrease in rumen pH rarely has a negative effect on calf health. Rather, it is beneficial for rumen development (Laarman et al., 2012; Suarez-Mena et al., 2015).

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Gluconeogenesis, β -oxidation, and acute phase response are interconnected hepatic processes. Hepatic β -oxidation of long-chain fatty acids supports energy required for gluconeogenesis (Zhao et al., 1998; Bradford and Allen, 2005; Lee et al., 2016), and both gluconeogenesis and β -oxidation meet the elevated glucose requirements of the activated immune system during hepatic acute phase response (Xu et al., 2015; Kvidera et al., 2017). Calves begin producing and utilizing SCFA from 3 to 8 wk of age (Martin et al., 1959), which coincides with weaning from milk in contemporary production systems (Winter, 1985; Quigley et al., 1991). Rumen development has been studied extensively during this phase to allow successful early weaning, but to our knowledge, no studies have measured changes in expression of hepatic energy and immune enzymes near weaning or explored their relationships with rumen SCFA and pH. In the current experiment, liver samples were used from calf studies previously published by Suarez-Mena et al. (2015). Our objectives were to determine the effects of physical form of oat grain (ground vs. whole) in a calf starter and rumen pH and SCFA on mRNA expression of key gluconeogenic and β -oxidation enzymes and haptoglobin, a positive acute phase response protein, in preweaned dairy calves. Previously, a decrease in mRNA expression of hepatic gluconeogenic enzymes has been related to functional development of rumen in early-weaned calves (Haga et al., 2008). In our experiment, we did not expect changes in mRNA expression of gluconeogenic enzymes because physical forms of oats in calf starter did not affect rumen development, as reported in a companion paper by Suarez-Mena et al. (2015). Rather, we hypothesized that differences in the calf rumen environment (SCFA and rumen pH) would be related to changes in hepatic mRNA expression of the rate-limiting gluconeogenic enzymes pyruvate carboxylase (*PC*) and cytosolic and mitochondrial phosphoenolpyruvate carboxykinase (*PCK1* and *PCK2*); the rate-limiting β -oxidation enzyme carnitine palmitoyltransferase I (*CPT1*); and the positive acute phase protein haptoglobin (*HPT*).

Liver samples were collected from calves after slaughter, flash frozen in liquid nitrogen, and stored at -80°C . Calves were randomly assigned to 1 of 2 treatments, differing in the physical form of oats included in the calf starter. Starter was either completely pelleted (containing ground oats) or a combination of whole oats and a protein pellet (Table 1). In both treatments, oats made up 25% of the total calf starter. Calves were cannulated by wk 2 of life in experiments 1 and 2 but were not cannulated in experiment 3. In all 3 experiments, a fixed amount of starter was offered on a daily basis, and refusals were fed through a rumen cannula in experi-

ments 1 and 2. Rumen contents were sampled weekly at -8 , -4 , 0 , 2 , 4 , 8 , and 12 h after grain feeding in experiments 1 and 2 for determination of pH and VFA. Calves were killed at 5 wk of age in experiment 1, 6 wk of age in experiment 2, and 7 wk of age in experiment 3 with captive bolt stunning and exsanguination. Liver tissue was obtained from the right lobe, flash frozen in liquid nitrogen, and stored at -80°C until further processing.

The mRNA expression of the gluconeogenic enzymes *PC*, *PCK1*, and *PCK2*, the fatty acid oxidation enzyme *CPT1*, and the positive acute phase protein *HPT* was measured by real-time quantitative reverse-transcriptase PCR. The expression of 3 candidate reference genes, *RPS9*, *ACTB*, and *GAPDH*, previously recommended for normalization of liver gene expression was also examined (Janovick-Guretzky et al., 2007). Briefly, approximately 75 mg of tissue was homogenized using an electric homogenizer (Polytron PT 10-35; Kinematica GmbH, Lucerne, Switzerland) in 1.5 mL of RNA-Solv (Omega Bio-Tek Inc., Norcross, GA). Total RNA was isolated using phenol-chloroform extraction buffer and purified on a silica column (EZNA Total RNA Mini Kit II, Omega Bio-Tek Inc.) with on-column DNase treatment (DNASE70-1SET, Sigma-Aldrich, St. Louis, MO). Purified RNA was quantified at 260 nm using a spectrophotometer (NanoDrop ND-1000, Thermo Fisher Scientific, Waltham, MA). Purity of RNA was assessed using an absorbance ratio of 260/280 (1.93 ± 0.13 ; mean $\pm$ SD), and its integrity (RNA quality indicator = 8.3 ± 0.21 ; mean $\pm$ SD) was measured with an Experion bioanalyzer (Bio-Rad Laboratories Inc., Hercules, CA). A total of 200 μg of RNA from each sample was reverse transcribed to cDNA (High Capacity Reverse Transcriptase kit, Applied Biosystems, Foster City, CA). The abundance of genes of interest was quantified in triplicate using real-time PCR (7500 Fast Real-Time PCR system, Applied Biosystems) with 400

Table 1. Number of calves, treatment length, slaughter age, and cannulation status within each experiment

Item	Experiment ¹		
	1	2	3
Treatment length (wk)	3	4	4
Slaughter age (wk)	5	6	7
Calves cannulated	Yes	Yes	No
Calves fed whole oats (no.)	4	3	4
Calves fed ground oats (no.)	3	3	3

¹Calves were fed either ground or whole oats in calf starters; 7 calves were slaughtered at 5 wk of age in experiment 1, 6 were slaughtered at 6 wk in experiment 2, and 7 were slaughtered at 7 wk in experiment 3.

Table 2. Adjusted regression coefficients between rumen pH, propionate, and mRNA expression of hepatic enzymes in calves in experiments 1 and 2

Variable ¹	pH	Acet	Prop	Buty	PC	PCK1	PCK2	HPT	CPT1
pH	1.00	0.62***	0.57***	0.37**	0.01	0.71***	0.35**	−0.09	0.18*
Acet		1.00	0.90***	0.61***	0.12	0.81***	0.75***	−0.06	0.16*
Prop			1.00	0.31**	0.14	0.76***	0.64***	−0.05	0.18*
Buty				1.00	−0.04	0.43***	0.47***	−0.08	−0.02
PC					1.00	0.36**	0.40**	−0.02	−0.09
PCK1						1.00	0.70***	−0.09	0.27**
PCK2							1.00	−0.08	0.06
HPT								1.00	0.32**
CPT1									1.00

¹Variables include rumen pH; rumen acetate (Acet), propionate (Prop), and butyrate (Buty) concentrations; pyruvate carboxylase (PC); phosphoenolpyruvate carboxykinase 1 (PCK1); phosphoenolpyruvate carboxykinase 2 (PCK2); haptoglobin (HPT); and carnitine palmitoyltransferase I (CPT1).

* $P < 0.10$; ** $P < 0.05$; *** $P < 0.01$.

nM forward and reverse primers using SYBR Green reagent as a reporter (PerfeCTa SuperMix with ROX, Quanta Biosciences, Gaithersburg, MD). Forward and reverse primers were designed to amplify a product length between 70 and 200 bp using Primer-BLAST (National Center for Biotechnology Information, <https://www.ncbi.nlm.nih.gov/tools/primer-blast/>; Supplemental Table S1, <https://doi.org/10.3168/jds.2019-16296>). All experimental samples for each gene were run on the same plot. The conditions for PCR reactions were as follows: enzyme activation, 10 min at 95°C; and cycling stages with 40 cycles, at 95°C for 15 s and 60 s at 60°C. Melt curve analysis was conducted after each run to verify the presence of single quantitative PCR product. Efficiencies of PCR for each primer pair and coefficient of determination for each standard curve were >0.99 and ≥ 0.98 , respectively (Supplemental Table S1). The expression of each reference gene was ranked by stability values using major computational programs (geNorm, Normfinder, BestKeeper, and the comparative Delta-Ct method) through RefFinder software (Xie et al., 2012; Supplemental Table S2, <https://doi.org/10.3168/jds.2019-16296>). The combination of *ACTB* and *GAPDH* was found to be the most stable. To analyze the variance in data, mRNA expression of all the enzymes was analyzed for principal component analysis using JMP software (SAS Institute Inc., Cary, NC). Principal component analysis confirmed low variability among the samples, with component 1 explaining 45.3% of the variability and component 2 explaining 30.2% of the variability (Supplemental Figure S1, <https://doi.org/10.3168/jds.2019-16296>).

In the companion paper by Suarez-Mena et al. (2015), rumen contents were sampled 1 d/wk at −8, −4, 0, 2, 4, 8, and 12 h relative to starter feeding from cannulated calves in experiments 1 and 2. A handheld pH meter was used to determine sample pH (pHTestr

10, Eutech Instruments, Vernon Hills, IL), and SCFA were measured using gas–liquid chromatography. In the current study, calf rumen pH and propionate were measured the last week before slaughter across 7 time points (−8, −4, 0, 2, 4, 8, and 12 h relative to starter feeding), averaged, and used for regression analysis with mRNA expression of hepatic enzymes (Table 2). Only 1 time point of rumen pH was collected for each animal, so its intrasample variation cannot be calculated. For propionate, intrasample variation was less than 10%. Papillae morphometry was studied using a Stereo Zoom 4 dissecting microscope (Bausch and Lomb, Rochester, NY) fitted with a measuring eyepiece at 11.25× magnification to observe rumen development.

Results were analyzed using the MIXED procedure in SAS (version 9.4, SAS Institute Inc.). Experiment, physical form of oats, and their interaction were used as fixed effects, whereas calf within physical form of oats was included as a random effect and geometric mean of *ACTB* and *GAPDH* reference genes was included as a covariate (Hussein et al., 2013). To account for intake differences between calves in different experiments, average daily intake of all calves was also included as a covariate in the statistical model. Gene expression data were normalized by natural logarithmic transformation using the Box Cox procedure in Minitab 18 (Minitab Inc., State College, PA), and least squares means were back-transformed for the presentation of results. Transformed data were confirmed for normal distribution by plotting residual plots (Supplemental Figure S2, <https://doi.org/10.3168/jds.2019-16296>). Least squares means were separated using PDIF in SAS when main effects were significant, and significant differences were declared at $P \leq 0.10$ (Grauward et al., 2013). The REG procedure in SAS was used to obtain regression coefficients between rumen pH, propionate, and mRNA expression of hepatic enzymes in experiments 1 and 2

(Supplemental Figures S3 and S4, <https://doi.org/10.3168/jds.2019-16296>).

In our current experiment, mRNA expression of *PC* ($P = 0.71$), *PCK1* ($P = 0.61$), *PCK2* ($P = 0.53$), and *CPT1* ($P = 0.39$) was not affected by physical form of oat in any of the 3 experiments (Figure 1), probably because physical form of oats in calf starter did not affect rumen SCFA concentrations in experiments

1 and 2, nor did it affect rumen development in any of the 3 experiments (Suarez-Mena et al., 2015). The enzymes *PC*, *PCK1*, and *PCK2* are the rate-limiting enzymes for liver gluconeogenesis, and *CPT1* is the rate-limiting enzyme for β -oxidation of fatty acids in the liver. Therefore, changes in these pathways provide a strong measurement of changes in the activity of these pathways.

Figure 1. The main effect of experiment (Exp; E1 = experiment 1; E2 = experiment 2; E3 = experiment 3) and physical form of oats (G = ground; W = whole) on relative mRNA expression of (A) phosphoenolpyruvate carboxykinase 1 (*PCK1*) and haptoglobin (*HPT*) and (B) pyruvate carboxylase (*PC*), phosphoenolpyruvate carboxykinase 2 (*PCK2*), and carnitine palmitoyltransferase I (*CPT1*) in liver tissue of preweaned Holstein calves. Values within experiment (E1, E2, and E3) and within oat diet (G vs. W) with different letters (a–c) differ ($P \leq 0.10$). Error bars represent standard error of means.

Hepatic expression of all enzymes (*PCK1*, *PCK2*, *HPT*, and *CPT1*) except *PC* differed between experiments *PC* ($P = 0.29$), *PCK1* ($P = 0.03$), *PCK2* ($P < 0.01$), *HPT* ($P = 0.01$), and *CPT1* ($P = 0.02$). The most prominent difference among experiments was slaughter age of the calves, which might be the biggest factor explaining differences in enzyme expression between the 3 experiments. In the first few weeks of life, when lactose from milk cannot meet energy needs of the calf, ontogenic maturation of hepatic gluconeogenic pathways plays a critical role in survival (Hammon et al., 2005, 2012). However, Lohakare et al. (2012) found no difference in the expression of hepatic gluconeogenic enzymes *PC*, *PCK1*, and *PCK2* between calves at 70 and 150 d of age, even with 50% restriction of concentrate feeding. This suggests that major gluconeogenic changes in calves happen before 70 d. In this study, calf slaughter age was 5 wk in experiment 1, 6 wk in experiment 2, and 7 wk in experiment 3. For enzymes *PCK1* and *CPT1*, greater expression was observed in 6-wk-old calves in experiment 2 compared with 5- and 7-wk-old calves in experiments 1 and 3, respectively ($P < 0.10$). Expression of *PC* was not different between all 3 experiments, and *PCK2* expression was similar in experiments 2 and 3 but greater than experiment 1. From these results, it appears that mRNA expression of rate-limiting gluconeogenic and β -oxidation enzymes may not follow a consistent increase or decrease with age in preweaning calves.

The greater expression of enzymes *PCK1*, *PCK2*, and *CPT1* in experiment 2 calves compared with experiment 1 calves may be an adaptive response of the liver to lower rumen pH. This is partly supported by the negative relationship between mRNA expression and rumen pH, including *PCK1* (adjusted $R^2 = 0.71$, $P < 0.01$, slope = -3.02), *PCK2* (adjusted $R^2 = 0.35$, $P = 0.02$, slope = -0.36), and *CPT1* (adjusted $R^2 = 0.18$, $P = 0.08$, slope = -0.26 ; Supplemental Figure S4, <https://doi.org/10.3168/jds.2019-16296>). However, *PC* (adjusted $R^2 = 0.01$, $P = 0.31$, slope = -0.06) and *HPT* (adjusted $R^2 = -0.08$, $P = 0.84$, slope = 0.07) were not correlated with rumen pH (Supplemental Figure S4, <https://doi.org/10.3168/jds.2019-16296>). Calves in experiment 2 experienced lower average ruminal pH compared with calves in experiment 1 (4.81 vs. 5.69; SEM = ± 0.18 , $P < 0.01$; Supplemental Figure S5, <https://doi.org/10.3168/jds.2019-16296>). Greater *PC* and *PCK2* expression indicates increased lactate conversion to glucose (Velez and Donkin, 2005; Aschenbach et al., 2010). In the current study, although *PC* expression was not affected by experiment, increased lactate production may have led to greater *PCK2* expression in experiment 2, resulting in greater gluconeogenesis from

lactate. Although rumen lactate concentration was not measured for calves in the current study, rumen pH below 5 (as in experiment 2) has been associated in other experiments with steers with increased rumen lactate production (Goad et al., 1998).

No negative effects of decreased ruminal pH on calf health were observed, suggesting that calves at this age can tolerate low rumen pH levels that would be detrimental to adult cattle health (Laarman et al., 2012; Suarez-Mena et al., 2015). In calves, low rumen pH has been proposed as a positive factor for rumen epithelial development (Laarman et al., 2012). In young ruminants, increasing rumen pH decreases SCFA absorption (Gray, 1948; Masson and Phillipson, 1951). A quadratic or linear decrease in rumen pH with age has been reported in preweaning calves (6.6–6.8 to 5.4–5.6) without disturbing calf health (Beharka et al., 1998; Lesmeister and Heinrichs, 2004). Young ruminants seem to have a better ability to tolerate low rumen pH by having greater capacity to metabolize lactate than adults (Donkin and Armentano, 1995). The liver of preweaned lambs possesses 2 to 3 times greater lactate-metabolizing capacity than the liver of postweaned sheep (Ballard and Oliver, 1965). This elevation in expression of gluconeogenic enzymes (*PCK2*) may protect calves from harmful effects of lactic acid in the rumen (Aschenbach et al., 2010). Because of the positive effects of low rumen pH on calf rumen development and its less-pronounced negative effects on calf health, there may be support for redefining ruminal acidosis criteria in developing ruminants.

Haptoglobin is the most prominent positive acute phase protein. In cattle, HPT secretion increases due to transportation stress, acute inflammation, and various digestive and respiratory infections (Petersen et al., 2004). Expression of *HPT* was similar in experiments 1 and 2 but greater than experiment 3. Calves in experiment 1 and 2 were cannulated, which may have contributed to stress and increased *HPT* expression compared with experiment 3, where noncannulated calves were used. In the current study, only *HPT* expression was affected by diet, and expression was greater in the whole-oat diet than in the ground-oat diet ($P = 0.05$). The calves on the whole-oat diet had greater fecal starch than calves on the ground-oat diet. The primary reason for the difference in fecal starch was the appearance of some unbroken oats in the feces of calves fed whole oats, which was also reflected in an increase in larger fecal particles. The starch in whole oats may not be available for complete hind gut fermentation, as there was no difference in fecal pH between calves fed whole oats and those fed ground oats. Greater hindgut exposure to starch through unbroken oats may be enough to

affect *HPT* (acute phase protein) response ($P = 0.05$) but not the expression of energy metabolism enzymes *PC* ($P = 0.71$), *PCK1* ($P = 0.61$), *PCK2* ($P = 0.53$), and *CPT1* ($P = 0.39$) between calves fed whole and ground oats. Similarly, Li et al. (2012) observed that lactating cows experiencing grain-based SARA challenge had greater starch and LPS concentration in cecal digesta and higher LPS-binding protein (acute phase response protein) in blood. In our study, ileal flow of starch was not measured, so hindgut starch digestion cannot be quantified. The difference between cecal and fecal starch might have been a better indicator of the extent of hindgut starch fermentation. More research is needed to investigate the effect of starch on hindgut fermentation and acute phase response of calves.

Gluconeogenesis from pyruvate requires both *PC* and *PCK*, whereas gluconeogenesis from propionate depends solely on *PCK*. Pyruvate can originate from lactate and certain AA. The presence of 2 forms of *PCK*, cytosolic (*PCK1*) and mitochondrial (*PCK2*), enhances the entry of different substrates into gluconeogenesis (Aschenbach et al., 2010). It has been well established that increased propionate leads to increased hepatic *PCK1* expression in cows (Karcher et al., 2007) and in calves (Zhang et al., 2015). However, lower ruminal propionate was measured in experiment 2 than in experiment 1. There could be 2 reasons for greater *PCK1* expression in experiment 2. First, SCFA profile does not indicate amount of SCFA absorbed, and a greater absorption rate of propionate at low pH may have increased propionate absorbed and utilized for gluconeogenesis and decreased the concentration in rumen fluid. Its production also may have decreased due to ruminal acidosis as indicated by a positive correlation with rumen pH (adjusted $R^2 = 0.57$, $P < 0.01$, slope = 0.03). Perhaps in this study the increase in *PCK1* expression is a negative feedback against decreased substrate concentration to provide sufficient oxaloacetate for gluconeogenesis. A negative correlation between propionate and *PCK1* was observed (adjusted $R^2 = 0.76$, $P < 0.01$, slope = -71.9). Propionate is the primary VFA metabolized by ruminant liver and contributes more than 70% toward hepatic gluconeogenesis in the adult cow (Dijkstra, 1994; Aschenbach et al., 2010). Unlike propionate, more than 80% of rumen butyrate is converted to ketone bodies by the rumen epithelium, so very little butyrate is available for hepatic metabolism (Weigand et al., 1975). However, in vitro, butyrate can inhibit hepatic gluconeogenesis from propionate in ruminants (Aiello et al., 1989), which may have caused the observed negative correlation between butyrate and gluconeogenic enzymes (*PCK1*: adjusted $R^2 = 0.43$, $P < 0.01$, slope = -24.2; *PCK2*: adjusted $R^2 = 0.47$, $P < 0.01$, slope

= -4.0). Unlike propionate and butyrate, acetate was positively correlated with *PCK1*, *PCK2*, and *CPT1*. In adult ruminants, liver mitochondria contain minimal amounts of acetyl-CoA synthetase, the enzyme responsible for the uptake of acetate. On the contrary, the liver of nonruminant animals has significant ability to utilize acetate (Ricks and Cook, 1981). In ruminants, acetate serves as an alternative energy source to glucose for extrahepatic tissues (Armentano, 1992). Prewaning calves are still in transition to become ruminants, which might be the reason why acetate positively correlates with mRNA expression of hepatic gluconeogenic and β -oxidation enzymes (*PCK1*: adjusted $R^2 = 0.81$, $P < 0.01$, slope = 105.9; *PCK2*: adjusted $R^2 = 0.75$, $P < 0.01$, slope = 16.5; *CPT1*: adjusted $R^2 = 0.16$, $P = 0.10$, slope = 8.2) in the current experiment.

The enzyme *CPT1* is a key liver enzyme regulating oxidation of long-chain fatty acids. Expression of *CPT1* was greater in calves in experiment 2 than in calves in experiments 1 and 3. Similarly, *CPT1* was increased in Holstein cows after 12 h of acute grain challenge (Xu et al., 2017) and after chronic SARA exposure on 60% concentrate (Xu et al., 2015). Increased expression of *CPT1* is thought to be a liver adaptation toward increased energy demand associated with LPS-mediated inflammatory acute phase response in ruminal acidosis (Xu et al., 2015). In the current study, there also was a positive correlation between *CPT1* and *HPT* (adjusted $R^2 = 0.32$, $P = 0.03$, slope = 0.28). The increased fatty acid oxidation in experiment 2 indicated by increased *CPT1* mRNA expression may have occurred to replace the energy deficiency created by decreased propionate oxidation (Bradford and Allen, 2005). Moreover, oxidation of fatty acids provides energy and reducing equivalents in the form of ATP and NADH to keep gluconeogenesis running (Bradford and Allen, 2005; Hammon et al., 2012).

This study had some limitations. Intakes were kept similar (voluntary intake plus refusals put through the cannula) between treatments in experiments 1 and 2. In experiment 3, although intakes were not statistically different between treatments, the calves on ground oats had 16% greater intake than calves on whole oats. Moreover, treatment length and slaughter age were different among experiments (3 wk in experiment 1 and 4 wk in experiment 2). To account for the differences in feed intake due to treatment length, slaughter age, and feeding regimen, average daily feed intake for each calf was used to statistically assess differences in hepatic enzyme expression.

In the preweaning phase, rate-limiting hepatic gluconeogenic and β -oxidation enzyme expression was not affected by the form of oats in calf starter. However,

these enzymes seemed to be modulated by the rumen environment, pH, and VFA. Decreased rumen pH may result in increased gluconeogenic and β -oxidation enzyme expression. This could be an adaptive response of calf liver toward countering negative effects of low ruminal pH at the times when this may be beneficial for rumen development. The long-term effects of low rumen pH on immune and metabolic processes of the calf liver warrant further investigation.

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