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Effects of Rumen Protected and Unprotected Choline on Energy-Related Biochemical Metabolites of Lactating Dairy Cows

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Abstract: Eight multiparous Holstein cows with an average milk production of 34.6±2.8 kg day^{-1} and body weight of 676±79 kg were used to evaluate the effect of rumen protected and unprotected choline on energy-related biochemical metabolites of lactating dairy cows. The experimental design was a balanced change over design with 4 treatments and 4 periods of 21 days. Experimental treatments were: No Choline (NC), Unprotected Choline (UC) fed at 50 g day^{-1}, Rumen Protected Choline (RPC 25) fed at 25 g day^{-1} and Rumen Protected Choline (RPC 50) fed at 50 g day^{-1}. Rumen protected choline was blended with 0.25 kg of ground corn and fed once per day as a top dress. Blood samples from coccygeal vessels were collected on last day of each period and analyzed for glucose, triglyceride, cholesterol, blood urea nitrogen, very low density lipoprotein, low density lipoprotein and high density lipoprotein. The result shows that blood metabolites such as glucose, triglyceride, cholesterol, blood urea nitrogen, very low density lipoprotein and low density lipoprotein not affected by treatments ($p>0.05$). Blood glucose concentration tendency increased by rumen protected and unprotected choline, but wasn't statistically significant. Unprotected choline decreased concentration of high density lipoprotein than control group ($p<0.05$). Rumen protected choline had no significant effect on high density lipoprotein levels. High density lipoprotein decreased by using unprotected choline, but other blood metabolites not changed by treatments.

Key words: Choline, blood metabolites, dairy cow, lipoprotein, glucose, triglyceride

INTRODUCTION

Choline is a quasi vitamin that has a variety of functions in mammalian metabolism. Its major functions are as the predominant phospholipid contained in the membranes of all cells in the body (as Phosphatydil choline), a component of the neurotransmitter acetyl choline and as a direct presource to betaine in methyl metabolism (Donkin, 2002; Pinotti *et al*., 2002). Choline has been classified as one of the B-complex vitamins, but it does not satisfy the standard definition of vitamin: It is synthesized endogenously and there is no evidence that it is an enzyme co-factor; furthermore, unlike other water soluble vitamins. It is difficult to identify a deficiency syndrome for choline in healthy mammals because of its interrelationship with methionine, folic acid and vitamin B_{12} (Pinotti *et al*., 2002). In lactating (dairy) ruminants, dietary choline availability remains low, but the output of methylated components in milk high and methionine and other methyl group source likely to be in short supply, particularly at the onset of lactation (Pinotti *et al*., 2002). Choline plays a major role in metabolism, particularly in lipid transport. It is a lipotropic agent because of its ability to prevent or correct excess fat deposition in the liver generally arising as a result of its dietary deficiency (Kuksis and Mookerjea, 1978; Zeisel, 1988).

Impaired triacylglycerol secretion to Very Low Density Lipoprotein (VLDL) is considered a major cause of fatty liver in dietary choline deficiency (Zeisel, 1988). The composition and metabolism of lipoprotein has been described in detail elsewhere (Eisenberg and Levy, 1975). Two main types of lipoprotein are involved in plasma triacylglycerol transport, chylomicron and VLDL (Eisenberg and Levy, 1975). In non-ruminants, VLDL are mainly synthesized and secreted by the liver, whereas chylomicron and small quantities of VLDV originate in the intestine (Moore and Christie, 1984). Choline in an essential component of VLDL and cannot be substituted by other phospholipid (Moore and Christie, 1984).

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The aims of the present study were to determine the effects of rumen protected and unprotected choline on energy-related biochemical metabolites of lactating dairy cows in early lactation.

MATERIALS AND METHODS

Experimental site: This research project was conducted at the Animal husbandry of Mughufat Malek (Mashhad) and department of animal science, Gorgan University of Agriculture Science and Natural Resources, Iran.

Animals and management: Eight multiparous Holstein cows selected for the 84 days experiment. Eight days before the experiment, the cows were moved from the herd to individual tiestalls and individually fed diets as Total Mixed Ration (TMR).

Experimental design and dietary treatment: The experimental design was a balanced change over design with 4 treatments and 4 periods of 21 days (14 days for adaptation and 7 days for data collection). Experimental treatments include: No Choline (NC), Unprotected Choline (UC) fed at 50 g day^{-1}, Rumen Protected Choline (RPC 25) fed at 25 g day^{-1} and Rumen Protected Choline (RPC 50) fed at 50 g day^{-1}. The average milk production and body weight of cows before experiment were 34.6±2.8 kg day^{-1} and 676±79 kg. RPC was supplied as CapShure choline (Balchem Corporation). This product manufactured by a patented micro encapsulation technique, which protects choline from ruminal degradation, yet releases it for absorption in the small intestine. RPC contains 25% choline chloride (w w^{-1}) and was blended with 0.25 kg of ground corn and feed as a top dress. The TMR sampled weekly during the experiment and analyzed for Dry Matter (DM), crude protein, neutral detergent fiber, acid detergent fiber, ether extract, calcium and phosphorus content (AOAC, 1990). Diets ingredients and composition are shown in Table 1.

Blood sampling and biochemical analysis: Blood samples from coccygeal vessels collected in 5 mL vacuntainer tubes containing 5 mg of potassium oxalate and 5 mg of sodium fluoride on last day of each period 1 h after the morning feeding. Plasma was obtained from blood after centrifugation at 2000× g for 20 min and then stored at -20°C, until analyzed for glucose, triglyceride, cholesterol, Blood Urea Nitrogen (BUN), VLDL, Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL). For these determinations, commercial kits (Parsazmoon, Tehran, Iran) were used by an automated biochemical analyzer (Biotecnica, Target, 3000, Rome, Italy).

Statistical analysis: The data were analyzed using mixed procedure of SAS (1996) by the following model:

Table 1: Ingredients and chemical composition of diet (DM basis)

Ingredients	Amount DM (%)	Chemical	Amount DM (%)
Alfalfa hay	16	CP	17.2
Corn silage	18	RDP	11.6
Cottonseed	8	RUP	5.6
Barley	26	NE_L (Mcal kg^{-2})	1.6
Ground corn	6	NDF	34.2
Cottonseed meal	12	ADF	21.3
Soybean meal	5	NFC[3]	40.9
Sugar beet pulp	4	Ca	0.7
Wheat barn	4	P	0.5
Calcium bicarbonate	0.6	DCAD[4] (mEq kg^{-1} DM)	144.0
Vitamin and minirals[1]	0.2	-	-
Salt	0.2	-	-

[1]Contained 20,000,000 IU of vitamin A kg^{-1}, 2,000,000 IU of vitamin D kg^{-1}, 15,000 IU of vitamin E kg^{-1}; 6,000 mg kg^{-1} of Mn, 6,000 mg kg^{-1} of Zn, 2,000 mg kg^{-1} of Fe, 1,500 mg kg^{-1} of Cu, 120 mg kg^{-1} of I, 50 mg kg^{-1} of Se and 20 mg kg^{-1} of Co; [2]Net energy for lactation calculated according to NRC (2001); [3]NFC (%) = 100 - (NDF (%) + CP (%) + fat (%) + ash (%)); [4]DCAD (Diet Cation-Anion Difference); mEq kg^{-1}: ((mEq Na + mEq K) - (mEq Cl + mEq S))

$$Y_{ijkl} = \mu + T_i + P_j + A_k + R_l + e_{ijkl}$$

Where:

Y_{ijkl} = Observation
μ = Overall mean
T_i = Treatment effects
P_j = Period effects
A_k = Animal effects
R_l = Residual effects from previous treatment
e_{ijkl} = Residual error. Differences with $p \leq 0.05$ were considered significant

RESULTS AND DISCUSSION

Blood metabolites such as glucose, triglyceride, cholesterol and blood urea nitrogen did not affected by treatments ($p>0.05$) (Table 2). The role of choline in glucose metabolism is not apparent; however, reducing the severity of lipid accumulation in liver would favour hepatic gluconeogenesis (Cadorniga-Valino *et al*., 1997) and act to increase blood glucose concentrations in the absence of changes in peripheral glucose utilization. In this experiment, the main reason that choline could not change blood glucose concentration is increase in Days In Milk (DIM), because choline mostly have positive effect on blood glucose in early lactating cows, when the cows are in negative energy balance (Hartwell *et al*., 2000), but in this study cows had passed the negative energy balance stage. Relationships between blood glucose with HDL, cholesterol, triglyceride and BUN are shown in Fig. 1-4. Figure 1-4 show that relationships between glucose with other blood metabolites are negative, indeed when the blood glucose concentration increases, other blood metabolites levels similarly decreases. These findings indicate that increase in blood

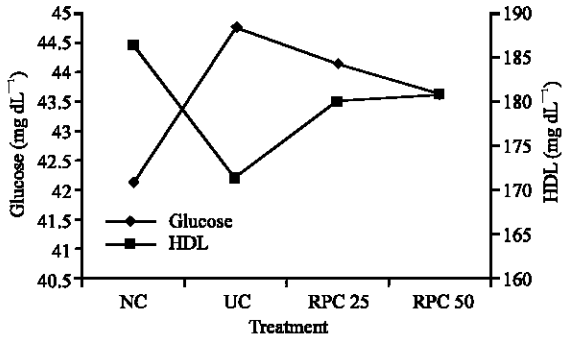

Fig. 1: Relationship between blood glucose and High Density Lipoprotein (HDL) levels. Values present the average of treatment effects. NC: No Choline, UC: Unprotected Choline, RPC 25: Rumen Protected Choline fed at 25 g day^{-1}, RPC 50: Rumen protected choline fed at 50 g day^{-1}

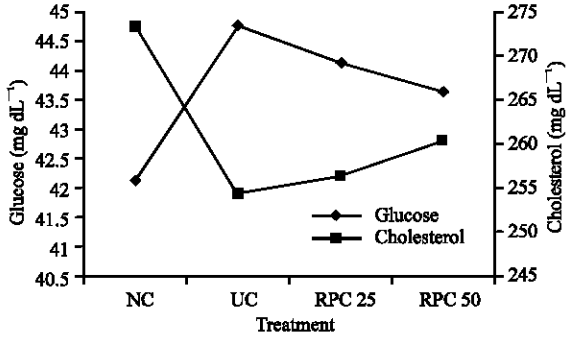

Fig. 2: Relationship between blood glucose and cholesterol levels. Values present the average of treatment effects. NC: No Choline, UC: Unprotected Choline, RPC 25: Rumen Protected Choline fed at 25 g day^{-1}, RPC 50: Rumen protected choline fed at 50 g day^{-1}

Table 2: Effects of treatments on blood glucose, triglyceride, cholesterol and BUN

	Treatments				
Item	NC	UC	RPC 25	RPC 50	SE
Glucose (mg dL^{-1})	42.12	44.75	44.12	43.62	1.70
Triglyceride (mg dL^{-1})	4.50	4.53	4.10	5.31	0.47
Cholesterol (mg dL^{-1})	273.25	254.50	256.50	260.38	7.60
BUN (mg dL^{-1})	17.12	16.75	16.00	17.25	0.40

Values with a different superscript in the same row are significantly different ($p<0.05$)

glucose concentration can reduce body fat mobilization and liver triglyceride accumulation. Inadequacy of choline supply is manifested by decreased concentrations of choline, betaine, phosphatidylcholine, methionine and S-adenosyl methionine and increased triacylglyceride concentrations in the liver (Pomfret *et al*., 1990). Deficiencies lead to reductions in circulating lipoprotein

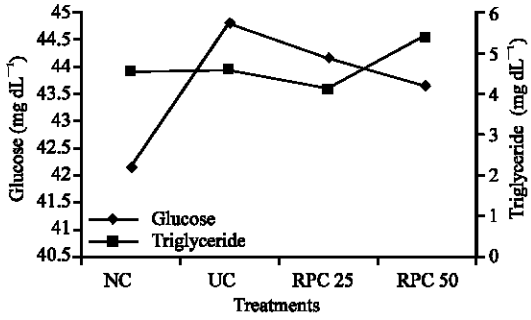

Fig. 3: Relationship between blood glucose and triglyceride levels. Values present the average of treatment effects. NC: No Choline, UC: Unprotected Choline, RPC 25: Rumen Protected Choline fed at 25 g day^{-1}, RPC 50: Rumen protected choline fed at 50 g day^{-1}

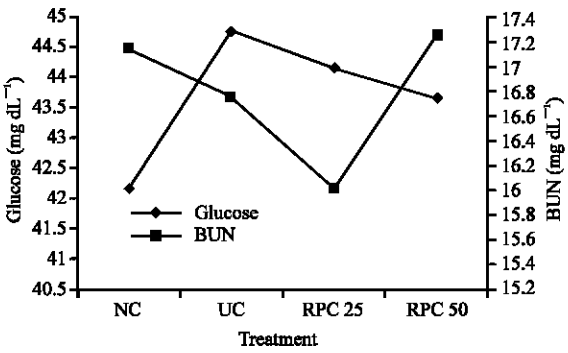

Fig. 4: Relationship between blood glucose and Blood Urea Nitrogen (BUN). Values present the average of treatment effects. NC: No Choline, UC: Unprotected Choline, RPC 25: Rumen Protected Choline fed at 25 g day^{-1}, RPC 50: Rumen protected choline fed at 50 g day^{-1}

as a direct result of impaired secretion by liver (Lombardi *et al*., 1968). Choline-deficient rats show threefold increase in hepatic triacylglyceride concentrations and reduced plasma methionine and phosphatidylcholine concentrations compared with controls (Pomfret *et al*., 1990; Yao and Vance, 1990). Fatty liver is a metabolic disorder that affects up to 30% of high producing periparturient cows and is frequently associated with impaired health, infertility and compromised milk production (Reid and Collins, 1980). Indeed in the experiment, blood TG wasn't changed, but tendency increased, when the cows fed with RPC, which is agree with the result of Erdman *et al*. (1984) that reported in their experiment blood serum TG did not affected by choline, whereas had a low increase in blood serum TG.

Table 3: Effects of treatments on blood VLDL, LDL and HDL

	Treatments				
Items (mg dL^{-1})	NC	UC	RPC 25	RPC 50	SE
VLDL	0.90	0.91	0.82	1.06	0.10
LDL	85.97	82.15	75.22	78.00	1.99
HDL	186.37[a]	171.25[b]	180.00[ab]	180.87[ab]	1.54

Values with a different superscript in the same row are significantly different ($p<0.05$)

Cholesterol concentration of blood was similar between treatments ($p>0.05$) and treatment without choline and unprotected choline had high and low amount of cholesterol, respectively. Bindel *et al*. (2000) observed numerical decreases in plasma NEFA in response to choline supplementation, but no response in plasma cholesterol, glucose, or insulin. The response of dairy cattle to supplemental choline has been attributed to its role as a lipotropic agent that can play a valuable part in decreasing liver adiposity, which is frequently observed in the periparturient period. Indeed, liver fat content has been shown to decrease numerically in response to choline supplementation in periparturient cows (Piepenbrink and Overton, 2003).

Table 3 shows that treatments had no any significant effect on VLDL and LDL concentration ($p>0.05$), but Unprotected Choline (UC) decreased HDL concentration than control group ($p<0.05$). Rumen protected choline had no significant effect on high density lipoprotein levels. As in nonruminant species, esterified triglyceride can be export from the liver as VLDL, but the rate of this process is limited in ruminants compared with other species (Grummer, 1993). Because phosphatidylcholine is required for VLDL assembly, the lake of sufficient dietary choline supply, compared with the increased demand for met for milk synthesis could render choline a limiting substrate for VLDL synthesis. This conditional deficiency of choline would further slow the rate of triglyceride export from liver, which could contribute to the development of fatty liver and limit milk production (Hartwell *et al*., 2000). In the experiment, RPC increased blood VLDL concentration, but this amount wasn't significant between control and RPC groups.

CONCLUSION

In lactating dairy cows, supplementing unprotected choline has significant effect on high density lipoprotein, but other blood metabolites not changed by treatments. The important result we found in this experiment that using rumen protected choline in mid-lactating dairy cows doesn't have significant effect on energy-related biochemical blood metabolites.

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