J. Dairy Sci. 100:1–9
https://doi.org/10.3168/jds.2017-12866

Reticulo-rumen mass, epithelium gene expression, and systemic biomarkers of metabolism and inflammation in Holstein dairy cows fed a high-energy diet

J. M. Arroyo,*† A. Hosseini,* Z. Zhou,*‡ A. Alharthi,* E. Trevisi,§ J. S. Osorio,#[1] and J. J. Loor*[1]
*Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana 61801
†Departamento de Nutrición Animal, Instituto de Producción Animal, Facultad de Veterinaria, Universidad de la Republica, Ruta 1 km 42.5, 80100, San José, Uruguay
‡Animal and Veterinary Sciences, Clemson University, 146 Poole Agricultural Center, Clemson University, Clemson, SC 29634
§Istituto di Zootecnica, Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore, 29122, Piacenza, Italy
#Department of Dairy Science, South Dakota State University, Brookings 57007

ABSTRACT

Feeding a higher-energy diet by increasing cereal grains at the expense of forage during the last 3 to 4 wk prepartum is a traditional approach to help the rumen "adapt" to the traditional diets fed at the onset of lactation. Increasing grain/concentrate in the diet changes ruminal fermentation and in sheep and goats elicits marked changes in mRNA expression of immune-related genes in ruminal epithelium. Whether such changes at the epithelial and systemic levels occur in dairy cows when the dietary energy content increases at a fixed level of concentrate is unknown. Fourteen nonpregnant, nonlactating Holstein cows were fed a control lower-energy (CON, 1.30 Mcal/kg of dry matter) diet to meet 100% of estimated nutrient requirements for 3 wk, after which half of the cows were assigned to a higher-energy diet (OVE, 1.60 Mcal/kg of dry matter) and half of the cows continued on CON for 6 wk. Levels of forage and concentrate for CON and OVE were 80 and 79% and 20 and 21%, respectively. Plasma samples were collected 1 d before slaughter to examine biomarkers of metabolism, liver function, inflammation, and oxidative stress. The reticulo-rumen mass was recorded at slaughter, and samples of epithelium were harvested from all cows. The expression of 29 genes associated with tight junctions, immune function, and nutrient transport (volatile fatty acids, urea, and trace minerals) was examined. Overfeeding energy led to consistently greater dry matter intake over time, and lowered plasma concentrations of haptoglobin, paraoxonase, bilirubin, fatty acids, and myeloperoxidase (secreted by neutrophils). In contrast, OVE resulted in greater hydroxybutyrate and cholesterol concentrations. A greater reticulo-rumen mass in cows fed OVE did not alter genes associated with tight junctions (*CDLN1*, *CDNL4*, *OCLN*, *TJP1*), immune function (*IL1B*, *IL10*, *NFKB1*, *TLR2*, *TLR4*, *TNF*), oxidative stress (*SOD1*, *SOD2*), or most nutrient transporters. However, feeding OVE upregulated the acute-phase protein SAA3 by 3.5-fold and downregulated a volatile fatty acid transporter (*SLC16A1*) and a Fe and Cu transporter (*SLC11A2*). The lack of effect on mRNA expression along with lower plasma concentrations of inflammation biomarkers indicates that long-term intake of a higher-energy diet ad libitum was not detrimental to ruminal epithelium integrity. In that context, a protective function of SAA3 could be envisioned with a role in opsonizing gram-negative bacteria that produce endotoxins. The long-term control of volatile fatty acid absorption and trace minerals from the rumen in cows overfed energy does not seem to be controlled at the gene transcription level. The relevance of these findings to the nutritional management of pregnant dry cows merits further research.

Key words: nutrition, gene expression, ruminal epithelium

INTRODUCTION

The rumen contains microorganisms which under "normal" conditions ferment carbohydrates and protein from the feed to gas (CO_2 and CH_4), short-chain fatty acids, and ammonia. It is well established that ruminal epithelium expresses transporters for short-chain fatty acids that allow for efficient absorption (Penner et al., 2009, 2011). Recent data, however, have underscored that feeding incremental levels of cereal grains induces the production of toxic, inflammatory, and unnatural compounds in the rumen (Saleem et al., 2012). The degree to which the ruminal epithelium responds under such conditions is unclear. Evidence indicates that ruminal epithelium could play an "immune role" in the

Received March 13, 2017.
Accepted August 3, 2017.
[1]Corresponding authors: johan.osorio@sdstate.edu and jloor@illinois.edu

context of preventing the translocation of commensal bacteria or its metabolites that could induce a systemic immune response (Trevisi et al., 2014).

To date, most studies of molecular mechanisms in ruminal epithelium have focused on the morphological and molecular adaptations induced by differences in total dietary concentrate (Penner et al., 2011; Minuti et al., 2015). However, there is a lack of research evaluating the molecular changes that occur in the rumen epithelium of cows when the level of dietary energy is increased while maintaining a similar level of dietary concentrate. Besides the focus on genes associated with energy metabolism and VFA transport under such dietary conditions, little information exists regarding the expression of transporters for trace minerals (Fe, Zn, Cu, and Mn) and immune-related genes. That information is important because increasing dietary energy content through the use of higher-fermentable feedstuffs can induce production of immune-related compounds that potentially could trigger a response by the ruminal epithelium (Trevisi et al., 2014).

The hypothesis in the present study was that increasing dietary energy content and intake through inclusion of rapidly fermentable carbohydrate while maintaining a similar level of concentrate would alter mRNA expression of genes associated with inflammation, permeability, immunity, and transport of trace minerals. Furthermore, changes in metabolism, immune, and inflammatory responses also would be altered in response to long-term energy overfeeding. Thus, transcriptome and systemic biomarker analyses were used to address the hypothesis and objectives.

MATERIALS AND METHODS

Animals and Treatments

All procedures were conducted under protocols approved by the University of Illinois Institutional Animal Care and Use Committee (protocol #12134). Fourteen nonlactating, nonpregnant multiparous (had completed 3.3 ± 0.8 lactations; mean ± SD) Holstein cows (initial BW = 717 ± 39 kg; initial BCS = 3.31 ± 0.14) were enrolled in the experiment and were fed for 3 wk a control diet (lower-energy, **CON**; NE_L = 1.30 Mcal/kg of DM) designed to meet 100% of NRC (2001) requirements. During this time, it was estimated that all cows consumed an average of 110 ± 5% of NRC requirements. At the end of the 3 wk, half of the cows were randomly assigned to a higher-energy diet (**OVE**; NE_L = 1.60 Mcal/kg of DM) and half of the cows continued on CON for 6 wk (Table 1). The diets fed as a TMR were designed to maintain a similar level of forage (~80%) and concentrate (~20%), while total energy content was varied. Cows were offered feed once daily at 0600 h and had unlimited access to fresh water. Cows in CON were feed restricted to consume only 100% of NRC requirements, whereas cows in OVE had ad libitum access to feed. It was estimated that OVE cows consumed nutrients at ~180% of NRC requirements. Cows were housed in ventilated indoor pens (10 m × 15 m; photoperiod of 8 h light and 16 h dark) equipped with individual electronic transmission gates and transponders (American Calan, Northwood, NH) for access to feed. Each pen had 10 sand-bedded free stalls with at least 1 stall per cow.

Sample Collection

Blood samples were collected before the morning feeding from the coccygeal vein or artery the day before slaughter. Samples were collected into evacuated

Table 1. Ingredient and analyzed nutrient composition of the control (CON) and higher-energy (OVE) diet fed to dry and nonpregnant Holstein cows for a period of 6 wk

	Diet	
Item	CON	OVE
Ingredient, % of DM		
Alfalfa hay	2.00	5.97
Alfalfa silage	8.88	13.61
Ground shelled corn	4.04	12.56
Corn silage	33.21	54.08
Dicalcium phosphate	0.79	0.70
Limestone	0.82	0.84
Magnesium chloride	0.46	0.70
Magnesium oxide	0.40	0.38
Magnesium sulfate	0.99	1.05
Mineral-vitamin premix[1]	0.20	0.21
Salt	0.20	0.14
Soybean meal, 48% CP	11.56	4.35
Urea	0.20	0.19
Vitamin A[2]	0.01	0.01
Vitamin D[3]	0.01	0.01
Vitamin E[4]	0.26	0.24
Wheat straw	35.97	—
Whole cottonseeds	—	4.98
Total forage, % of DM	80.06	78.64
Total concentrate, % of DM	19.94	21.38
Chemical analysis		
NE_L,[5] Mcal/kg	1.30	1.60
CP, % of DM	14.08	14.45
ADF, % of DM	34.40	26.30
NDF, % of DM	50.40	38.30

[1]Contained a minimum of 5% Mg, 10% S, 7.5% K, 2.0% Fe, 3.0% Zn, 3.0% Mn, 5,000 mg/kg of Cu, 250 mg/kg of I, 40 mg/kg of Co, 150 mg/kg of Se, 2,200 IU/kg of vitamin A, 660 IU/kg of vitamin D_3, and 7,700 IU/kg of vitamin E.

[2]Contained 30,000 kIU/kg.

[3]Contained 5,009 kIU/kg.

[4]Contained 44,000 IU/kg.

[5]Calculated using the Dairy Cattle NRC (2001) model. Inputs were 8.5 kg of DMI for CON or 14.4 kg of DMI for OVE. A BW of 717 kg was the input for both diets.