

# The relationships between milk production and some blood metabolites and their effects on returning to estrus in lactating Holstein dairy cows

Fallahnezhad Anarjan, N<sup>1</sup>.; Moghaddam, G.A<sup>2\*</sup>

*1 Department of Animal Science, Islamic Azad University, Shabestar Branch, Shabestar, Iran*

*2 Department of Animal Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran*

*\*Corresponding author: [ghmoghaddam@tabrizu.ac.ir](mailto:ghmoghaddam@tabrizu.ac.ir)*

## Abstract

In this study, the relationships and correlations between milk production and some blood metabolites related to energy and protein balance and their effects were investigated on returning to estrus after AI in lactating Holstein dairy cows. Blood and milk samples were collected from sixty lactating Holstein dairy cows based on their reproductive status in 13-17 d post AI. BUN, MUN, glucose, triglyceride, cholesterol and BHBA were measured by enzymatic colorimetric method and blood progesterone concentration was measured by ELISA method. Between 18-25 d post AI, 27 cows returned to estrus (RE group), but 33 cows did not (NRE group). In RE group, there were significant correlations between DIM and Monthly Milk yield Record (MMR) ( $p < 0.05$ ), BUN ( $p < 0.01$ ) and glucose ( $p < 0.01$ ). Furthermore, MMR was correlated with BUN ( $p < 0.05$ ), MUN ( $p < 0.05$ ), glucose ( $p < 0.05$ ) and cholesterol ( $p < 0.05$ ). In addition, BUN in this group was correlated with MUN ( $p < 0.01$ ) and glucose ( $p < 0.05$ ) and the correlation between glucose and BHBA ( $p < 0.05$ ) was significant. In NRE group, there were significant correlations between DIM and MMR ( $p < 0.01$ ) and between MMR and glucose ( $p < 0.05$ ). Also parity was correlated with glucose ( $p < 0.05$ ). BUN and MUN concentrations correlation was also significant ( $p < 0.05$ ). Except progesterone concentrations ( $p < 0.01$ ), there was not any difference between the mean of the two groups variables. In conclusion, the result of this study suggested that in animals returned to estrus after AI and failed to continue pregnancy, there were significant correlations between milk yield and some blood metabolites (BUN, MUN, glucose, triglyceride, cholesterol and BHBA) related to physiological imbalance. In animals that continued pregnancy and did not return to estrus, there was not any significant interpreted correlation between the variables. Therefore, despite adverse effect of negative energy balance on physiological imbalance and blood metabolites, progesterone concentration had the key role in stability of the pregnancy.

**Keywords:** blood metabolites, milk production, physiological imbalance, reproduction

## Introduction

Genetic selection programs, based solely on increased milk production, have resulted in cows that are genetically predisposed to a greater degree of Negative Energy Balance (NEB) in early lactation. The NEB occurs because increased Dry Matter Intake (DMI) accounts for only about half of the milk yield response to selection. Additional substrate required to support milk synthesis is provided through enhanced mobilization of adipose reserves and skeletal muscle (Veerkamp, 1998).

An antagonistic relationship exists between genetic merit for milk yield and reproduction, with increasing NEB in early lactation being cited as an underlying causal factor (Jorritsma et al., 2003; Pryce et al., 2004). Failure to establish a successful pregnancy may arise from failure of cows to exhibit estrus, failure to establish an appropriate pattern of ovarian steroidogenesis and follicular growth, or from embryo mortality (Royal et al., 2000). Increasing severity of NEB in early lactation is associated with impaired ovarian function and delayed resumption of estrous cycles (Jolly et al., 1995).

Recently, in an extensive point of view, NEB and other metabolic and hormonal imbalances were proposed as Physiological Imbalance (PI). PI is defined as cows whose physiological parameters deviate from the normal and consequently have an increased risk of developing production diseases (clinical or subclinical) and reduced production or reproduction (Ingvartsen, 2006). Reducing the degree of PI in individual cows causes to reduce the risk of disease and, thereby, improve production and reproductive performance (Ingvartsen, 2006; Moyes et al., 2010).

Estimation of energy status under field conditions is difficult, because energy content of the feed depends highly on environmental factors, such as climate, processing, and storage. Furthermore, estimates of DMI are inaccurate because there is variation with physiological state of the individual cow, ambient temperature, photoperiod, feeding strategy, and forage quality (Allen, 2000; Ingvartsen & Andersen, 2000). In addition, digestibility of feed and of energy-yielding substances and the efficiency of their further use depend on many factors. Therefore, assessments of energy ingested at the animal level might provide more reliable information. Studies have shown that some metabolites that characterize NEB are associated with reduced fertility (Walsh et al., 2011). Several reviews (Ingvartsen et al., 2003; Ingvartsen, 2006; LeBlanc, 2010) identified plasma NEFA, BHBA, and glucose as the major metabolites that relate to degree of PI. However, evaluating the benefits of including other variables in blood, BUN or MUN, cholesterol and triglycerides in the index for PI to improve the use of PI as a risk factor for disease and reproductive failure is warranted. (Bjerre-Harpøth et al., 2012; Moyes et al., 2013) Previous studies have focused solely on the effect of NEB or protein nutrition on reproductive performance; but, in this study, the effect of both energy and protein

related to metabolites on returning to estrus and pregnancy status of lactating Holstein dairy cows was investigated. Therefore, the objectives of this study were 1) to determine the relationships and correlations between some blood metabolites that, based on previous studies, are related to energy and protein balance and 2) to determine their effects on insemination and pregnancy failure in high producing lactating dairy cows.

In the same vein, the hypotheses were as follows 1) there is a correlation between milk production and protein and energy balance related metabolites and 2) there is a difference between mean of these metabolites in different groups based on their reproductive statuses.

## **Material and methods**

### *Farm management*

A commercial dairy farm in North West of Iran, Tabriz, with 1300 lactating Holstein cows was used in this study. Cows were housed in a free stall system and fed a TMR. The same diet was fed to all milking herds, except cows in their first 20 days postpartum, which were fed a ration containing additional effective fibers. Cows were milked 3 times daily (at approximately 05:30, 13:30, and 21:30 o'clock) and fed following each milking. To determine the diet chemical composition, 5 random samples were collected (approximately every 3 to 4 weeks) during the experimental period and were analyzed in feed analysis laboratory. Samples prepared for analysis (according AOAC 2000, method 934.01) and Dry Matter, Crude Protein (according AOAC 2000 method 976.5 by Kjeldatherm, Gerhardt; Germany) Etheric Extract (according AOAC 2000, method 920.39 by Soxtherm, Gerhardt; Germany) Crude Fiber (according AOAC 2000, method 969.09) Ash and Nitrogen Free Extract were analyzed. Results are shown in Table 1. Reproductive management consisted of a

voluntary waiting period of 60 days. After that, the cows were identified in estrus by visual observation. Pregnancy diagnosis was performed by herd veterinarians by touché rectal method approximately 56 to 63 days after insemination.

#### *Experimental animals*

Sixty Holstein dairy cows (19 at first, 16 at second, 10 at third and 15 at fourth and higher Parity) were used in the study. They were at 70- 120 DIM. Based on reproductive status blood and milk samples were collected during 4 months (August to December) from the cows that, were in 13-17 days post AI, , approximately at 10:00 o'clock after milking and feeding. Between 18-25 days post AI cows that returned to estrus (n=27) were named "Returned to Estrus" (RE) group and those that did not return to estrus (n=33) were named "Non-Return to Estrus" (NRE) group.

#### **Sampling and measurements**

Blood samples were collected from tail vein at approximately 1000h (after milking and feeding time) by evacuated Venoject tubes (without anti coagulant), placed on Ice, carried to the laboratory and centrifuged (15Min at 3500 rpm) and reserved in 2 cc micro tubes at -20 c°. At the same time, milk samples were collected from one cartier by 50cc Falcon capped vials, placed in +4 c° refrigerator and carried to the laboratory. Then, the milk serum was separated by TCA (Tri Chloro Acetic Acid) method and reserved at -20 c°. Blood serum glucose, urea, triglyceride, cholesterol and milk serum urea were measured by enzymatic colorimetric method with spectrophotometer (Shimadzo; Japan) and Ziest Chem kits. Blood serum Beta Hydroxy Butiric Acid (BHBA) was measured by Ultra Violet (UV) method with spectrophotometer and Ranbut kits (RANDOX

laboratories; UK, RT294QY). Blood serum progesterone was measured by ELISA method with ELISA Reader (Anthos 2020; Salzburg, Austria) and DRG ELISA kits (EIA-1561 DRG Instruments GmbH, Germany, 18, D-35039 Marburg).

#### **Statistical analysis**

One of the objectives of this study was to determine the relationships between variables that, based on previous studies, are related to energy and protein balance. For this propose before correlation analysis, the measured data were tested for skewness and kurtosis. In spite of the significant test results, there was no difference in regression analysis of converted and measured data. Therefore, the measured data were used in the statistical analysis. Another objective of this study was to determine the effect of variables related to energy and protein balance on reproduction and pregnancy status that was performed by comparing the means of the variables by t-Test in the two groups divided by cow's reproductive statuses. SAS and SPSS software was used for statistical analysis.

#### **Results**

As mentioned earlier, cows were sampled in 13-17 days post AI and based on their reproductive statuses, between 18-25 days post AI were divided into two groups "Returned to Estrus" (RE) and "Non-Return to Estrus" (NRE).

#### *Correlations coefficients*

Simple correlations between variables in RE group are mentioned in Table 2. In this group, there was a negative correlation between Days In Milk (DIM) and Monthly Milk Record (MMR) ( $r=-0.532$ ;  $p<0.05$ ), Blood Urea Nitrogen (BUN) ( $r=-0.577$ ;  $p<0.01$ ) and a positive correlation between DIM and glucose ( $r= 0.653$ ;  $p<0.01$ ).

In this group, there was a positive correlation between MMR and BUN ( $r=0.515$ ;  $p<0.05$ ), Milk Urea Nitrogen (MUN) ( $r=0.499$ ;  $p<0.05$ ), cholesterol ( $r=0.499$ ;  $p<0.05$ ) but a negative correlation with glucose ( $r=-0.521$ ;  $p<0.05$ ). Furthermore, BUN was also positively correlated with MUN ( $r=0.684$ ;  $p<0.05$ ) but negatively correlated with glucose ( $r=-0.549$ ;  $p<0.05$ ). Correlation of glucose, in this group, with Beta Hydroxy Butyric Acid (BHBA) ( $r=-0.478$ ;  $p<0.05$ ) was positive.

Simple correlations in NRE group are shown in Table 3. As can be seen there was a negative correlation between DIM and MMR ( $r=-0.594$ ;  $p<0.01$ ) and between MMR and glucose ( $r=-0.384$ ;  $p<0.05$ ) and

positive correlations between parity and glucose ( $r=0.381$ ;  $p<0.05$ ) and between BUN and MUN ( $r=0.416$ ;  $p<0.05$ ).

#### Statistical Analysis (t-Test)

RE and NRE groups means of measured variables and progesterone concentrations were compared by t-Test exam, the results are of which shown in Table 4. No difference was observed in variables except in progesterone concentrations (RE=2.49, NRE=7.10 Ng/dl;  $t=0.0270$ ,  $p<0.01$ ).

Table 1: Ingredients and chemical composition of diet fed during study on dairy cows

Ingredient				
Alfalfa hay; chopped, g/kg of DM	218			
Corn silage, g/kg of DM	209			
Barley, g/kg of DM	221.2			
Cottonseed meal, g/kg of DM	120.4			
Wheat bran, g/kg of DM	100			
Corn; whole grain g/kg of DM	48			
Cottonseed; whole grain, g/kg of DM	30			
Corn gluten meal, g/kg of DM	24			
Sugar beet pulp, g/kg of DM	12			
Dicalcium phosphate, g/kg of DM	8.7			
Vitamin and Mineral mix, g/kg of DM	5.8			
Salt, g/kg of DM	2.9			
Chemical composition	Mean	SD	Max	Min
Dry matter %	55.81	1.17	57.33	54.11
Crude protein %	18.25	0.84	18.83	16.68
Crude fiber %	41.59	0.74	42.65	40.57
Ether extract %	5.97	0.11	6.13	5.83
Ash %	10.13	0.48	10.64	9.37
Nitrogen free extract %	23.96	0.21	24.29	23.73

\*Net Energy for Lactation, 1.55 Mcal/kg of DM.

\*Vitamin and Mineral mix contained a minimum of 90000 mg/kg of P, 180000 mg/kg of Ca, 60000 mg/kg of Na, 25000 mg/kg of Mn, 3000 mg/kg of Cu, 2000 mg/kg of Mg, 3000 mg/kg of Zn, 3000 mg/kg of Fe, 100 mg/kg of Co, 30 mg/kg of Se, 200 mg/kg of I, 3000 mg/kg of Antioxidant, 560000 IU/kg vitamin A, 100600 IU/kg vitamin D3 and 1030 IU/kg vitamin E.

Table 2: Simple correlation coefficients of animal variables (DIM, MMR, and parity), protein balance related metabolites (BUN and MUN) and energy balance related metabolites (glucose, triglyceride, cholesterol and BHBA) in RE group. (n=27)

	DIM	MMR	Parity	BUN	MUN	Glucose	Triglyceride	Cholesterol
DIM	1							
MMR	<b>-0.532*</b>	1						
Parity	-0.416	0.215	1					
BUN	<b>-0.577**</b>	<b>0.515*</b>	0.079	1				
MUN	-0.359	<b>0.499*</b>	-0.044	<b>0.684**</b>	1			
Glucose	<b>0.653**</b>	<b>-0.521*</b>	-0.327	<b>-0.549*</b>	-0.285	1		
Triglycerides	-0.053	-0.042	-0.011	0.079	0.035	-0.088	1	
Cholesterol	-0.166	<b>0.499*</b>	-0.380	0.343	0.275	0.013	-0.197	1
BHBA	-0.192	0.242	0.096	0.179	-0.006	<b>-0.487*</b>	-0.248	-0.110

DIM= Days in Milk; MMR= Monthly Milk Record; BUN = Blood Urea Nitrogen; MU=Milk Urea Nitrogen; TG= Triglycerides; BHBA= Betahydroxy Butyric Acid  
 Values in bold are significant. \*P< 0.05, \*\*P<0.01.

Table 3: Simple correlation coefficients of animal variables (DIM, MMR, and parity), protein balance related metabolites (BUN and MUN) and energy balance related metabolites (glucose, triglyceride, cholesterol and BHBA) in NRE group. (n=33)

	DIM	MMR	Parity	BUN	MUN	Glucose	Triglyceride	Cholesterol
DIM	1							
MMR	<b>-0.594**</b>	1						
Parity	-0.244	0.072	1					
BUN	-0.053	0.066	0.094	1				
MUN	0.071	0.134	0.121	<b>0.416*</b>	1			
Glucose	0.113	<b>-0.384*</b>	<b>0.381*</b>	0.033	-0.102	1		
Triglycerides	0.204	-0.223	-0.266	0.132	-0.062	0.119	1	
Cholesterol	0.031	0.167	-0.061	-0.217	0.118	-0.309	0.034	1
BHBA	0.276	-0.109	-0.015	0.059	-0.068	0.161	0.119	0.001

DIM= Days in Milk; MMY= Monthly Milk Yield; BU = Blood Urea; MU=Milk Urea; TG= Triglycerides; BHBA= Betahydroxy Butyric Acid  
 Values in bold are significant. \*P< 0.05, \*\*P<0.01.

Table 4: Comparison Between NPG and PG measured Variables Means by t-Test method in 13 to 17 days after AI.

	DIM day	MMR Kg/d	Parity	BUN mg/dl	MUN mg/dl	Glucose mg/dl	Triglyceride mg/dl	Cholesterol mg/dl	BHBA mmol/l	Progesterone ng/dl
RE group	94.37	31.31	2.95	17.95	15.84	58.86	18.20	178.03	0.567	2.49
NRE group	103.63	33.46	3	16.72	14.98	54.20	16.69	177.50	0.619	7.10
t-value	0.0044	0.8486	0.069	-0.8946	-0.7093	-0.7738	-0.6008	-0.0323	1.02291	0.027**

**Discussion**

The development of metabolic diseases and infertility in the dairy industry is multifaceted, and the

definition of physiological imbalance (Ingvarsten, 2006) assumes an association between PI and increased risk of infertility and disease for dairy cows throughout lactation. However, information based on which

physiological parameters should be included in a system to predict PI at different stages of lactation and production levels is lacking. In this study, the relationships and correlations between some blood metabolites which are related to PI, were investigated. For this propose BUN and MUN as an adequate indicator of metabolic status, especially in the early lactation (Wattiaux & Karg, 2004) and glucose, triglyceride, cholesterol and BHBA as indicators of energy balance (Bjerre-Harpøth et al., 2012; Moyes et al., 2013) with considering some animal variables like DIM, MMR and parity within RE and NRE groups were selected for measurement as variables.

As shown in Tables 2 and 3, similar results were obtained for both RE and NRE groups, i.e. a positive correlation between MUN and BUN (for RE;  $r= 0.684$ ;  $p<0.01$  and for NRE;  $r=0.416$ ;  $p<0.05$ ) that is similar to the results reported by Broderick and Clayton (1997) and Hof et al. (1997). These results were expected because urea is the metabolic end product of protein catabolism in the body and is easily measured by the nitrogen content (i.e., urea nitrogen concentration). Urea nitrogen concentrations circulating in the bloodstream are measured in either plasma or serum fractions (PUN or SUN, respectively) or are often referred to generically as BUN. Typically, BUN peaks about 4 to 6 hours after meals because of Rumen Degradable Protein (RDP) catabolism; the metabolism of Rumen Undegradable Protein (RUP) contributes to BUN continuously throughout the day. The fluctuations in BUN during the day are usually smaller (2 to 3 mg/100 dl) in cows fed with a TMR than in cows fed with concentrates and forages separately. Urea is a small, water-soluble molecule that permeates all cells and tissues in the body and passes easily between the blood and milk within the mammary gland. With a lag time of less than 1 hour for equilibration with blood concentrations, MUN provides

a rapid, noninvasive, and inexpensive means of assessing the dynamics of BUN (Butler, 1998).

Furthermore, regarding both RE and NRE groups, a negative correlation was observed between DIM and MMR (for RE;  $r= -0.532$ ;  $p<0.05$  and for NRE  $r=-0.594$ ;  $p<0.01$ ). It is well considered that, in 305 DIM, milk yield curve, declines up to the end of lactation period after reaching the peak at about 60 DIM; in this study, by considering experimental animals that were at least in 70 DIM, decrease in MMR increase in DIM was expected.

#### *NRE group*

As shown in Table 3, regardless of the above mentioned correlations, although there was a weak and negligible positive correlation between parity and glucose( $r=0.381$ ;  $p<0.05$ ) and also a weak negative correlation between MMR and glucose( $r=-0.384$ ;  $p<0.05$ ), there is not any physiological background for interpretation of these weak correlations. Other variables were not correlated with each other. It means in animals with steady status of pregnancy there was no physiological imbalance to act as a factor to deteriorate the status of pregnancy.

#### *RE group*

Some interesting obtained results, in the present study include significant correlations between metabolites in RE group. In this group in which animals failed to continue pregnancy and returned to estrus, protein and energy imbalance related metabolites are highly correlated with milk production variables containing MMR and DIM. Parity was not correlated

with other variables in this group. In RE group, there was positive correlation between MMR and MUN ( $r=0.499$ ;  $p<0.05$ ) and BUN ( $r=0.515$ ;  $p<0.05$ ). Furthermore, DIM is highly correlated with BUN ( $r=-0.577$ ;  $p<0.01$ ) that means by increasing DIM and decreasing MMR, BUN and MUN concentrations decrease. Regarding the experimental animals that are in more than at least 70 DIM, the findings agree with the previous reports by Rajala-Schultz and Saville (2003) for high-producing herds. Other studies examining the relationship between milk yield and MUN level found either no significant correlation between these parameters (Butler et al., 1995; Godden et al., 2001; Gustafsson & Carlsson, 1993) or a negative link (Broderick & Clayton, 1997).

On the physiological basis, lactating cows in early lactation faced NEB. It occurs because increased DMI accounts for only about half of the milk yield. Additional substrate required to support milk synthesis is provided through enhanced mobilization of adipose reserves and skeletal muscle (Veerkamp, 1998). Therefore, the early lactation period is characterized by the mobilization of body reserves of different tissues to cover the energy demand, and include various metabolic and endocrine adaptations. On the other hand a decrease in energy supply results in decrease of ammonia fixation by microbes in the rumen as well as increased amino acids catabolism in the liver and, consequently, leads to an increase in urea synthesis (Oltner & Wictorsson, 1983).

It is worth noting that most of the previous studies focused on various nutrition, management and environment perspectives of factors that affect BUN and MUN concentrations; however, in this study, in addition to other blood metabolites as physiological imbalance parameters under invariable and same condition for diet and animals with an emphasis on reproduction MUN

and BUN were investigated. In animals that returned to estrus, MUN and BUN concentrations and milk yield are highly correlated. Therefore, high milk yield and BUN and MUN concentrations have detrimental effect on reproduction. These results agree with the previous studies (Guo et al., 2004; Melendez et al., 2000; Rajala-Schultz et al., 2001; Vallimont et al., 2003).

To explain the reasons for these results, Butler (1998) concluded that embryo survival and growth depend upon the quality of the uterine luminal environment, and high BUN concentrations alter uterine secretions seem to be related to the detrimental effects on fertility by the dynamic changes in uterine pH with BUN. In conclusion, the poor fertility of dairy cows especially in early lactation period reflects the combined effects of a uterine environment; then, antecedent effects of negative energy balance or postpartum health problems will be further compromised by the effects of high BUN concentrations. MMR in this group had a negative correlation with glucose ( $r=-0.521$ ;  $p<0.05$ ) and a positive correlation with cholesterol ( $r=0.499$ ;  $p<0.05$ ). Also DIM was highly correlated with glucose ( $r=0.653$ ;  $p<0.01$ ). Furthermore, among blood metabolites, glucose had significant negative correlation with BUN ( $r=-0.549$ ;  $p<0.05$ ) and BHBA ( $r=-0.478$ ;  $p<0.05$ ). In return to estrus animals, by increasing milk yield, a decrease in blood glucose concentration and an increase in blood cholesterol concentration were observed. These results were expected because Moyes et al. (2013) and Bjerre-Harpøth et al. (2012) explained in their experiments that milk production affects blood metabolites which are assumed as indicators of energy balance like glucose, cholesterol and BHBA. Also Wattiaux and Karg (2004) stated that BUN is an adequate indicator of metabolic status, especially in the early lactation. Therefore by considering DIM variable in the early lactation, because of low energy consumption and high milk yield, animals

face negative energy balance that caused directly by energy deficiency and indirectly by altering optimal conditions for several metabolic and endocrine factors. All factors mentioned above directly or indirectly have adverse effects on reproductive physiology and eventually cause reproductive failure and return to estrus in this group.

There are numerous studies about direct effect of metabolites on reproductive tract and pregnancy failure. For example, Rabiee et al. (1999) recognized glucose as a major source of energy for the ovary; Canfield and Butler (1990) have reported a relationship between plasma glucose concentrations and pulsatility of LH. Spicer et al. (1993b) have documented the importance of cholesterol as a precursor of ovarian steroidogenesis, and concluded that understanding the variables that contribute to changes in plasma cholesterol concentrations may help understand factors that may contribute to reproductive success in postpartum dairy cows. Rabiee and Lean (2000) found that there was a positive and highly significant cross correlation ( $r = 0.5$ ) between the uptake of glucose and cholesterol and suggested that glucose may promote cholesterol uptake into ovarian cells or vice versa. Glucose and BUN concentrations were negatively correlated ( $r=-0.549$ ;  $p<0.05$ ). Oltner and Wictorsson (1983) stated that if we consider glucose concentration as energy balance metabolite, the decrease in energy supply results in decrease ammonia fixation by microbes in the rumen as well as increased amino acids catabolism in the liver which, consequently, leads to increase in urea synthesis.

Glucose and BHBA concentrations were negatively correlated ( $r=-0.478$ ;  $p<0.05$ ); these results agree with Bjerre-Harpøth et al. (2012) who showed that plasma concentration of BHBA is negatively associated with plasma glucose. Therefore, in relation to the first

hypothesis of the present study, it should be noted that in RE group there are significant relationships between milk production and energy and protein balance variables.

#### *RE and NRE group's t-Test*

As shown in Table 4, mean and t values for progesterone concentrations were (for RE=3.56 and for NRE=16.74 Ng/dl;  $t=2.499$ ,  $p<0.01$ ). Despite of significant correlations between different variables in RE and NRE groups no difference was observed in means of variables except in progesterone. These results agree with Folman et al. (1973) who suggested that plasma progesterone concentration is positively correlated with fertility. The results are also in line with Sklan et al. (1991) who have found positive associations between blood progesterone concentration and pregnancy rate. Also, Spicer et al. (1990, 1993a) observed a negative correlation between plasma progesterone concentration and NEB. This explains significant correlations of blood metabolites and production in RE group.

#### **Conclusion**

In conclusion, according to the results of the present study, in animals that returned to estrus after AI and failed to continue pregnancy, there was a significant correlation between milk yield and some blood metabolites (BUN, MUN, glucose, triglyceride, cholesterol and BHBA) which were related to physiological imbalance. Therefore, these metabolites directly or indirectly had an adverse effect on

reproductive tract that eventually caused reproductive failure and return to estrus. In animals that continued pregnancy and did not return to estrus, there was not any significant correlation between these variables. Also in spite of the significant correlations among variables in return to estrus group, except progesterone concentration, there was no difference between RE and NRE groups' variables means compared by t-Test method. Therefore, despite adverse effect of negative energy balance on physiological imbalance and blood metabolites, progesterone concentration had a key role in stability of pregnancy. Our research clarified the integrity of protein and energy balance related metabolites on individual animal in farm condition and investigated their effects on reproduction in this situation.

### References

- Allen, M. S., (2000). Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *J. Dairy. Sci.* 83, 1598–1624.
- AOAC International. (2000). *Official Methods of Analysis*. 17th ed. AOAC International, Washington, DC.
- Bjerre-Harpoth, V., Friggens, N. C., Thorup, V. M., Larsen, T., Damgaard, B. M., Ingvarsen, K. L., & Moyes, K. M. (2012). Metabolic and production profiles of dairy cows in response to decreased nutrient density to increase physiological imbalance at different stages of lactation. *J. Dairy. Sci.* 95, 2362–2380.
- Broderick, G. A., & Clayton, M. K. (1997). A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. *J. Dairy. Sci.* 80, 2964–2971.
- Butler, W.R. (1998). Review: Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. *J. Dairy. Sci.* 83, 2533-2539.
- Butler, W. R., Cherney, D. J. R., & Elrod, C. C. (1995). Milk urea nitrogen (MUN) analysis: field trial results on conception rates and dietary inputs. In *Proceeding of Cornell Nutrition Conference*, Rochester, NY. Cornell University, Ithaca, NY, page 89.
- Canfield, R. W., & Butler, W. R. (1990). Energy balance and pulsatile LH secretion in early postpartum dairy cattle. *Domest. Anim. Endocrin.* 7, 323–330.
- Folman, Y. Rosenberg, M. Herz, Z., & Davidson, M. (1973). The relationship between plasma progesterone concentration and conception in postpartum dairy cows maintained on two levels of nutrition. *J. Reprod. Fertil.* 34, 267–278.
- Godden, S. M.; Lissemore, K. D.; Kelton, D. F.; Leslie, K. E.; Walton, J. S., & Lumsden. J. H. (2001). Relationships between milk urea concentrations and nutritional, management, production and economic variables in Ontario dairy herds. *J. Dairy. Sci.* 84, 1128–1139.
- Guo, K., Russek-Cohen, E., Varner, M. A., & Kohn. R. A. (2004). Effects of milk urea nitrogen and other factors on probability of conception of dairy cows. *J. Dairy. Sci.* 87, 1878–1885.
- Gustafsson, A. H., & Carlsson, J. (1993). Effects of silage quality, protein evaluation systems and milk

- urea content on milk yield and reproduction in dairy cows. *Livest. Prod. Sci.* 37, 91–105.
- Hof, G., Vervoorn, M. D., Lenaers, P. L., & Tamminga, S. (1997). Milk urea nitrogen as a tool to monitor the protein nutrition of dairy cows. *J. Dairy. Sci.* 80, 3333–3340.
- Ingvarstsen, K. L. (2006). Feeding- and management-related diseases in the transition cow, physiological adaptations around calving and strategies to reduce feeding-related diseases. *Anim. Feed. Sci. Tech.* 126, 175–213.
- Ingvarstsen, K. L., & Andersen, J. B. (2000). Integration of metabolism and intake regulation: A review focusing on periparturient animals. *J. Dairy. Sci.* 83, 1573–1597.
- Ingvarstsen, K. L., Dewhurst, R. J., & Friggens, N. C. (2003). On the relationship between lactational performance and health: Is it yield or metabolic imbalance that cause production diseases in dairy cattle? A position paper. *Livest. Prod. Sci.* 83, 277–308.
- Jolly, P. D., McDougall, S., Fitzpatrick, L. A., Macmillan, K. L., & Entwistle, K. (1995). Physiological effects of under nutrition on postpartum anestrus in cows. *Journal of Reproduction and Fertility Supplement* 49, 477–492.
- Jorritsma, R., Wensing, T., Kruip, T., Vos, P., & Noordhuizen, J. (2003). Metabolic changes in early lactation and impaired reproductive performance in dairy cows. *Vet. Res.* 34, 11–26.
- LeBlanc, S. (2010). Monitoring metabolic health of dairy cattle in the transition period. *J. Repro. Develop.* 56(Supplement):S29–S35.
- Melendez, P.; Donovan, A.; Hernandez, J. (2000). Milk urea nitrogen and infertility in Florida Holstein cows. *J. Dairy. Sci.* 83, 459–463.
- Moyes, K. M., Friggens, N. C., & Ingvarstsen, K. L. (2010). Nutritional strategies to combat physiological imbalance of dairy cows during early lactation: The effect of changes in dietary protein and starch ratios. *Acta. Vet. Scand.* 60, 166–174.
- Moyes, K. M., Larsen, T., & Ingvarstsen, K. L. (2013). Generation of an index for physiological imbalance and its use as a predictor of primary disease in dairy cows during early lactation. *J. Dairy. Sci.* 96, 2161–2170.
- NRC. (2001). *Nutrient Requirements of Dairy Cattle*. National Academy Press, Washington, DC.
- Oltner, R., & Wiktorsson, H. (1983). Urea concentration in milk and blood as influenced by feeding varying amount of protein and energy to dairy cows. *Livest. Prod. Sci.* 10, 457–467.
- Pryce, J. E., Royal, M. D., Garnsworthy, P. C., & Mao, I. L. (2004). Fertility in the high-producing dairy cow. *Livest. Prod. Sci.* 86, 125–135.
- Rabiee, A. R., & Lean, I. J. (2000). Uptake of glucose and cholesterol by the ovary of sheep and cattle and the influence of arterial LH concentrations. *Anim. Reprod. Sci.* 64, 199–209.

- Rabiee, A. R. Lean, I. J. Gooden, J. M., & Miller, B.G. (1999). Relationships among metabolites influencing ovarian function in the dairy cow. *Journal of Dairy Science* 82, 39–44.
- Rajala-Schultz, P. J., Saville, W. J. A., Frazer, G. S., & Wittum, T. E. (2003). Association between milk urea nitrogen and fertility in Ohio dairy cows. *J. Dairy. Sci.*84, 482–489.
- Royal, M. D., Darwash, A. O., Flint, A. P. F., Webb, R., Woolliams, J. A., & Lamming, G. E. (2000). Declining fertility in dairy cattle: Changes in traditional and endocrine parameters of fertility. *Anim. Sci.* 70, 487–502.
- SAS Institute. (2006). *User's Guide: Statistics. Version 9.1.* SAS Institute, Cary, NC.
- Sklan, D., Moallem, U., & Folman, Y. (1991). Effect of feeding calcium soaps of fatty acids on production and reproductive responses in high producing lactating cows. *J. Dairy. Sci.*74, 510–517.
- Spicer, L. J., Alpizar, E., & Echternkamp, S. E. (1993a). Effects of insulin, insulin-like growth factor-I, and gonadotropins on bovine granulosa cell proliferation, progesterone production, estradiol production, and (or) insulin-like growth factor-I production in vitro. *J. Anim. Sci.*71, 1232–1241.
- Spicer, L. J., Tucker, W. B., & Adams, G. D. (1990). Insulin-like growth factor-I in dairy cows: Relationships among energy balance, body condition score, ovarian activity, and estrous behavior. *J. Dairy. Sci.* 73, 929–937.
- Spicer, L. J., Vernon, R. K., Tucker, W. B., Wettemann, R. P., Hogue, J. F., & Adams, G. D. (1993b). Effects of inert fat on energy balance, plasma concentrations of hormones and reproduction in dairy cows. *J. Dairy. Sci.* 76, 2664–2673.
- Vallimont, J. E., Rogers, G. W., Holden, L. A., O'Connor, M. L., Cooper, J. B., Dechow, C. D., & Clay, J. S. (2003). Milk urea nitrogen and fertility: A population study using test-day records. *J. Dairy. Sci.* 81(Supplement 1):239. (Abstract)
- Veerkamp, R. F. (1998). Selection for economic efficiency of dairy cattle using information on live weight and feed intake: A review. *J. Dairy. Sci.* 81:1109–1119.
- Walsh, S. W., Williams, E. J., & Evans, A. C. (2011). A review of the causes of poor fertility in high milk producing dairy cows. *Anim. Reprod. Sci.* 123,127–138.
- Wattiaux, M. A., & Karg, K. L. (2004). Protein level for alfalfa and corn-silage based diets: I. Lactational response and milk urea nitrogen. *J. Dairy. Sci.* 87, 3480–3491.

# بررسی ارتباط بین تولید شیر و برخی متابولیت‌های خونی و تأثیر آن‌ها بر بازگشت به فحلی

## در گاوهای شیری هلشتاین

نادر فلاح‌نژاد انرجان<sup>۱</sup>، غلامعلی مقدم<sup>۲\*</sup>

۱- گروه علوم دامی دانشگاه آزاد واحد شبستر، شبستر، ایران

۲- گروه علوم دامی دانشکده کشاورزی، دانشگاه تبریز، تبریز، ایران

\* نویسنده مسئول: ghmoghaddam@tabrizu.ac.ir

### چکیده:

در این مطالعه، ارتباط و همبستگی بین تولید شیر و برخی متابولیت‌های مرتبط با بالانس انرژی و پروتئین و اثرات آن‌ها بر بازگشت به فحلی پس از تلقیح مصنوعی در گاوهای شیری هلشتاین مورد بررسی قرار گرفت. بدین منظور از ۶۰ رأس گاو شیری هلشتاین بر اساس وضعیت تولیدمثلی‌شان بین روزهای ۱۳ تا ۱۷ پس از تلقیح مصنوعی نمونه‌های خون و شیر گرفته شد. غلظت ازت اوره‌ای خون، ازت اوره‌ای شیر، گلوکز، تری‌گلیسرید، کلسترول و بتا‌هیدروکسی بوتیریک‌اسید با استفاده از روش آنزیمی - کالری متری و غلظت پروژسترون خون با استفاده از روش الایزا اندازه‌گیری شد. بین روزهای ۱۸ تا ۲۵ پس از تلقیح مصنوعی، در ۲۷ رأس گاو، بازگشت به فحلی (گروه RE) و در ۳۳ رأس گاو، عدم بازگشت به فحلی (گروه NRE) مشاهده گردید. در گروه RE همبستگی معنی‌داری بین روزهای شیردهی DIM و رکورد تولید شیر ماهانه MMR ( $p < 0/05$ )، ازت اوره‌ای خون ( $p < 0/01$ ) و گلوکز ( $p < 0/01$ ) مشاهده شد. علاوه بر این رکورد تولید شیر ماهانه همبستگی معنی‌داری با ازت اوره‌ای خون ( $p < 0/05$ )، ازت اوره‌ای شیر ( $p < 0/05$ )، گلوکز ( $p < 0/05$ ) و کلسترول ( $p < 0/05$ ) داشت. همچنین در این گروه ازت اوره‌ای خون همبستگی معنی‌داری با ازت اوره‌ای شیر ( $p < 0/01$ ) و گلوکز ( $p < 0/05$ ) داشت و همبستگی بین گلوکز و بتا‌هیدروکسی بوتیریک‌اسید ( $p < 0/05$ ) نیز معنی‌دار بود. در گروه NRE همبستگی معنی‌داری بین روزهای شیردهی و رکورد تولید شیر ماهانه ( $p < 0/01$ ) و بین رکورد تولید شیر ماهانه و گلوکز ( $p < 0/05$ ) وجود داشت. همچنین همبستگی شکم زایش با گلوکز ( $p < 0/05$ ) و غلظت ازت اوره‌ای خون با شیر ( $p < 0/05$ ) معنی‌دار بود. به غیر از غلظت‌های پروژسترون ( $p < 0/01$ ) تفاوت معنی‌داری بین میانگین متغیرهای دو گروه مشاهده نشد. در نتیجه، نتایج به دست آمده از این تحقیق نشان می‌دهد که در گاوهایی که بازگشت به فحلی داشته و قادر به ادامه آبستنی نبودند، همبستگی‌های معنی‌داری بین تولید شیر و برخی متابولیت‌های خونی (ازت اوره‌ای خون، ازت اوره‌ای شیر، گلوکز، تری‌گلیسرید، کلسترول و بتا‌هیدروکسی بوتیریک‌اسید) که با عدم تعادل فیزیولوژیک نیز مرتبط هستند، مشاهده شد. در گاوهایی که عدم بازگشت به فحلی داشته و قادر به ادامه آبستنی بودند، همبستگی‌های معنی‌دار قابل تفسیری بین متغیرها مشاهده نشد. بنابراین، علیرغم تأثیرات منفی بالانس منفی انرژی بر عدم تعادل فیزیولوژیک و متابولیت‌های خونی، غلظت پروژسترون نقش کلیدی در تداوم آبستنی دارد.

واژه‌گان کلیدی: متابولیت‌های خون، تولید شیر، عدم تعادل فیزیولوژیک، تولیدمثلی