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THE EFFECT OF ORAL ADMINISTRATION OF CHOLINE ON SOME LIVER FUNCTION CHARACTERIZED BLOOD PLASMA ENZYMES OF EARLY LACTATING DAIRY COWS

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Sixteen early lactating primiparous and multiparous Holstein cows were used for four weeks to investigate the effects of feeding choline on the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and γ-glutamyltransferase (GGT) in the plasma of the cows during early lactation period. Cows were randomly assigned to one of the following treatments: no supplement (control) and 90 g/d of rumen-protected choline (RPC) product. Treatments did not affect significantly ALT, AST and GGT during early lactation period, but for ALP there was a significant difference between the groups. The research results showed significant influence of feeding protected choline on ALP.

AST – ALT – ALP – GGT – lactating cows – blood plasma

Առաջնածին և բազմածին Հոլշտեյն ցեղի 16 կովերի վրա ուսումնասիրվել է կտրիչի ազդեցությունից պաշտպանված խոլինի ազդեցությունը լյարդի ֆունկցիան արտահայտող արյան պլազմայի ասպարտատ- և ալանինտրանսամինազ (ԱՍՏ, ԱԼՏ), հիմնային ֆոսֆատազ (ՀՖ) և γ-գլյուտամիլտրանսֆերազ (ԳԳՏ) ֆերմենտների ակտիվության վրա: Կովերը բաժանվել են 2 խմբերի՝ I — ստուգիչ (առանց հավելումի), II — փորձնական (օրական ստացել են 90գ խոլին) վաղ լակտացիայի շրջանի 4 շաբաթվա ընթացքում: Խոլինի կերակրումը վաղ լակտացիայի շրջանում չի ազդում արյան պլազմայի նշված ֆերմենտների ակտիվության վրա, բացի ՀՖ-ից, որի ակտիվությունը հավաստիորեն նվազում է:

ԱՍՏ —ԱԼՏ —ՀՖ, ԳԳՏ —կաթնաբեր կովերը — արյան պլազմա

В опытах были использованы 16 первотелок и многократно рожавших коров породы Голштейн, которым в ранний период лактации вскармливали холин в течение 4-х недель. Коровы были разбиты на 2 группы: I-контрольная (без добавок), II-получавшая 90 г холина в день. В плазме крови определяли активность аспартат- и аланинтрансаминаз (АСТ и АЛТ), щелочной фосфатазы (ЩФ) и γ-глутамилтрансферазы (ГГТ) в ранний период лактации. Добавка холина не отражалась на активности трансаминаз и ГГТ, однако вызывала статистически достоверное уменьшение активности ЩФ в ранний период лактации.

АСТ – АЛТ – ЩФ – ГГТ - лактирующие коровы - плазма крови

In the higher standards of milk production, the priority in modern breeding is keeping dairy cows in high lactation and healthy. The control of their feeding and metabolic status is important for the health of the herd. The priority for high milk production is prevention of metabolic diseases and other disorders. The test of metabolic profiles by analyzing biochemical parameters in the blood of dairy cows is a significant contribution to veterinary medicine [10]. Determining some enzyme activities in blood would be useful in identifying liver function including: aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) and γ-glutamyl transferase (GGT).

Aspartate aminotransferase (AST) is a widely distributed enzyme, which is found in many tissues and organs, with high activity in liver [17]. Increased AST activity is a sensitive marker of fatty liver [6], and appearance of ketosis in dairy cows during early lactation [12]. Unlike AST, horse, pig, and ruminant liver cells do not show high ALT activity, and the increased activity of that enzyme during liver damage, even in necrosis, is insignificant [4].

Alkaline phosphatase (ALP) belongs to hydrolases which causes hydrolysis of monoesters of phosphoric acid [2; 8] and is known as cholestatic liver enzyme as well. It is localized mainly in the cellular membrane of hepatocytes [15]. ALP activity increases in case of hepatitis and biliary disorders [2].

γ-glutamyltransferase (GGT) is a membrane-bound enzyme in organs with emphasized functions in secretion and resorption. In the plasma it is significant as a sign of hepatobiliary system diseases connected with cholestasis and is used in diagnosing liver disease. Its activity is relatively high in livers of cows, horses, sheep and goats [13].

Choline is an essential nutrient involved with the transport of fat from the liver and required for the synthesis of phosphatidylcholine, a phospholipid found in the membranes of very low density lipoprotein that transport lipids in the blood [9]. Hence, choline deficiency in lactating cows may be associated with hepatic lipidosis. This transport of lipids in the blood is an important factor in preventing fatty liver disease and ketosis. So providing supplemental choline may improve the transport of lipids, thus reducing the risk for ketosis. Because most dietary choline is degraded in the rumen by microbial populations [11], and not much is available for absorption; therefore, choline must be rumen protected when fed. Cooke et al. (2007) reported that RPC can prevent and alleviate fatty liver induced by feed restriction in dairy cattle [3].

The aim of this study was to determine the effect of feeding supplemental choline on the activity of liver enzymes including AST, ALT, ALP and GGT in the blood plasma of early lactating cows.

Materials and methods. **Cows, treatments and experimental design.**

Sixteen early lactating primiparous and multiparous Holstein cows were used from October 2011 to November 2011 in our study beginning five weeks postpartum.

Cows were housed in individual tie stalls and cared for under experimental procedures and protocols approved by the veterinary organization of Iran. Selection of cows was based on parity, milk yield of previous lactation (milk yield of dams for the cows in their first lactation) and body condition score (BCS). Eight cows per treatment (number of lactation = 2.56; mean) were randomly assigned to receive one of the following treatments: no supplement (control), and 90 g/d of rumen-protected choline (RPC) product. The RPC product (Reashure Choline, Balchem, USA)) is a rumen-protected source of choline chloride. Reashure choline is produced by encapsulating choline chloride with a coating matrix able to resist rumen breakdown and release choline in the intestine and contained 0·25 choline. Cows were fed a total mixed ration (TMR) *ad libitum*. The meal was adjusted to production intensity, and consisted of ordinary alfalfa hay, silage, beet pulp, and concentrates (including barley, corn, canola meal, cottonseed, wheat bran, cottonseed meal, wheat grain, corn glutton meal, soybean meal, sodium bicarbonate, fat meal, limestone and vitamin-mineral supplement). The RPC was top-dressed onto the TMR.

Blood sampling and statistical analysis

Blood samples were obtained before morning meal from the coccygeal vein on the last day and then were collected in heparinized Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). Blood samples were placed on ice immediately following collection. Plasma was harvested after centrifugation of the blood at 3000 g for 15 min. Plasma was stored at −20 °C until subsequent analysis for aspartate aminotransferase (AST), alanin aminotransferase (ALT), alkalin phosphatase (ALP) and Gamma glutamyl transferase (GGT). AST, ALT, ALP and GGT activities were measured on “BT 1500 auto-analyzer” through spectrophotometer method, using kits produced by “Farasamed Co, Ltd. Tehran, Iran.

Statistical analysis of the 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. Significant level were declared at $P<0.05$.

Results and Discussion. The effects of feeding supplemental choline on liver enzymes of lactating dairy cows are shown (in tab. 1). Feeding RPC affects plasma AST, ALT and ALP during the early lactation period. The measured activities showed a decrease of the tested enzyme activity values in the blood plasma of the groups which received choline comparing to the control group. The effect was significant for ALP ($p<0{\cdot}05$), and insignificant for AST and ALT ($p<0{\cdot}05$). Therefore, ALP activity in the blood plasma differs between the groups significantly. There was a decrease of the tested enzyme activity values in choline group compared to the control group.

Table 1. The effects of supplemental choline on plasma metabolites of lactating cows

	Control	Choline	SEM	P-Value
AST (units/l)	74.00	71.50	3.82	0.65
ALP (units/l)	60.62 [a]	42.87 [b]	3.22	0.0016
ALT (units/l)	40.25	38.25	2.98	0.64
GGT (units/l)	29.00	26.50	1.26	0.18

AST, aspartate aminotransferase; ALP, alkaline phosphatase; ALT, alanin aminotransferase; GGT, Gamma-glutamyl transferase

The early lactation period is when liver function is most likely to become impaired because of triglyceride infiltration into hepatocytes; therefore, higher levels of the liver enzyme in early lactation period may be indicative of reduced liver function [5]. Modern milk production often puts the production capabilities of cows at risk, which can result in metabolic disorders. Lactation has a great impact on biochemical parameters in blood of cows. The activity of aminotransferases in blood is very important. Aminotransferases act as a catalyst in connecting the metabolism of amino acids and carbohydrates. Accordingly, changes in their activity in the blood can be a consequence of their increased activity in cells (primarily liver), but also a reflection of cell structure damage.

Some liver enzymes are included on most routine laboratory tests. They are aspartate aminotransferase (AST), and alanine aminotransferase (ALT), which are known as transaminases; and alkaline phosphatase (ALP), and Gamma glutamyl transferase (GGT) which are known as cholestatic liver enzyme. Elevations of these enzymes can indicate the presence of liver dysfunction.

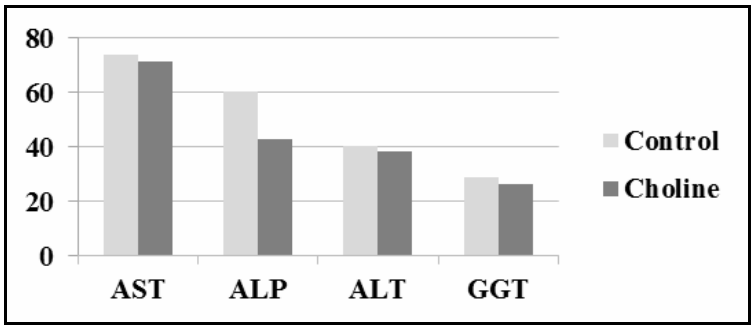

Fig.1. The effect of supplemental choline on plasma metabolites of lactating cows

In our study, the activity of AST, ALT, ALP and GGT for the control group was higher than those of choline group (fig 1). But it was merely significant for ALP. Therefore, elevated levels of ALP in the control group indicates that there was something wrong with the liver [7].

In this study, feeding RPC did not reduce concentration of plasma AST, ALT and GGT significantly. In comparison, other researchers reported that the concentration and the activity of AST, ALT and GGT in cows and goats were not affected by choline supplementation [1, 14, 16].

Blood was taken in the morning. Cows were clinically healthy and in good production capability, so we consider that the obtained results are reflections of metabolic events. We can assume that the difference of enzyme activity in blood per certain group arises from the increased or decreased activity of these enzymes in cells.

In conclusion, feeding RPC decreased ALP significantly. Lower ALP suggests that supplemental RPC can improve liver function of dairy cows.

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