Advances in Bioscience and Biotechnology, 2017, 8, 68-77
http://www.scirp.org/journal/abb
ISSN Online: 2156-8502
ISSN Print: 2156-8456

Effects of Supplemental Dietary Energy Source on Feed Intake, Lactation Performance, and Serum Indices of Early-Lactating Holstein Cows in a Positive Energy Balance

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How to cite this paper: Lin, X.Y., Liu, G.M., Yin, Z.Y., Wang, Y., Hou, Q.L., Shi, K.R. and Wang, Z.H. (2017) Effects of Supplemental Dietary Energy Source on Feed Intake, Lactation Performance, and Serum Indices of Early-Lactating Holstein Cows in a Positive Energy Balance. *Advances in Bioscience and Biotechnology*, **8**, 68-77.
https://doi.org/10.4236/abb.2017.82005

Received: January 13, 2017
Accepted: February 10, 2017
Published: February 13, 2017

Abstract

The present study investigated the effects of the supplemental dietary energy source on early lactating cows in a positive energy balance. Cows in the control group were fed a basal total mixed ration containing high-quality hay as forage, and the dietary concentrate to forage ratio was 45:65. The corn supplementation resulted in a significant decrease in the milk fat content ($P < 0.05$) and a low milk fat yield ($P = 0.15$), whereas the fat supplementation resulted in a decreasing trend of the milk protein and lactose content ($P \leq 0.1$). Additionally, the corn supplementation significantly decreased the serum 5-hydroxytryptamine level ($P < 0.05$). The results support the proposal that the source of supplemental dietary energy has varying effects on feed intake, lactation performance, and the intermediate metabolism of early lactating cows in a positive energy balance. 5-Hydroxytryptamine secretion may be associated with the varying effects of the source of supplemental dietary energy.

Keywords

Dairy Cow, Lactation, Energy Source, 5-Hydroxytryptamine

1. Introduction

Research significance: Postpartum dairy cows have an increased lactation yield and dry matter intake (DMI), with a peak lactation yield of 50 - 70 days in milk (DIM) (Struken *et al.* 2011) [1] and a peak DMI at 70 - 98 DIM [2] (Meng *et al.* 2002). Because the lactation yield increases before the DMI, postpartum cows are in a state of negative energy balance and need to manage body tissues to meet

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the energy demands of lactation and maintenance [3] (Block *et al.* 2001). In cow rearing, the dietary energy level can be improved through fat or concentrate supplementation to alleviate the negative energy balance. Research advances: Dietary fat or concentrate supplementation affects rumen fermentation. Certain types of fatty acids, especially long-chain unsaturated fatty acids, inhibit rumen microbial growth and lower the rumen degradation rate of dietary fiber [4] (Grummer, 1991). Concentrate contains high levels of rumen fermentable carbohydrates (RFC). Excess concentrate supplementation will decrease the rumen pH value, increase the risk of subclinical ruminal acidosis (SARA), and lower the rumen degradation rate of dietary fiber and feed conversion efficiency [5] (Zebeli *et al.* 2010). The adverse effects of fat supplementation on rumen fermentation can be prevented with rumen inert fats, such as fatty acid calcium salts and saturated fat [6] (Bernard and Kertz 2009). To eliminate the adverse effects of concentrate supplementation, the dietary physically effective neutral detergent fiber (peNDF) and RFC contents must be balanced. To control the rumen pH value, Zebeli *et al.* (2008) suggested that the peNDF > 1.18 to RFC ratio of the cow diet should be no less than 1.45. Dietary fat only undergoes limited metabolism in the rumen, primarily through the rumen microbial hydrogenation of unsaturated fatty acids [7]. The resultant saturated fat subsequently enters the small intestine for digestive absorption, which provides very little effective available energy for the rumen microbes. In contrast, concentrate has a higher rumen degradation rate and can therefore provide a large amount of available energy for the rumen microbes during the degradation process. The resultant volatile fatty acids can be absorbed through the rumen wall. Thus, fat and concentrate are two distinct sources of energy that have demonstrated substantial differences in their absorption site, form of absorbed energy, and ability to provide available energy to the rumen microbes. Additionally, lactating cows have demonstrated varying responses to these two types of supplemental dietary energy sources. Studies have shown that fat supplementation increases the milk yield [8] (Hammon *et al.* 2008), while concentrate supplementation increases the milk protein yield [9] (Lohrenz *et al.* 2010). One potential explanation for these findings is that fat supplementation reduces the amount of glucose used for milk fat synthesis and as an oxidation energy supply and targets the glucose for lactose synthesis, thereby improving the milk yield [4] (Grummer, 1991). Concentrate produces more propionate (a precursor for gluconeogenesis) during rumen fermentation. Both glucose and the precursor for gluconeogenesis play roles in targeting the amino acids in the blood for metabolism in the breast and promoting milk protein synthesis [10] (Rulquin *et al.* 2004). Additionally, it has been shown that supplementation with rumen inert fat or the abomasal infusion of long-chain fatty acids reduces the blood glucose level [8] (Hammon *et al.* 2008). Research gap: The effects of the supplemental dietary energy source on rumen fermentation in cows have been extensively studied, but the effects on post-ruminal metabolism have not been as extensively investigated. Research question: The aim of this study was to examine the effects of the supplemental dietary energy source on the lactation performance and serum indices in early lactating cows. An emphasis was placed on observing the changes in the sugar and fat metabolism associated with the serum indices and serum hormone levels. To control the effects of rumen environment changes on the experimental results, the feeding trial was conducted using high-quality hay as forage and the dry matter (DM) content of the concentrate in the basal diet was limited to 45% or less.

2. Materials and Methods

2.1. Experimental Animals

The experimental animals included twelve healthy disease-free postpartum Holstein cows in early lactation (36 ± 14 DIM) with an average parity of 3.4 ± 1.4 from Taian Lubao dairy farm. The cows were randomly divided into three groups of four cows each (Table 1).

2.2. Experimental Design

Cows were fed the same basal TMR (Table 2). Feedings were performed at 8:30 in the morning once per day. The amount of feed offered was determined based on ~10% of the remaining feed. Cows in the control group were only fed the basal diet, and those in the treatment groups were supplemented with either 680 g/d fatty acid calcium salts (Megalac) or 2.5 kg/d corn flour [Corn, DM 83.8%] per individualwhich was based on DM. The fatty acid calcium salts (Megalac, VOLAC, UK) contained 6.5 Mcal/kg of net energy for lactation, and the corn flour contained 2.13 Mcal/kg DM of net energy for lactation. The two treatment groups were iso-energetically supplemented before the once daily basal TMR morning feeding. The amount of fat and corn supplementation gradually increased during a 7-d transition period. Thereafter, the trial commenced and lasted 21 d. Samples were collected during the last 2 d of the trial. Cows had free access to water throughout the trial period. Milking was performed twice a day at 07:00 and 18:00.

2.3. Sample Collection and Analysis

All animal work was approved by the Animal Welfare and Health Committee of

Table 1. General condition of the three groups of experimental cows.

	Group			SEM	*P*
	1	2	3		
n[1]	4	4	4		
Parities	3.2	2.8	4.2	0.67	0.32
Days in milk	38	37	35	7.59	0.92
Daily milk yield, kg/d	25.6	24.9	24.8	1.57	0.61
Body condition score	2.78	3.10	2.81	0.19	0.39

[1]Multiparous Holstein cows with averaging 618 ± 39 kg of BW.

Table 2. Ingredients, composition, and nutrient levels of the basal experimental diet (dry matter basis).

Ingredients	Content (%)
Ingredients	
Alfalfa hay	19.9
Leymus chinensis hay	13.4
Oat grass hay	22.4
Mixed concentrate[1]	44.3
Total	100.0
Nutrient levels	
Dry matter	89.45
Net energy for lactation, NE_L/(Mcal/kg)[2]	1.45
Crud protein	14.02
Crude fat, ether extracts	2.82
Organic matter	91.05
Neutral detergent fiber	41.81
Acid detergent fiber	27.47

[1]Commercial product purchased from Dacheng-Land O'Lakes (Tianjin) Feed Co., Ltd. (Lot# 80028); [2]NE_L is calculated as: NE_L (Mcal/kg DM) = 0.5501 × DE (Mcal/kgDM) - 0.0946 (r = 0.9172). Other nutrient levels are measured values.

Shandong Agricultural University. During the trial period, the weights of the feed offered and feed remaining were recorded daily, and the data from the last 2 d were used for the statistical analysis of feed intake. The feed samples offered and remaining were collected during the last 2 d. The milk yield was recorded during the trial period, and the data obtained from the last 2 d were used for statistical analysis. The milk samples were collected during the last 2 d of the trial. The morning and evening milk samples were mixed at a ratio of 3:2.

Blood samples were collected from the vein of the tail root of the cows during the last 2 d of the trial at AM 5:00 in the morning. Serum glucose, triglyceride (TG), and β-hydroxybutyrate (BHBA) detection kits were purchased from Mike Biotech. Co., Ltd., Sichuan, China. A non-esterified fatty acid (NEFA) detection kit was purchased from Nanjing Jiancheng Bioengineering Institute, China. The serum insulin, glucagon, insulin-like growth factor-1 (IGF-1) levels were measured by enzyme-linked immunosorbent assay (ELISA) using a kit purchased from Jiuding, Tianjin, China. The serum cholecystokinin (CCK), leptin, apolipoprotein A-IV (apoA-IV), glucagon-like peptide-1 (GLP-1), melanocortin (MC), and 5-hydroxytryptamine (5-HT) levels were measured by ELISA assay using a commercial kit purchased from Xinran, Shanghai, China.

2.4. Data Analysis

One-way ANOVA was performed using SAS 9.0 with the following statistical model: $Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$, where μ is the mean value, α_i is the treatment effect, and

ε_{ij} is the random error. Differences between the groups were considered statistically significant at $P < 0.05$. A P value between 0.05 and 0.10 was considered a trend toward statistical significance.

3. Results

3.1. Feed Intake, Lactation Performance, and Body Condition Score (BSC)

Supplementation with either 680 g/d fat or 2.5 kg/d corn alone before the morning feeding did not significantly affect the DMI of the basal TMR of the individual early lactating cows (Table 3). No significant differences were found in the DMI between the treatment and control groups or between the two treatment groups ($P > 0.05$). In contrast, the source of supplemental dietary energy strongly affected the milk fat and NFS content. Corn supplementation reduced the milk fat content, while fat supplementation reduced the milk lactose content; both values were significantly different from the control values ($P < 0.05$). Additionally, the fat supplementation resulted in decreasing trends the milk protein ($P = 0.10$) and lactose contents ($P = 0.08$), with a relatively high milk yield ($P = 0.15$), milk fat yield ($P = 0.15$), TS content ($P = 0.12$), and post-trial BSC ($P = 0.13$). Compared with the pre-trial values, the post-trial BSCs of the control, fat-supplemented, and corn-supplemented cows increased by 0.07, 0.25, and 0.09, respectively.

Table 3. Effects supplemental dietary energy source on dry matter intake (DMI) of the basal total mixed ration, milk yield, and body condition score (BSC) of early lactating Holstein cows.

	Treatment			SEM	P
	Control	Megalac	Corn		
DMI (kg/d)	22.09	21.20	23.47	1.04	0.38
Milk yield (kg/d)	27.01	29.14	25.28	1.89	0.15
Milk fat/%	4.06^{a}	3.99^{ab}	3.59^{b}	0.19	0.05
g/d	1086.51	1160.20	914.18	81.62	0.15
Milk protein/%	3.24	3.04	3.24	0.09	0.10
g/d	873.42	889.56	823.85	76.03	0.82
Milk lactose/%	4.59^{a}	4.50^{b}	4.61^{a}	0.50	0.08
g/d	1240.49	1313.29	1168.32	97.97	0.60
Non-fat solids/%	8.97^{a}	8.68^{b}	8.98^{a}	0.13	0.04
Total solids/%	10.95	12.43	10.75	0.85	0.12
Pre-experiment BSC	2.78	3.10	2.81	0.19	0.39
Post-experiment BSC	2.85	3.35	2.90	0.17	0.13
BSC difference	+0.07	+0.25	+0.09		

Note: Different superscript letters in the same row indicate statistically significant difference; uppercase letter for $P < 0.01$ and lowercase letter for $P < 0.05$.

3.2. Serum Indices

Corn supplementation significantly reduced the serum 5-HT level ($P < 0.05$) (**Table 4**), whereas fat supplementation resulted in an increasing trend in the serum ApoA-IV level ($P = 0.09$). The serum CCK levels were higher in the cows supplemented with fat and lower in those supplemented with corn ($P = 0.15$). Additionally, the serum levels of TG ($P = 0.12$) and leptin ($P = 0.19$) were numerically higher in the fat-supplemented cows. The source of supplemental dietary energy had no significant effect on the other serum indices tested ($P > 0.05$).

4. Discussion

4.1. Feed Intake, Lactation Performance, and BCS

Fat supplementation is generally considered to reduce the feed intake of cows even though the results reported have been inconsistent. For example, fat supplementation reduced the DMI of cows in certain trials [11] [12] [13] (Benson *et al.* 2001; Onetti and Grummer 2004; Weiss and Pinos-Rodriguez 2009) but had no effect in others [14] (Mosley *et al.* 2007). The inconsistency in the results from these different trials may be related to the varied lactation stages of the experimental animals. For mid-late lactating cows in a positive energy balance, fat supplementation had little effect on feed intake, especially in the case of a longer supplementation period [15] (Chiiliard 1993). This finding may be explained by the general mechanism of feed intake regulation in cows introduced previously by Conrad *et al.* (1964) [16]. Through this mechanism, the feed intake of cows is

Table 4. Effects of the supplemental dietary energy source on the serum indices in early lactating Holstein cows.

	Treatment			SEM	P
	Control	Megalac	Corn		
Blood glucose/(mmol/L)	1.15	0.92	1.36	0.25	0.50
Triglyceride/(mmol/L)	0.06	0.11	0.08	0.01	0.12
β-hydroxybutyrate /(mmol/L)	0.72	0.65	0.74	0.07	0.63
Non-esterified fatty acid/(mmol/L)	414.97	487.24	449.24	56.36	0.67
Insulin/(μIU/ml)	4.68	3.20	4.37	0.99	0.57
Glucagon/(pg/ml)	282.24	378.59	256.63	62.30	0.38
Insulin-like growth factor-1/(ng/ml)	39.80	30.78	26.69	14.49	0.49
Glucagon-like peptide-1/(pmol/L)	4.62	4.82	4.19	0.60	0.39
Cholecystokinin/(pg/ml)	229.61	251.81	172.46	24.98	0.15
Leptin/(ng/ml)	3.63	4.36	3.25	0.36	0.19
Apolipoprotein A-IV/(ng/ml)	17.44^{ab}	20.34^{a}	12.66^{b}	2.03	0.09
5-Hydroxytryptamine/(ng/L)	783.12^{a}	764.17^{a}	456.88^{b}	55.28	0.005
Melanocortin/(pg/ml)	47.18	52.48	42.95	4.82	0.47

Note: Different superscript letters in the same row indicate a statistically significant difference; uppercase letter indicates a $P < 0.01$ and a lowercase letter indicates a $P < 0.05$.

mainly influenced by the degree of fullness (DF) of the digestive tract and the energy demands of cows. Moallem *et al.* (2010) [17] observed that dietary fat supplementation caused a decline in the DMI of cows for three consecutive weeks, whereas an iso-energetic dietary concentrate supplementation had no such result. Isoenergetically supplemented lactating cows with dietary fat or concentrate in the hot summer season and found that the DMI decline was due to fat supplementation rather than concentrate supplementation. This finding indicates that fat is more likely than concentrate to reduce the DMI possibly because of the fat-induced changes in gastrointestinal hormone secretion and hepatic oxidative metabolism (Relling and Reynolds 2007) [18]. The effects of dietary fat supplementation on internal secretion and *in vivo* metabolism were the primary focus of the present study. High-quality hay was used as forage to avoid the effect of a rumen pH decrease on the experimental results. Although early lactating cows were used as experimental animals, the control group demonstrated a 0.07 incremental increase in the BSC by the end of the trial. This result indicates that the cows were in a state of positive energy balance by the end of the study.

The experimental treatments had no significant effects on the milk yield. The fat supplementation numerically improved the milk yield by 2.13 and 3.86 kg/d compared with the control and corn supplementation, respectively. This result is consistent with previous findings that demonstrated an improvement in the milk yield with fat supplementation [8] [19] (Van Knegsel *et al.* 2005; Voigt *et al.* 2005; Hammon *et al.* 2008). The corn supplementation significantly reduced the milk fat content, reducing the milk fat yield by 172.33 and 246.02 g/d compared with the control and fat supplementation, respectively. This trend is consistent with previous results from the intravenous infusion of glucose in lactating cowsand the abomasal perfusion [20] [21] (Lin *et al.* 2010; Zhang *et al.* 2014) of glucose in lactating milk goats. A milk fat decrease due to corn supplementation may be related to a substantial propionate production resulting from corn fermentation in the rumen. Propionate is a precursor for gluconeogenesis, and its increased production promotes hepatic gluconeogenesis, thereby stimulating insulin secretion and distributing more precursors of fat synthesis in the circulating blood to body tissues other than those involved in milk fat synthesis [21] (Zhang *et al.* 2014). It should be noted that fat supplementation reduces the glucose consumption related to tissue metabolism, while concentrate supplementation promotes hepatic gluconeogenesis.

4.2. 5-HT

Relling and Reynolds (2007) supplemented the diet of lactating cows with calcium salts of saturated, monounsaturated, or polyunsaturated fatty acids, which resulted in elevated plasma GLP-1 and CCK levels. GLP-1, CCK, leptin, ApoA-IV, 5-HT, and MC play roles in regulating appetite [18]. In this study, the fat supplementation was associated with relatively high serum CCK and leptin levels and an increasing trend in the ApoA-IV level. In contrast, the corn supplementation significantly reduced the serum 5-HT level. Taken together, these results suggest that dietary fat and starch have different effects on the secretion of appetite-related hormones. The effects of the concentrate supplementation on the blood 5-HT level were generally not observed because 5-HT plays a role in regulating appetite and energy metabolism (Launay *et al.* 1994) [22]. In the present study, the serum 5-HT level was measured and the experimental result was an incidental finding worthy of attention. 5-HT was isolated and identified from the serum and is also known as serotonin. It acts as a hormone and neurotransmitter and has a wide range of physiological functions. Currently, seven 5-HT receptor subtypes have been identified [22] (Launay *et al.* 1994). In cows, 5-HT may act as a negative feedback inhibitor of lactation (Hernandez *et al.* 2008). The source of supplemental dietary energy affects the energy distribution for in vivo metabolism in cows. Fat supplementation (a fat-producing diet) targets more energy toward lactation, while concentrate supplementation (a sugar-producing diet) targets more energy to body tissues [19] (Van Knegsel *et al.* 2014). It is generally believed that sugar-producing diets stimulate insulin secretion. 5-HT can cause vasoconstriction, and the variation in the blood 5-HT level due to dietary concentrate supplementation is associated with the change in energy distribution for in vivo metabolism caused by different sources of supplemental dietary energy.

5. Conclusions

1) The iso-energetic supplementation of fat or corn had different effects on the milk yield of early lactating cows in a positive energy balance and fed a high-quality forage diet. The fat supplementation resulted in a higher milk yield but a lower feed intake and milk protein content, while the corn supplementation was associated with a lower milk fat content and milk fat yield.

2) The fat and corn supplementations had different effects on the serum levels of appetite-related hormones in early lactating cows. Fat supplementation resulted in higher serum CCK, leptin, and ApoA-IV levels, while corn supplementation was associated with a higher 5-HT level and a lower CCK level.

Acknowledgements

Financial support was provided by the fund for Modern Agro-industry Technology Research System of China (CARS-37), Modern Agro-industry Technology Research System of Shandong (SDAIT-12-011-06), Shandong province science technology development plan (2012NC11103), natural science fund of China (31072050) (31372340).

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