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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 12 (1): 69-73, 2009
ISSN 1028-8880

Investigation on the Effect of Supplementing Rumen-Protected Forms of Methionine and Choline on Health Situation and Reproductive Performance of Holstein Dairy Cows

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Abstract: Twenty Holstein dairy cows in their first and second lactation were used from 4-week prepartum through 20-week postpartum. The aim was to investigate the effect of feeding different levels of ruminally protected methionine and choline on health situation and reproductive indices of Holstein dairy cows. Cows were randomly assigned to receive one of the following treatments: 18 g day^{-1} of rumen protected methionine (RPM), 60 g day^{-1} of rumen protected choline (RPC), 18 g day^{-1} of RPM +60 g day^{-1} of RPC and neither supplement (control). The treatments significantly affected services per conception and open days of lactating dairy cows, but did not significantly affect on days to first estrus and number of pregnant cows. RPM+RPC-fed cows had the lowest open days, days to first estrus and services per conception compared with other groups. Although no statistical differences were noted for any given health category, the overall incidence of health-related disorders was numerically lowest for cows fed RPM+RPC. Results indicate that the supplementation of RPM and RPC have been improved reproductive performance and health situation of dairy cows.

Key words: Rumen-protected methionine, protected choline, transition period, metabolic disorders, milk production

INTRODUCTION

Management and nutrition during the transition period influence milk production, the incidence of peripartum metabolic disorders and reproductive performance. Therefore, management of cows during this critical stage is crucial for the productivity of dairy cattle (Overton and Waldron, 2004). The periparturient period of dairy cattle is characterized by dramatic changes in nutrient demand that necessitate remarkable coordination of metabolism to meet requirements for energy, glucose and amino acid (AA) by the mammary gland following calving (Bell, 1995). Dairy cows mobilize large amounts of fatty acids from adipose tissue to meet their energy requirements during the periparturient period and early lactation, resulting in increased circulating concentrations of nonesterified fatty acids (NEFA) in the bloodstream.

Choline is a nutrient involved with the transport of fat from the liver, synthesized in part from methionine and required for the synthesis of phosphatidylcholine, a phospholipid found in the membranes of very low density lipoprotein. Hence, choline deficiency in transition dairy cattle may be associated with hepatic lipidosis. Choline is considered a quasi-vitamin due to its necessity for maintaining health in certain species or when methyl precursors are low in the diet (Combs, 1992). Phosphatidylcholine, derived from choline, is involved in intracellular signaling and is an integral constituent of all cell membranes and is an abundant source of labile methyl groups for transmethylation reactions (Hartwell *et al.*, 2000). There is an estimated requirement for gram quantities of choline for normal tissue metabolism and milk production in lactating dairy cows (Erdman and Sharma, 1991) and because very little dietary choline escapes rumen degradation, choline may limit milk production. The microbial populations in the rumen quickly degrade dietary choline; therefore, the only practical means of increasing choline to the dairy cows is to feed it in a rumen-protected form (RPC) (Atkins *et al.*, 1988). If synthesis of choline and related compounds during the periparturient period is insufficient for maximal hepatic metabolism of NEFA, the severity of hepatic lipid infiltration may be exacerbated by providing additional choline through the diet. The benefit could be similar to the effects reported when hepatocytes isolated from rats fed a choline-free diet were supplemented with choline or methionine *in vitro* (Yao and Vance, 1988). Supplementation with either nutrient increased concentrations of phosphatidylcholine in media and export of triglycerides from the hepatocytes as VLDL.

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Methionine is a sulfur-containing AA that is involved in many pathways including the synthesis of phospholipids, carnitine, creatine and the polyamines (Bequette *et al.*, 1998). In addition to being used for protein synthesis, methionine can be used to provide methyl groups for a variety of reactions and to provide sulfur groups from the synthesis of cysteine. Methionine has been identified as one of the most limiting AA for the synthesis of milk and milk protein by dairy cows fed diets based on corn (Overton *et al.*, 1998). One approach that has been used to supply additional methionine to the cow is to protect it from ruminal degradation for subsequent absorption in the small intestine. The pathways of methionine and choline metabolism are interrelated. Methionine is the source of the methyl donor S-adenosylmethionine, the metabolite that provides methyl groups for the *de novo* synthesis of choline.

Choline and methionine metabolism are closely associated and as much as 28% of absorbed methionine is used for choline synthesis (Emmanuel and Kennelly, 1984). Methionine plays a direct role in very low-density lipoprotein (VLDL) synthesis in bovine and acts to reduce plasma ketones during early lactation (Durand *et al.*, 1992). Active synthesis of phosphatidylcholine is necessary for VLDL secretion from rat hepatocytes (Yao and Vance, 1988). Thus, supplying choline directly may enhance synthesis of phosphatidylcholine and increase VLDL synthesis or serve to increase methionine availability for lipoprotein synthesis to indirectly alter liver triacylglyceride clearance as VLDL. The effects of choline, in this regard, may depend on the supply and profile of AA absorbed from the small intestine of the dairy cow. The primary fuels for the developing fetus include glucose, lactate and AA and during periods of reduced feed intake, AA may be important sources of carbon for gluconeogenesis (Bell, 1995). Increasing the availability of AA may increase glucose production, increase hepatic AA oxidation, or directly promote protein synthesis. Brüsemeister and Südekum (2006) reported that supplementary choline may improve the yield of dairy cows by elevating the export of triglycerides from the liver and by sparing methionine as a methyl donor and the demand for methyl groups still lacks adequate consideration in the design of diets for dairy cows. Davidson *et al.* (2008) reported that milk yield was higher in multiparous cows fed rumen-protected choline compared with the multiparous cows fed rumen-protected methionine or control diet. Also, multiparous cows fed rumen-protected choline had higher milk protein yield than cows fed control but was not different from cows fed rumen-protected methionine. Zahra *et al.* (2006) reported that cows which received rumen-protected choline produced 1.2 kg day^{-1} more milk in the first 60 day of lactation. The mechanism by which rumen-protected choline increased milk production was not revealed in this study. Socha *et al.* (2005) reported methionine + lysine supplementation increased yield of energy-corrected milk, fat and protein and tended to increase production of 3.5% fat-corrected milk compared with basal and methionine-supplemented diets. Girard *et al.* (2005) reported dietary supplement of rumen-protected methionine modified milk crude protein and casein concentrations but not yield in mid- and late-lactation.

An improved understanding of the mechanisms that regulate these overlapping pathways is needed so that these compounds may be fed to lactating dairy cows in order to improve lactation performance and reduce the incidence of ketosis and fatty liver. This hypothesis was that the supplementation of ruminally protected forms of methionine and choline in the diet of dairy cows can be improved the health situation and reproductive performance of these animals.

Therefore, the objective of this research was to study the effect of feeding different levels of ruminally protected methionine and choline from 4 week prepartum through 20 week postpartum on some reproductive indices and health situation of Holstein dairy cows in Iran.

MATERIALS AND METHODS

Cows, treatments and data collection: Twenty Holstein dairy cows in their first and second lactation from December 2007 to June 2008 were used in a lactation study from 4 week prepartum (body condition score, BCS = 3.6±0.04; Mean±SE) through 20 week postpartum in a large dairy farm around Tehran (Tehran Province, Iran). Selection of cows for initializing this experiment was based on parity, milk yield of earlier lactation (milk yield of dams for the cows in their first lactation) and body condition score. Cows (3 cows in 1st lactation and 2 cows in 2nd lactation per treatment) were randomly assigned to receive one of the following treatments: 18 g day^{-1} of rumen-protected methionine (RPM), 60 g day^{-1} of rumen-protected choline (RPC), 18 g day^{-1} of RPM +60 g day^{-1} of RPC and neither supplement (control). The RPM product [SmartamineM™ (Adisseo, Antony, France)] was a white solid granular powder coated with a polymer (2-vinyl pyridine-co styrene) sensitive to acidic pH in abomasum and contained 75% DL-methionine. The RPC product [COL 24® (Soda Feed Ingredients, Monaco, France)] is a rumen-protected source of choline chloride. COL 24® is produced by encapsulating choline chloride with a coating matrix able to resist rumen breakdown and release choline in the intestine and contained 24% choline chloride. The DM-based forage to concentrate ratio was 57:43 for dry period diet and 44:56 for lactation diet. The RPM and RPC were top dressed onto the TMR. Cows were fed for *ad libitum* intake the total mixed diets and feed refusals were recorded once daily, allowing 5% orts at each feed delivery. Prepartum and postpartum diets (Table 1, 2) were formulated using the CNCPS program. The CNCPS program is a model designed to evaluate diets and animal performance for all classes of cattle in unique production situations, using science-based principles of rumen function, microbial growth, feed digestion and passage and physiological state (Fox *et al*., 2004). Reproductive data were recorded, including days open, days to first estrus, number of pregnant cows and services per conception. Also, health problems were recorded during the trial, including incidence of dystocia, mastitis, retained placenta, displaced abomasum, ketosis, milk fever, foot/leg problems, twin births and uterine problems.

Table 1: Ingredient composition of pre- and postpartum diets (DM %)

Ingredients	Prepartum TMR	Postpartum TMR
Alfalfa hay	36.00	17.81
Corn silage	20.89	25.84
Barley grain	10.44	17.39
Corn grain	5.22	7.61
Soybean meal	10.68	7.79
Wheat bran	10.56	6.60
Whole cottonseed	-	6.82
Canola meal	-	6.82
Beet pulp	3.60	-
Minerals and vitamins supplement[1]	0.78	0.61
Salt	0.16	0.49
Calcium carbonate	-	0.37
Sodium bicarbonate	-	0.62
Fat supplement	-	1.23
Ammonium chloride	0.27	-
Calcium chloride	1.16	-
Magnesium sulfate	0.23	-

[1]Contained 180 g kg^{-1} Ca, 70 g kg^{-1} P, 30 g kg^{-1} Mg, 4 g kg^{-1} Fe, 0.5 g kg^{-1} Cu, 5 g kg^{-1} Mn, 4 g kg^{-1} Zn, 0.1 g kg^{-1} Co, 0.1 g kg^{-1} I, 0.03 g kg^{-1} Se, 0.4 g kg^{-1} antioxidant, 5×10^5 IU kg^{-1} of vitamin A, 10^5 IU kg^{-1} of vitamin D and 2×10^3 IU kg^{-1} of vitamin E

Table 2: Chemical composition of pre- and postpartum diets

Items	Prepartum TMR	Postpartum TMR
DM (%)	66.00	62.00
CP (%)	16.50	18.00
Ash (%)	8.90	7.10
Total fat (%)	2.40	5.10
NDF (%)	39.70	34.70
ADF (%)	27.80	20.80
NFC[1] (%)	32.50	35.10
peNDF (%)	27.00	22.00
Ca (%)	0.89	0.56
P (%)	0.41	0.45
NE_L (Mcal kg^{-1})	1.43	1.63
RUP (% of CP)	27.10	34.50
RDP (% of CP)	72.90	65.50
Met (%)	1.94	1.82
Lys (%)	6.60	6.42

[1]NFC = 100 -(CP + Ash + Total fat + NDF)

Statistical analysis: Statistical analysis of reproductive data was performed using the general linear models procedure (Proc GLM) of SAS and the statistical model included the effects of treatment, parity and treatment×parity. Also, because the incidences of health problems were coded as a dichotomous variable (0 = health; 1 = disease), a logistic regression model was used to analyze the effect of treatments on the incidence of health problems, using the maximum likelihood method of the Logistic procedure of SAS. Significant levels were declared at $p<0.05$.

RESULTS AND DISCUSSION

The treatments significantly affected services per conception and open days of lactating dairy cows ($p<0.05$), but did not affect significantly on days to first estrus and number of pregnant cows (Table 3). Higher-producing cows are usually may experience longer periods of negative energy balance than lower-producing cows. As a result of a more negative energy balance, the higher producing cows may be at greater risks of reproductive/conception failure than lower-producing cows (Ravagnolo and Misztal, 2002). Therefore, providing RPM was expected to enable the higher-producing cows to maintain productivity but at reduced expenses of reproductive malfunction.

Polan *et al*. (1991) reported that supplementation of rumen-protected forms of lysine and methionine had not significant effects on days to first service, services per conception and calving interval in dairy cows. Erdman and Sharma (1991) reported that although not

Table 3: The effects of supplemental rumen-protected methionine and choline on reproductive indices of Holstein dairy cows

	Treatment					p-value		
Items	Control	RPM[1]	RPC[1]	RPM+RPC	SEM[2]	Trt[1]	Parity	Trt×Parity
Days to first estrus	61.8	48.8	64.0	31.6	7.4	0.325	0.008	0.364
Open days	115.4[a]	88.6[ab]	106.6[ab]	73.8[b]	9.3	0.002	0.663	0.739
Services per conception	3.0[b]	2.2[ab]	2.4[ab]	2.0[a]	0.2	0.034	0.096	0.425
Number of pregnant cows	1.0	3.0	3.0	4.0	0.6	0.064	0.125	0.534

[1]Trt: Treatment; RPM: Rumen-protected methionine; RPC: Rumen-protected choline, [2]SEM: Standard error of means, [a, b]: Means with different superscripts differ significantly ($p<0.05$).

Table 4: Incidence of health problems in Holstein cows fed supplemental rumen-protected methionine and choline from 4 week prepartum through 20 week postpartum

	Treatment			
Items	Control	RPM	RPC	RPM+RPC
Retained placenta	2	1	2	0
Mastitis	1	1	0	0
Displaced abomasum	0	0	0	0
Uterine problem	1	0	0	0
Milk fever	0	0	0	0
Dystocia	2	1	2	0
Ketosis	0	0	0	0
Foot/Leg problems	0	0	0	0
Twin births	0	1	0	0

significantly different, the number of pregnant cows during the experiment tended to be lower as choline intake increased. Services per cow and per conception tended to be higher for choline-treated cows bred or pregnant prior to week 2. Days open also were greater for choline treatments using data up to week 44 postpartum. Also, there was no apparent trend due to RPC on number pregnant, services, or days to first breeding. They were proposed that, because of the higher actual milk yield, reproductive responses were more related to differences in milk yield than to the effect of RPC. Present results are contrary to the results of Erdman and Sharma (1991), but in the case of number of pregnant cows, this result is consistent with the result of them.

Although no statistical differences were noted for any given health category, the overall incidence of health-related disorders was numerically lowest for cows fed RPM+RPC (Table 4).

Janovick Guretzky *et al.* (2006) reported the incidence of twinning tended to be greater for cows in the RPC group compared with the control group which likely contributed to the tendency for more cows in the RPC group to have a retain placenta. The tendencies for increased twinning and associated retained placentas would not be related to supplemental RPC because number of fetuses was established long before initiating RPC supplementation. Other health problems occurred predominantly postpartum and incidence was not different between treatments. Piepenbrink and Overton (2003) reported a larger number of cows fed higher amounts of RPC treatment seemed to have more difficult transitions, having numerically more incidence of displaced abomasum compared to the cows fed the other treatments. Xu *et al.* (1998) reported the overall incidence of health-related disorders was numerically lowest for cows fed high amount of rumen-protected lysine and methionine. Erdman and Sharma (1991) reported that the supplementation of RPC to the diet of lactating dairy cows had no significant effect on the incidence of clinical mastitis. Their result was consistent with the result of our study.

CONCLUSIONS

The treatments significantly affected services per conception and open days of lactating dairy cows, but did not affect significantly on days to first estrus and number of pregnant cows. RPM+RPC-fed cows had the lowest open days, days to first estrus and services per conception compared with other groups. The overall incidence of health-related disorders was numerically lowest for cows fed RPM+RPC. The results of this study indicate that the supplementation of ruminally protected forms of methionine and choline in the diet can be beneficial and recommendable for dairy producers, because from economical perspective, it was expected that the supplementation of rumen-protected methionine and choline in the diets of dairy cows can decrease the costs of health problems, reproductive disorders and feed because the incidence of metabolic and reproductive disorders and negative energy balance would be lower in these cows due to the improvement in health situation and reproductive performance.

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