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Original Article

The Influence of Rumen-Protected Choline and α-tocopherol Supplementation on Early Lactating Dairy Cows Metabolism

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Abstract

It is well documented that choline is known as one of the essential ingredients of phospholipids. Choline acts as a determinative element for appropriate cell membrane functions. On the other hand α-tocopherol (Vit E) is a fat-soluble vitamin. This vitamin acts as a strong antioxidant in the living body's defense system against oxidative stress. Lipid peroxidation in peripartum and early lactating cows is significantly increased while the level of serum Vit E is decreases dramatically. These concomitant physiological changes demonstrate a higher level of oxidative stress subsequently leads to serious health issues in dairy cows. Therefore, the present research was designed to investigate the following items in dairy cattle: 1) evaluation of the possible changes in serum protein fractions, and 2) comparing the oxidative status of orally RPC and vitamin E supplementation in dairy cows in early lactation period. In the current study 30 early lactating primiparous and multiparous Holstein cows (body condition score (BCS)=2.51 ± 0.10) were used beginning five weeks postpartum. All the animals were randomly divided in to three groups (n=10) (number of lactation=2.61). The animals were randomly assigned to receive one of the following treatments. Group 1 served as control group were not received any supplement. The second group was supplemented with 90 g/d of RPC (Reashre Choline, Balchem, USA). The third group was administrated 4400 IU/d vitamin E (Roche, Vitamins Ltd; Switzerland). In the current study, serum protein electrophoresis showed four main fractions as follows: albumin, α-globulin, β-globulin, and γ-globulin. The recorded data showed that the percentages of albumin and γ-globulin fractions were higher in treated groups compared to the control group. In the animals supplementing with RPC and vitamin E the percentages of serum albumin increased to the value of 37. 70±1.63 and 38.21±1.28 respectively compare to the control group (34.69±1.21), which were significant *(P<0.05)*.

Keywords: electrophoresis, serum proteins, RPC, vitamin E

1. Introduction

It is well documented that the blood proteins, excluding the γ-globulin fraction, are mainly synthesized in the liver (1). Electrophoresis of the protein content of a normal plasma or serum consequently leads to identifying two important protein fractions called albumin and globulin. The relative amount of these proteins is significantly linked to the animal species, for instance in laboratory animals (guinea pigs, rats, rabbits), humans, sheep, and goats the albumin level is higher than globulin. While in cows the ratio of albumin/globulin is almost equal (2). As well as animal species some other physiological and environmental factors such as animal age, gender, body mass index (BMI), endocrine status, lactation, pregnancy, ambient temperature, photoperiod, nutritional state, and estrus condition have significant effects on the level of serum protein (3, 4). Different disease and health complication conditions lead to a significant reduction in the level of serum albumin. On the other hand, the level of α-globulin in serum samples obtained from the injured animals with traumas significantly increased, also the metabolism alterations in the case of lipoprotein metabolism lead to the changes in the level of serum β-globulin fraction.

However, since there have been several factors that can be responsible for the changes in the levels of serum proteins, it seems impossible to recognize all these responsible effectors (5, 6). The lactation period has been known as one of the most anabolic and challenging conditions in the life cycle of female animals. During this important period, protein plays a pivotal role as an essential ingredient for milk production.

The classical and conventional assays based on the electrophoretic methods for serum protein fraction measurements are as follows: protein separation on agarose gel, and cellulose acetate membrane. By the way, capillary zone electrophoresis (CZE) has been proposed as the preferred technique for separation and quantification of serum proteins (7). In this method, the segregation of proteins and other charged molecules is performed in a capillary tube filled with a special buffer solution with the aid of high voltage electrical power (8).

Thiols have been known as one of the most potent reducing authoritative factors acting as antioxidants (9). Antioxidant status, especially the thiol status, is considered one of the most prominent factors for normal body function and physiology. It has been well documented that the possible changes in thiol status lead to induction of programmed cell death (Apoptosis) (10), this situation has been apperceived in some diseases such as renal failure and vascular disease (9).

During the respiratory oxidation procedure free radicals, oxidant agents, are produced. These oxidative elements can harm different macromolecules such as nucleic acids, fat, and proteins which can consequently lead to serious health problems (11). The natural body defense system which hampers the lesion of free radicals and neutralizes them have been known as total antioxidant capacity (TAC) (12).

Phospholipids are known as the main components of living cells. Choline is known as one of the essential ingredients of phospholipids. This acts as a determinative element for appropriate cell membrane functions. Also, choline plays a pivotal role in lipids transport in the blood. The proper blood lipid transport is considered an essential element in the prevention of fatty liver and ketosis in the lactating dairy cow. Therefore, fortifying diets with the choline supplement may ameliorate the lipids metabolism and transport in the blood and consequently reduce the peril of ketosis and fatty liver disorders. Unfortunately, one of the difficulties in providing dietary choline is the rumen microbial degradation of choline (13). Therefore, the rumen-protected form of choline (RPC) must be used in the diet formulation. It is well documented that choline is essential for tissue metabolism (14). In a study on dairy cattle, conducted by Cooke (25) it is established that the RPC consumption could hinder

fatty liver disorder induced by feed restriction (15). α-tocopherol also known as vitamin E (Vit E) is a fat-soluble vitamin. This vitamin acts as a strong antioxidant in the living body's defense system against oxidative stress (16). Therefore, Vit E can ameliorate the oxidative stress and improve the health of dairy cows (17). In the same way as absorption and metabolisms of lipids, Vit E is absorbed from the intestine and transported to the liver. In the liver, α-tocopherol is wrapped in lipoproteins and via blood plasma, spread out from the liver to the other tissues of the living body (18). The consumption of diet formulation which is fortified with Vit E seriously leads to the dairy cows' health betterment (19, 20). Lipid peroxidation in peripartum and early lactating cows is increased significantly (21, 22) while the level of blood serum Vit E is decreases dramatically (23). These concomitant physiological and metabolic changes demonstrate a higher level of oxidative stress which subsequently leads to serious health issues in dairy cows (24). Previously published works in cattle, mice, and rats (35, 36) showed that Vit E plays a key role in recovering from postpartum-related oxidative stress and alleviating the lipid peroxidation which occurred in liver (25, 26). In a study conducted by Soltys (27), it was shown that Vit E supplementation could improve the liver antioxidant capacity and the TAC in fatty liver induced mice. There have been a few published works investigating the role of Vit E supplementation on liver metabolism and the incidence of metabolic disorders in dairy cattle.

Therefore, the present research was designed to investigate the following items in dairy cattle: 1) evaluation of the possible changes in serum protein fractions, and 2) comparing the oxidative status of orally RPC and vitamin E supplementation in dairy cows in early lactation period. The expected results may demonstrate a contribution to a better comprehension of biochemical processes in early lactating cows.

2. Materials and Methods

2.1. Animals

In the current study 24 early lactating primiparous and multiparous Holstein cows (body condition score (BCS)=2.51 ± 0.10) were used beginning five weeks postpartum. The animals were healthy, with a normal appearance, and housed in individual tie stalls. The animals' selection criteria were based on parity, previous lactation milk yield, and BCS.

2.2. Study Design

All the 24 animals were randomly divided in to three groups (n=8) (number of lactation = 2.61). The animals were randomly assigned to receive one of the following treatments. Group 1 served as control group were not received any supplement. The second group was supplemented with 90 g/d of RPC (Reashre Choline, Balchem, USA). The third group was administrated 4400 IU/d vitamin E (Roche, Vitamins Ltd; Switzerland). The animals were fed total mixed rations (TMR) *ad libitum*. The diet formulation was based on the nutrient requirements of dairy cattle using the National Research Council (NRC) recommendation as shown in table 1 (28). The RPC and Vit E were top-dressed onto the TMR.

2.3. Blood Sampling

Before the morning meal on the last day of the experiment, the blood samples were obtained from the coccygeal vein (tail vein) in heparinized and non-heparinized Vacutainers (Becton Dickinson, Franklin Lakes, NJ) tubes. Following the blood sampling, all the samples were placed on ice immediately.

2.4. Measurement of Total Antioxidant Capacity and Plasma Total Thiol Molecules (TTM)

The FRAP test (ferric reducing antioxidant power) was used for plasma TAC evaluation according to the previously described method by Benzie (29).

As previously described by Hu and Dillared (30) total sulfhydryl content was determined in plasma.

2.5. Capillary Zone Electrophoresis

The Capillary system (Sebia, Issy-les-Moulineaux, France) was operated according to the manufacturer's instructions under software version 1.4.1.

2.6. Statistical Analyses

Statistical analyses were performed using SAS. All dependent variables and their residuals were evaluated for normality using Proc GLM procedure by inspection of standardized residuals plotted against the predicted residuals. Significant level were declared at *P<0.05.*

Table 1. Ingredients and nutrient composition of the diet

Ingredient (g/kg of DM)		Composition	
Alfalfa hay (medium chopped)	204.7	DM (g/kg)	590
Corn silage	175.8	CP (g/kg)	171.7
Beet pulp	41.3	Ash (g/kg)	57.6
Ground barley grain	198.8	Total fat (g/kg)	43.8
Ground corn grain	58.7	NDF (g/kg)	304.8
Ground wheat grain	28.5	ADF (g/kg)	183.8
Solvent extracted soybean meal	79.9	NFC ᶜ (g/kg)	382.1
Wheat bran	7.1	Ca (g/kg)	8.1
High lint whole cottonseed	29.5	P (g/kg)	5.0
Canola meal	100.2	NEL (Mcal/kg)	1.66
Corn gluten meal	11.5	RUP (g/kg of CP)	314.5
Fat supplement (energy booster)	15.9	RDP (g/kg of CP)	685.5
Minerals and vitamins supplement	6.3	Met (g/kg MP)	22.1
Salt	2.5	Lys (g/kg MP)	76.9
Calcium carbonate	3.2		
Sodium bicarbonate	10.2		
Di calcium phosphate	3.7		
Magnesium oxide	1.9		
Mycosorb	0.6		
Biotin premix	0.7		
Zeolit	19.0		

3. Results

In the current study, serum protein electrophoresis showed four main fractions as follows: albumin, α-globulin, β-globulin, and γ-globulin. The percentages of the measured serum albumin and globulins fractions of the animals are presented in table 2. The recorded data showed that the percentages of albumin and γ-globulin fractions were higher in treated groups compared to the control group. In the animals supplementing with RPC and vitamin E the percentages of serum albumin increased to the value of 37. 70±1.63 and 38.21±1.28 respectively compare to the control group (34.69±1.21), which were significant *(P<0.05)*. Whereas the treatments did not affect different fractions of globulin significantly, but there were numerical increases in the percentages of γ-globulin in both treated groups (Table 2).

The recorded data in the current study showed that the amounts of TTM in treated groups, RPC (1.45±0.67) and Vit E (1.52±0.98) were affected significantly compared to the control group (1.24±0.76) (*P<0.05*) (Table 3). While the results revealed that RPC and Vit E did not affect TAC significantly (*P>0.05*). The recorded data for TTM and TAC are tabulated in table 3.

Table 2. Serum protein fractions

Protein Fraction	Control	RPC	Vit E
Albumin	34.69±1.21^{b}	37. 70±1.63^{a}	38.21±1.28^{a}
α-globulin	14.21±1.11^{a}	13.34±1.01^{a}	12.60±1.18^{a}
β-globulin	9.31±1.43^{a}	7.11±1.00^{a}	6.52±0.98^{a}
γ-globulin	33.65±1.87^{a}	33.86±1.21^{a}	34.64±1.32^{a}

^{ab} different superscripts are indicating significant differences (P<0.05)

Table 3. The amount of TTM and TAC in early lactating cows supplementing with RPC and vitamin E

	Control	RPC	Vit E
TTM	1.24±0.76[b]	1.45±0.67[a]	1.52±0.98[a]
TAC	0.115±0.01[a]	0.126±0.08[a]	0.130±0.06[a]

[ab] different superscripts are indicating significant differences (P<0.05)

4. Discussion

During the lactation period, dairy cattle tolerate several physiological alterations. All these changes take place in relation to the animals' blood composition in case of alteration in metabolites levels and transport. In reality, lactating animals adapt their body metabolism and physiology to furnish sufficient sources of nutrients for milk production (31).

The recorded data in the current study revealed that the serum proteins in the treated animals were separated into 4 major fractions such as albumin, α, β, and γ globulin. The results of a study conducted by Saun indicated that the albumin is associated with postpartum diseases, they mentioned that the level of albumin in the serum of the early lactating animal could be used for prediction of disease (32). Despite the concerns regarding variables befuddle albumin altered level interpretation, it acts as a proper indicator for some metabolic diseases in early lactating cattle with an ability to reflect the availability of amino acids from the labile protein reservoirs. The results of the current study revealed that supplementation of RPC and Vit E had significant effects on level of albumin in the serum samples of the treated animals. Some beneficial effects of RPC and Vit E were related to the betterment of the liver function, on the other hand, it might be a consequence of the induction of insulin secretion, which lowers plasma NEFA concentration via the reduced lipolysis. The insulin secretion is induced by the increased level of glucose. Consequently, the fat metabolization from adipose tissue was suppressed and the amount of NEFA was significantly reduced. Hence the liver load for NEFA oxidation is reduced, and the fat accumulation decreased in the liver. Commonly, in the early lactating cows, the blood NEFA content originating from adipose tissues increased significantly due to negative energy balance (33).

Reduction in the amount of NEFA and triglycerides in the blood serum due to choline administration leads to the betterment in the liver health condition. It is well documented that choline act as a donor of the methyl group and exerts its beneficial effects through the betterment of lipid metabolism.

A study conducted by El-Shahat in ewes showed that supplementation of Vit E leads to the increment in the levels of albumin, globulin, and total serum protein (34). Similarly, Helal showed that Vit E administration in buffaloes leads to the increment in the levels of albumin, globulin, and total serum protein (35).

The results of a study conducted by Deihl showed that Vit E deficiency in rabbits leads to a reduction in the levels of serum albumin and a dramatic increase in the globulin levels, while the total protein concentration remains constant (1). Some previously published research showed that the blood metabolites such as albumin and globulin in cows and goats were not affected by choline supplementation (36-40).

Authors' Contribution

Study concept and design: Y. S. K. and A. D. H.
Acquisition of data: S. K. H. and I. S. H.
Analysis and interpretation of data: A. A. A. and Z. H. A.
Drafting of the manuscript: A. A. B. and H. A. L.
Critical revision of the manuscript for important intellectual content: M. A. Y. and H. A. K.
Statistical analysis: S. K. H.
Administrative, technical, and material support: Y. S. K.

Ethics

All the steps of this study were performed in accordance with the guidelines for the use and care of animals and approved by the animal ethical committee

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Conflict of Interest

The authors declare that they have no conflict of interest.

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