



## Effects of dietary supplements of rumen-protected folic acid on lactation performance, energy balance, blood parameters and reproductive performance in dairy cows



H.Q. Li<sup>a</sup>, Q. Liu<sup>a,\*</sup>, C. Wang<sup>a</sup>, Z.M. Yang<sup>a</sup>, G. Guo<sup>a</sup>, W.J. Huo<sup>a</sup>, C.X. Pei<sup>a</sup>,  
Y.L. Zhang<sup>a</sup>, S.L. Zhang<sup>a</sup>, H. Wang<sup>b</sup>, J.X. Liu<sup>c</sup>, Y.X. Huang<sup>a</sup>

<sup>a</sup> College of Animal Sciences and Veterinary Medicines, Shanxi Agricultural University, Taigu, Shanxi 030801, PR China

<sup>b</sup> Animal Husbandry and Veterinary Bureau of Yuci County, Yuci, Shanxi Province 030600, PR China

<sup>c</sup> College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang 310058, PR China

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### ABSTRACT

The objective of this study was to evaluate the effects of dietary supplements of rumen-protected folic acid (RPFA) on lactation performance, energy balance, reproductive performance and blood parameters of dairy cows. Ninety-six multiparous Holstein cows were assigned to four groups of 24 each according to their previous 305-days milk production. The treatments were: control, low RPFA (LRPFA), medium RPFA (MRPFA) and high RPFA (HRPFA) with 0–3 g RPFA/cow per day, respectively. Supplements of RPFA were top-dressed and manually mixed into the upper one third of the ration during the morning feeding from 3 weeks before the expected calving date to 15 weeks after parturition. Dry matter (DM) and net energy (NE) intakes, milk yield, milk protein content and production increased linearly ( $P < 0.05$ ) with increasing RPFA supplementation, whereas milk fat content and production only tended to be linearly increased. Cows supplemented with RPFA showed a similar pattern ( $P < 0.05$ ) of decreased loss of body weight (BW) and improved energy balance (expressed in MJ/cow/day) during the 105 days in milk (DIM) period. Concentrations of glucose in plasma, folate and methionine in serum increased linearly ( $P < 0.05$ ), whereas concentrations of non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHBA) and serum homocysteine linearly ( $P < 0.01$ ) decreased. Total conception rate and the percentage of cows pregnant at 150 DIM linearly increased ( $P < 0.05$ ), DIM at first breeding and breeding per conception tended to change linearly with increasing RPFA supplementation. These results indicated that supplementary RPFA in cow diets had positive effects on feed intake and milk production, increased concentrations of plasma glucose and serum folates, decreased plasma concentrations of NEFA and BHBA and serum concentration of homocysteine, and improved energy balance and reproductive performance. These data suggest that RPFA improves lactation, energy balance and reproductive performance in a dose-dependent manner.

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**Abbreviations:** ADF, acid detergent fibre; BHBA, beta-hydroxybutyrate; BW, body weight; CP, crude protein; DIM, day in milk; DM, dry matter; HRPFA, high rumen-protected folic acid; LRPFA, low rumen-protected folic acid; MRPFA, medium rumen-protected folic acid; aNDF, neutral detergent fibre; NEFA, non-esterified fatty acids; NE, net energy; NE<sub>M</sub>, net energy required for body maintenance; NE<sub>L</sub>, net energy required for lactation; OM, organic matter; RUP, rumen-undegradable protein; TMR, total mixed ration; 5-methyl-THF, 5-methyl- tetrahydrofolate; THF, tetrahydrofolate.

\* Corresponding author. Fax: +86 354 628 8052.

E-mail address: [liuqiangabc@163.com](mailto:liuqiangabc@163.com) (Q. Liu).

## 1. Introduction

Folic acid, as a B-complex vitamin, plays an important role in mammals to transfer one carbon unit (Choi and Mason, 2000) to meet the requirements for the synthesis of purines and pyrimidines, the synthesis, repair and methylation of DNA, the synthesis of phosphatidylcholine and the biosynthesis of proteins and nucleotides (Bailey and Gregory, 1999). It is well known that ruminal bacteria can synthesize folic acid (NRC, 2001). However, dairy cows have greatly increased their average milk and milk component yields with the gradual breeding of animal species and intensification in the past decades. It is likely that folic acid requirements of cows have increased according to the improved lactation performance and that ruminal synthesis of folic acid may not be sufficient to meet these new needs. Santschi et al. (2005) and Schwab et al. (2006) estimated apparent ruminal synthesis of folic acid to be between 16.5 and 21.0 mg/day, while the requirement of folic acid estimated in NRC (2001) for a cow weighing at 650 kg and producing 35 kg of 40 (g/L) fat-corrected milk was 35 mg/day: 33 mg/day for tissue utilization and 2 mg/day for milk production. Therefore, ruminal synthesis of folic acid was not sufficient to meet the requirements of high-producing dairy cows, especially around parturition (Girard and Matte, 1999). Moreover, several studies have documented that folic acid supplementation in dairy cow diets has positive effects on milk production, milk protein concentrations and yields (Girard et al., 1995; Girard and Matte, 1998). Supplementary folic acid at 2.6 g/day to cows weighing 755 kg increased milk production and milk crude protein yields (Graulet et al., 2007). Preynat et al. (2009) also found that milk production was increased by a combined supplement of folic acid and vitamin B12 with or without a supplement of rumen-protected methionine. Juchem et al. (2012) reported a significant improvement in conception rates 42 days after first breeding with a dietary supplement of B vitamins protected from ruminal degradation in dairy cows. These results suggested that folic acid requirements increased in highly productive dairy cows, especially in early lactation. However, more than 95% of folic acids supplemented in dairy diet is directly degraded by rumen microflora (Santschi et al., 2005). Thus, research on lactation performance, energy balance, blood parameters and reproductive performance influenced by the supplements of RPFA is necessary. In addition, one previous research found the first breeding postpartum for multiparous cows occurred 3.8 days earlier in cows given a combined supplement of folic acid and B12 (Duplessis et al., 2014). However, other studies reported no differences in milk production during early lactation in cows administered folic acid by injections (Girard et al., 1995) or dietary supplements (Girard et al., 2005).

Considering the inconsistency of results from earlier studies and the limited number of studies on the effects of folic acid on energy balance and reproduction of dairy cows, we hypothesized that a RPFA supplement would improve the lactation performance, energy balance and reproductive performance of lactating dairy cows. The aim of this study therefore was to determine the effects of increasing RPFA supplements from 3 weeks prepartum to 15 weeks of lactation on DM intake, milk production, energy balance and reproductive performance of cows.

## 2. Materials and methods

Experimental protocol was approved by the Animal Care and Use Committee of Shanxi Agriculture University (Taigu County, Shanxi Province).

### 2.1. Animals and experimental design

Ninety-six multiparous ( $2.5 \pm 0.26$  parity) Holstein cows with similar BW ( $629 \pm 17.3$  kg) and previous milk production ( $8,764 \pm 243.8$  kg) were used from 3 weeks before the expected calving date to 15 weeks after parturition. Cows were blocked by lactation number, previous 305 days milk production and expected calving date and were assigned into four groups of 24 of each in a randomized block design. The treatments were: control, low RPFA (LRPFA), medium RPFA (MRPFA) and high RPFA (HRPFA) with 0–3 g RPFA per cow per day, respectively. Supplements of RPFA were top-dressed and manually mixed into the upper one third of the ration during the morning feeding. Degradability of RPFA in the rumen and in the small intestine was determined in situ using rumen and duodenal cannulated cattle, and they were 300 g/kg and 500 g/kg, respectively. Therefore, dairy cows in control, LRPFA, MRPFA and HRPFA treatments received 0, 35, 70 and 105 mg of folic acid per cow per day, respectively. Cows were housed in a naturally ventilated tie-stall barn and allowed to exercise for 2 h in an open dry lot before each milking. Cows had *ad libitum* access to feed and water. They were milked at 07:00 and 19:00 h daily and meals were served as a total mixed ration (TMR; Table 1) after each milking. Diet was formulated based on NRC (2001) recommendations for a 630 kg cow producing 32 kg/day of milk containing 40 g/kg of milk fat and 30 g/kg CP.

### 2.2. Measurements and collection of samples

Feed offered and refusals were measured for each cow and recorded daily through the experimental period to calculate daily dry matter (DM) intake. Milk yields were recorded daily from parturition through 105 days in milk (DIM). Body weights were recorded on 2 consecutive days at parturition and at 35, 70 and 105 DIM after the 07:00 h milking. Data for DIM at first and second breedings, first-breeding conception rate, second-breeding conception rate, and number of breedings per conception were recorded timely. First-breeding conception rate was defined as the percentage of cows confirmed pregnant after the first service, whereas second-breeding conception rate represented the percentage of cows confirmed

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