Austin Journal of Veterinary Science & Animal Husbandry

Open Access

Austin Publishing Group

Research Article

Circadian Intake Timing in Ruminants: Nitrogen Metabolism and Milk Fat Properties

Akbar Nikkhah*

Department of Animal Sciences, University of Zanjan, Iran

***Corresponding author:** Akbar Nikkhah, Chief Highly Distinguished Professor, Department of Animal Sciences, University of Zanjan, Foremost Principal Highly Distinguished Elite-Generating Scientist, National Elite Foundation, Iran

Received: August 06, 2015; **Accepted:** September 24, 2015; **Published:** September 28, 2015

Abstract

The objective was to establish effects of providing a Total Mixed Ration (TMR) at either 0900 h or 2100 h on nitrogen partitioning and milk fatty acids profiles in lactating cows. Four multiparous and four primiparous Holstein cows were used in a cross-over design study with two 6-week periods, each with 3-week adaptation. Total fecal and urine were collected during a sampling week in each period to measure nutrient digestibility and nitrogen partitioning. Milk proportions of total short, medium, and long chain fatty acids were not significantly affected by eating time. Feeding at 2100 h *vs.* 0900 h decreased ($P \leq 0.05$) milk proportions of $C_{10:0}$, $C_{12:0}$, $C_{12:1}$, $C_{13:0}$, $C_{13:1}$ and $C_{18:3\ n-3}$, and tended to decrease ($P \leq 0.10$) proportions of $C_{8:0}$ and $C_{18:1\ trans-9}$, while increasing that of $C_{18:0}$. Evening fed cows tended to realize a greater rumen volume than morning fed cows (107 *vs.* 90 L, $P<0.01$). Evening vs. morning feeding reduced the proportion of the apparently digested N that was excreted in urine (480 *vs.* 550 g/g, $P<0.05$). Therefore, feed delivery at 2100 h *vs*. 0900 h improved nitrogen dynamics and milk energy output while to some extent manipulating milk fatty acids profile. Future studies are required to establish other aspects of the multi-science of eating time in ruminant ecology.

Keywords: Feeding time; Evening eating; Rumen; Physiology; Holstein cow

Introduction

Over the last few decades, most recently and in Latin square design studies with 14-d adaptation periods, feeding TMR at 2100 *vs.* 0900 h increased rumen digestion and milk fat yield [1]. The results have contributed to the emergence of a multi science now known as "ruminant chronophysiological management", particularly related to the timing of eating [2,3]. The objective was to determine milk fatty acids profiles and nitrogen (N) partitioning with 21-d adaptation periods in response to feeding at 2100 h *vs*. 0900 h.

Materials and Methods

Four multiparous (645 ± 75 kg body weight; 77 ± 25 days in milk; mean ± SD) and four primiparous (576 ± 46 kg BW; 90 ± 33 days in milk) lactating Holstein cows were monitored in a cross-over design experiment with two 42-d periods. Each period had 21-d of adaptation. Four cows were rumen-cannulated. Cows received a TMR with forage to concentrate ratio of 50.2:49.8 (DM basis) ad libitum for the entire experiment, permitting 5-10% orts. The average outside temperature and relative humidity during sampling weeks were -3.7°C and 78.9%, respectively. Lights were turned on at 03:45 just before morning milking, and were turned off at 22:45 h. Experimental treatments were feeding a TMR either at 0900 h or at 2100 h. The forage portion of the TMR was a 50:50 mixture of alfalfa silage and barley silage.

On the first day of week-4, urinary catheters were placed in the urethra 24-h before connection to the collection tubing. Total urine excretions were collected into polyester containers via indwelling bladder catheters. Urine was weighed twice daily at 0900 and 2100 h during week-4. To minimize N escape as ammonia, 100 ml of concentrated sulfuric acid was added to urine containers before each collection. A 50 ml sample of mixed urine was taken at each weighing and frozen at -20°C for later N determination.

Cows were milked twice daily at 0400 and 1600 h in their stalls. Milk was aliquoted into 50 ml vials at four consecutive milking for all cows during sampling weeks. One milk sample was preserved with 2-bromo-2-nitropropane-1,3-diol, stored at 4°C, and analyzed for milk components by near infrared using the Milk-o-Scan 303AB (Føss Electric, Hillerød, Denmark). Another milk sample (10 ml) was taken with no preservative and frozen at -20°C for subsequent analysis of fatty acid profiles. Milk fatty acid profiles were determined using a gas chromatograph (Hewlett Packard HP5890A, Agilent Technologies, Inc., Santa Clara, CA). The GC was equipped with a capillary column (0.25 mm ID, J&W Scientific HP88 100m, Agilent Technologies, Inc., Santa Clara, CA) with a film thickness of 0.2 µm. The injector and detector temperatures were set at 220°C and 290°C, respectively.

Data were analyzed as linear MIXED MODELS [4].The models for N partitioning included fixed effects of treatment (evening vs. morning feeding), parity (primiparous vs. multiparous), and their interaction. Least square means were estimated with the Restricted Maximum Likelihood (REML) method, and degrees of freedom were calculated using Satterwaith method [4]. Fixed effects were declared significant at $P<0.05$, and trends were discussed at $0.05<P\leq 0.10$. Results were reported as least square means ± difference standard errors.

Citation: Nikkhah A. Circadian Intake Timing in Ruminants: Nitrogen Metabolism and Milk Fat Properties. Austin J Vet Sci & Anim Husb. 2015; 2(3): 1014.

Results

Feeding at 2100 h increased N intake in primiparous cows. Feeding multiparous cows at 2100 *vs.* 0900 h reduced (P<0.01) total N output (513.6 *vs*. 575.4 g). Feed delivery at 2100 h instead of 0900 h reduced (P<0.01) daily urinary N excretion (177 *vs.* 194 g) in primiparous cows. As a proportion of N intake, urinary N excretion tended to be lower (P=0.06) and fecal N excretion (P=0.01) and milk N secretion (P=0.03) were lower for 2100 h than for 0900 h feeding. As a proportion of N apparently digested, feeding at 2100 h *vs.* 0900 h reduced urinary excretion (P=0.05) and milk secretion (P<0.01) of N.

Feeding at 2100 *vs*. 0900 h increased the proportion of $C_{18:0}$, but did not affect proportions of total short, medium, and long chain fatty acids in milk (Table 1). Feed delivery at 0900 h instead of 2100 h increased (P≤0.05) milk proportions of $C_{10:0}$, $C_{12:0}$, $C_{12:1}$, $C_{13:0}$, $C_{13:1}$ and $C_{18:3\ n-3}$, and tended to increase (P≤0.10) proportions of $C_{8:0}$ and $C_{18:1trans-9}$ (Table 1). Treatments did not significantly affect milk protein percentage and yield. The changes in body weight and body condition score were not affected by feeding time.

Discussion

Feeding at 2100 *vs*. 0900 h significantly increased N intake in primiparous cows. The increased N intake may most likely represent increased DM intake within 3 h of feeding [2,3]. Similarly, DM intake in feedlot cattle was increased by feeding at 2100 h rather than at 0900 h [5]. If cows anticipate the feed delivery time, they may show a more intense eating activity [6]. There is a possibility that 2100 h-fed cows in the current study could anticipated feeding time and thus were craving for the fresh feed. Melatonin secretion usually increases in dark period [7]. The increased nocturnal melatonin secretion has recently been shown to interact with diurnal variation in glucose metabolism in rats and humans [8]. Melatonin is involved in the evening insulin resistance in non-ruminants. Glucose uptake and oxidation are known to contribute to satiety [9]. Hence, a possible interaction among melatonin, insulin, and glucose uptake might attenuate satiety in the 2100 h-fed cows.

Furthermore, the rumen fill controls feed intake differently during day vs. night [10]. The inhibitory effect of rumen fill on feed intake begins at a greater rumen volume overnight than in the day, which could be related evolutionary diurnal patterns in rumination, rumen muscle contractions, and rumen fill [10-12]. The greater nutrient intake, larger rumen volume, and higher rumen VFA and ammonia shortly post-feeding [1] for 2100 h *vs*. 0900 h feeding lend support to the evolutionary trend of the 24-h patterns in feeing behavior and nutrient assimilation.

The greater N intake of primiparous cows due to feeding at 2100 h was in agreement with [13] who found that cows fed a protein-meal at 0030 *vs*. 0830 h ate more of it. The decreased urinary N partitioning by evening feeding is in accordance with the lower rumen ammonia concentrations for the 2100 *vs*. 0900 h feeding. The TMR delivery at 2100 h *vs*. 0900 h reduced the proportion of the digested N secreted in milk, but did not significantly affect (P=0.17) daily secretion (g/d) of milk N. This can be explained by the tendency for greater N intake with feeding at 2100 h. An increased proportion of N retained would automatically reduce the proportion secreted in milk even if the absolute amount of milk N secretion would not change.

Table 1: Effects of Time of Feeding (TF) on milk fatty acid profiles [1].

	TF			P-value		
Item	0900 h	2100 h	SE	TF	Parity	TF × Parity
8:0	0.52	0.42	0.06	0.10	0.22	0.19
10:0	1.70	1.58	0.05	0.01	0.45	0.01
12:0	2.37	2.08	0.14	0.05	0.32	0.82
12:1	0.07	0.06	0.003	0.01	0.77	0.43
13:0	0.09	0.08	0.003	0.01	0.94	0.93
13:1	3.98	3.65	0.14	0.03	0.42	0.72
14:0	8.64	8.71	0.41	0.86	0.27	0.03
15:1	0.016	0.02	<0.01	0.05	0.03	0.40
18:0	13.50	14.87	0.45	0.01	0.14	0.91
18:1 *trans-9*	2.16	1.03	0.62	0.10	0.33	0.06
18:3 *n-3*	3.14	3.01	0.06	0.03	0.59	0.97
19:0	0.22	0.23	<0.01	0.01	0.02	0.87
CLA, *cis9 trans11*	1.20	1.06	0.13	0.27	0.21	0.58
CLA, *trans10 cis12*	0.025	0.028	0.003	0.37	0.59	0.06
20:4	0.09	0.10	0.003	0.18	0.04	0.70
22:3	0.05	0.05	0.006	0.91	0.06	0.71
22:5	0.090	0.096	0.004	0.19	<0.01	0.44
SCFA[2]	9.96	9.48	0.51	0.36	0.09	0.55
MCFA[2]	34.65	34.56	0.41	0.83	0.99	0.04
LCFA[2]	54.37	54.98	0.54	0.27	0.42	0.28

[1]The individual fatty acid peak area divided by the total fatty acids peak area multiplied by 100. Approximately 92 g/100 g of total fatty acids measured were reported.

[2] LCFA = long chain fatty acids or > C18; SCFA = short chain fatty acids or C4-C13; MCFA = medium chain fatty acids or C14-C17; CLA = conjugated linoleic acid.

Based on the original theory of Davis and Brown (1970, cited by Bauman and Griinari, 2003), a relationship exists between milk fat depression and milk content of trans octadecenoic acids ($C_{18:1trans}$). In the current study, the 0900 h-fed cows had lower milk fat percent (3.0% *vs*. 3.5%) and yield (1050 *vs*. 1220 g/d) than the 2100 h-fed cows [2]. Nonetheless, the proportion of trans isomers of $C_{18:1}$ was similar between groups (Table 1), suggesting that other factors than only the rumen-derived trans fatty acids were likely responsible for lower milk fat yield in the 0900 h-fed cows.

Linoleic acid ($C_{18:2}$) can be biohydrogenated to stearic acid ($C_{18:0}$) in the rumen. Linoleic acid is first converted to *cis-9, trans-11* CLA and then to $C_{18:1\ trans-11}$ before producing stearic acid [14]. The mammary desaturation of $C_{18:1\ trans-11}$ is the major contributor to milk *cis-9, trans-11* CLA [15]. As a result, changes in milk fat content of *cis-9, trans-11* CLA and that of $C_{18:1\ trans-11}$ are expected to be parallel. In continuous cultures, a lower rumen pH (5.5 *vs*. 6.5) increased the oleic acid conversion to stearic acid [16] which implies a reduced rumen production of $C_{18:1\ trans-11}$ and mammary synthesis of *cis-9, trans-11* CLA. Additionally, the lower *vs*. normal pH reduced 13C enrichment of $C_{18:1\ trans-10}$ and abolished detection of *trans* isomers beyond C_{10} [16]. Milk fat proportions of $C_{18:1\ trans-11}$ and CLA *cis-9, trans-11* were similar between 0900 h and 2100 h feed deliveries, which concurs with their similar average rumen pH. It can be suggested that the transitory

lower rumen pH at 5-6 h post-feeding in the 2100 h-fed than in the 0900 h-fed cows may have not altered the rumen biohyrogenation pathways of $C_{18:2}$.

Conclusion

Milk proportions of total short, medium, and long chain fatty acids were not significantly different. Feed delivery at 0900 h *vs.* 2100 h increased milk proportions of $C_{10:0}$, $C_{12:0}$, $C_{12:1}$, $C_{13:0}$, $C_{13:1}$ and $C_{18:3\ n-3}$, and tended to increase proportions of $C_{8:0}$ and $C_{18:1trans-9}$, while decreased that of $C_{18:0}$. Evening feeding increased milk fat and energy outputs. As a proportion of N apparently digested, feeding at 2100 *vs.* 0900 h reduced urinary milk N, thereby improving N retention and reducing N losses that have environmental implications.

Acknowledgment

The supports of the Iranian Ministry of Science, Research and Technology, National Elite Foundation, and University of Zanjan, Iran, are acknowledged.

References

1. Nikkhah A, Furedi C, Kennedy A, Crow G, Plaizier J. Effects of feed delivery time on feed intake, rumen fermentation, blood metabolites and productivity of lactating cows. J. Dairy Sci. 2008; 91: 1-12.
2. Nikkhah A. Eating timing an evolutionary manager of postmodern rumen physiology and health: a review. Open Access Anim Physiol. 2011; 3: 13–19.
3. Nikkhah A. Chronophysiology of ruminant feeding behavior and metabolism: an evolutionary review. Biol Rhythm Res. 2012; 44: 197-218.
4. SAS Institute, Inc., SAS/STAT user's guide. V. 9.1. SAS Institute, Inc., Cary, NC. 2003.
5. Schwartzkopf- Genswein KS, Beauchemin KA, McAllister TA, Gibb DJ, Streeter M, Kennedy AD, et al. Effect of feed delivery fluctuations and feeding time on ruminal acidosis, growth performance, and feeding behavior of feedlot cattle. J. Anim. Sci. 2004; 82: 3357-3365.
6. Phillips CJ, Rind MI, The Effects of frequency of feeding a total mixed ration on the production and behavior of dairy cows. J. Dairy Sci. 2001; 84: 1979-1987.
7. Illnerova H, Sumova A. Photic entrainment of the mammalian rhythm in melatonin production. J. Biol. Rhythms. 1997; 12: 547–555.
8. la Fleur SE, Kalsbeek A, Wortel J, Fekkes MI, Buijs RM. A daily rhythm in glucose tolerance: a role for the suprachiasmatic nucleus. Diabetes. 2001; 50: 1237-1243.
9. Anika SM, Houpt TR, Houpt KA. Insulin as a satiety hormone. Physiology. Behavior. 1980; 25: 21.
10. Taweel HZ, Tas BM, Dijkstra, J, Tamminga, S. Intake regulation and grazing behavior of dairy cows under continuous stocking. J. Dairy Sci. 2004; 87: 3417-3427.
11. Hancock J. Studies of grazing behavior in relation to grassland management. I. Variations in grazing habits of dairy cattle. J. Agric. Sci. 1954; 44: 420-429.
12. Gordon J.G, McAllister IK. The circadian rhythm of rumination. J. Agric. Sci.1970; 291-297.
13. Robinson PH, Gill M, Kennelly JJ. Influence of time of feeding a protein meal on ruminal fermentation and forestomach digestion in dairy cows. J. Dairy Sci. 1997; 80: 1366-1373.
14. Bauman DE, Griinari JM. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. Livest. Prod. Sci. 2001; 70: 15-29.
15. Corl BA, Baumgard LH, Dwyer DA, Griinari JM, Phillips BS, Bauman DE, et al. The role of 9-desaturase in the production of cis-9, trans-11 CLA. J. Nutr. Biochem. 2001; 12: 622-630.
16. AbuGhazaleh AA, Riley MB, Thies EE, Jenkins TC. Dilution rate and pH effects on the conversion of oleic acid to trans $C_{18:1}$ positional isomers in continuous culture. J. Dairy Sci. 2005; 88: 4334-4341.

Citation: Nikkhah A. Circadian Intake Timing in Ruminants: Nitrogen Metabolism and Milk Fat Properties. Austin J Vet Sci & Anim Husb. 2015; 2(3): 1014.