

EFFECT OF LACTATION ON ENERGY METABOLISM IN DAIRY COWS FROM DIFFERENT CATEGORIES

Celeska Irena¹, Ulčar Igor¹, Stojkovski Velimir^{2, 3}, Dovenski Toni³,
Mitrov Dine⁴, Džadžovski Igor⁴

¹*Department of Pathophysiology, Faculty of Veterinary Medicine – Skopje;*

²*Department of Biochemistry and Cell Biology, Faculty of Veterinary Medicine – Skopje;*

³*Department of Reproduction, Faculty of Veterinary Medicine – Skopje;*

⁴*Department of Internal Medicine of Farm Animals, Faculty of Veterinary Medicine – Skopje*

e-mail: iceleska@fvm.ukim.edu.mk

ABSTRACT

The aim of this article was determination of the energy status of Holstein-Friesian cows in three dairy farms in our country. For that purpose, blood samples were taken from three different farms with similar diet for dairy cows. Blood samples were taken from clinically healthy cows, from 2nd to 7th lactation. Several biochemical parameters were measured for determination of the energy status: glucose, non-esterified fatty acids, β -hydroxybutyrate (BHB), triacylglycerols and total cholesterol. Total of N=378 samples were taken from multiparous Holstein-Friesian dairy cows from all categories. Cows were divided into three main groups. The group 1 was formed of dry cows, and it was divided into two subgroups: “far from calving” (n=64) and “close up to calving” (n=62). The animals in the group 2 - early lactation cows, were divided into three subgroups as follows: up to 14 days after calving (n=66), up to 60 days after calving (n=63) and up to 100 days after calving (n=62). The group 3 was with cows that were in middle lactation, more than 100 days after calving (n=61). Biochemical parameters were analyzed with standard colorimetric methods, using Sentinel and Randox reagents, on photometer Stat Fax 3300 (Awareness Technology Inc.). These results have shown that dairy cows developed hypoglycemia and early lipolysis (high level of serum NEFA and BHB), during the dry period. The obtained results confirm the ability of the dairy cows for adaptation in various physiological stages of lactation, but the actual system of feeding does not allow them to achieve their genetic potential. Finally, the evident health problems, occurring as a result of managerial and nutritional errors on the dairy farms, defined as “production diseases” are the most serious cause for decreased production effects.

Key words: dairy cows, energy balance, transition period, biochemical profile, production disease

INTRODUCTION

Dairy cattle are at increased risk for many disease and disorders during periparturient period. At this time there is incising milk production, but a lag in feed intake. These combinations create negative energy balance (1). Energy balance is by and far

one of the most critical nutritional factors impacting on animal health, lactation and reproductive performance (2, 3). The animals attempt to supply the needs for milk production by drawing on body fat reserves. Thus, the animal experience a period of negative energy balance and it has to mobilize tissue lipid and protein in order to sustain productive

function (4). Plasma glucose and non-esterified fatty acids (NEFA) decline with age and are lower during 5 to 8 week in early weaned calves (5). This mobilization of fatty acids results in the production of major ketone bodies, acetone, acetoacetate and β -hydroxybutyrate (BHB). These compounds are important as a source of energy when carbohydrate levels are reduced. However, the accumulation of these compounds can lead to ketosis. Ketosis has been shown in dairy herds with significantly reduced milk yield (1). Marked decrease in dry matter intake immediately before calving is extremely important in the development of excessive negative energy balance (6). Metabolic disorders are highly multifactorial and a wide range of animal, managerial and fed factors may lead to such problems (7). Energy metabolism was analyzed by the measurement of blood concentrations of glucose, acetoacetate and NEFA (8). Glucose is the primary metabolic fuel, and is absolutely required for vital organ function, fetal growth and milk production (9). Glucose represents an overriding metabolic demand during the transition period because of the requirements of the mammary gland for lactose synthesis (10). The homeoeretic mechanisms have role to maintain the high level of milk production, via mobilization of body fat store, which results in elevated levels of circulating ketone bodies in early lactation. Ketone bodies are intermediate metabolic products. They provide available energy to peripheral tissues when carbohydrates levels are limited (1). Cows with precalving energy deficit would start mobilizing energy reserves in the last week before parturition. This could be measured via serum or plasma NEFA (11). The NEFA could undergo a variety of different metabolic paths within the liver. Liver becomes infiltrated with fat during ketosis, although a significant fraction of assimilated NEFA could be reesterified. Also, a substantial portion of NEFA are converted in ketone bodies. Evidence from other species indicates that the rate of hepatic ketogenesis from NEFA is determined both by the rate of supply of NEFA and by carbohydrate status of liver (12). The estimation of NEFA/BHB ratio elucidate the relative significance of lipolysis and ketogenesis (13). The "gold standard" test for subclinical ketosis is determination of blood BHB. This ketone body is more stable in blood than acetone and acetoacetate. Clinical ketosis generally involves much higher levels of BHB (14). Hepatic ketogenesis may be

augmented by diminishing carbohydrate status (12). Blood parameters that may reflect nutrition status of the cow, such as glucose, NEFA, BHB, and total cholesterol, also enzymes and proteins that reveal liver status are of interest to be included in the transition period profiles (7). Clinical ketosis typically occurs spontaneously in susceptible high-yielding dairy cows between the 2nd and 7th week of lactation (12). The more obvious biochemical features of the condition are hyperketonemia, with minimum blood acetoacetate concentration, hypoglycemia, and elevated concentration of NEFA in the blood, fatty infiltration of the liver and loss of liver glycogen (12). Subclinical ketosis in dairy cattle can lead to economic losses through decreased milk production, decreased reproductive performance, increased risk of abomasum dislocation and increased risk of clinical ketosis (15).

MATERIAL AND METHODS

Blood samples from dairy cows included in the analysis, were taken by puncture of v. jugularis in the period from May to September at the same time of day, as well as from 09:00 am to 14:00 or 4 hours after milking and feeding.

Blood was taken carefully with no disturbing the animal. Blood was collected in serological test glass tubes. After spontaneous coagulation at room temperature, the tubes were centrifugated in the Sanyo Mistral 2500 centrifuge of 2500 rpm during 5 minutes. Blood samples were kept in a freezer at a temperature of -18°C to -20°C . Determination of glucose, triacylglycerols and total cholesterol in serum, was performed with enzymatic colorimetric methods, with commercial reagent manufactured by SENTINEL (Italy) in accordance with IFCC. Determination of NEFA and BHB was performed with enzymatic colorimetric methods, with a commercial reagent manufactured by RANDOX (UK) in accordance with IFCC. Total of N=378 samples were taken from multiparous Holstein-Friesian dairy cows from all categories. Cows were divided into three main groups. The group 1 covered dry cows. This group was divided into two subgroups: "far from calving" (n=64) and "close up calving" (n=62). The animals in the group 2 - early lactation cows, were divided into three subgroups as follows: up to 14 days after calving

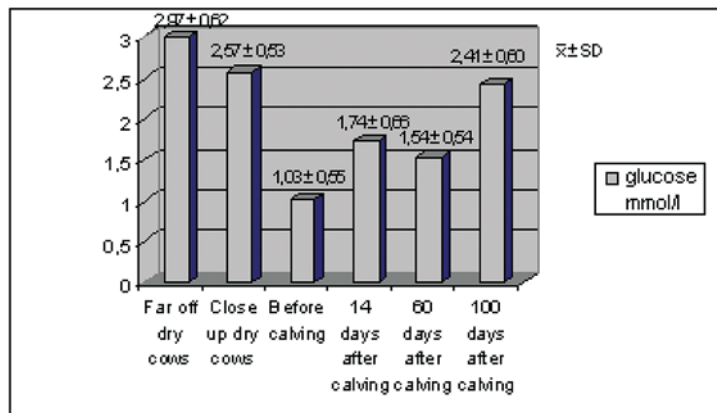
(n=66), up to 60 days after calving (n=63) and up to 100 days after calving (n=62). The group 3 contained cows that were in middle lactation, more

than 100 days after calving (n=61). Results of all samples were statistically analyzed by STATISTICA program for Windows 5.0 (Stat Soft Inc).

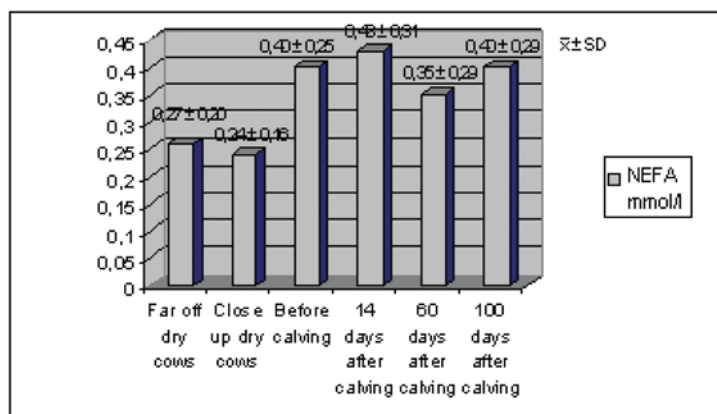
RESULTS AND DISCUSSION

From the research we get following results for biochemical parameters as represent of negative energy balance (Histograms 1-5):

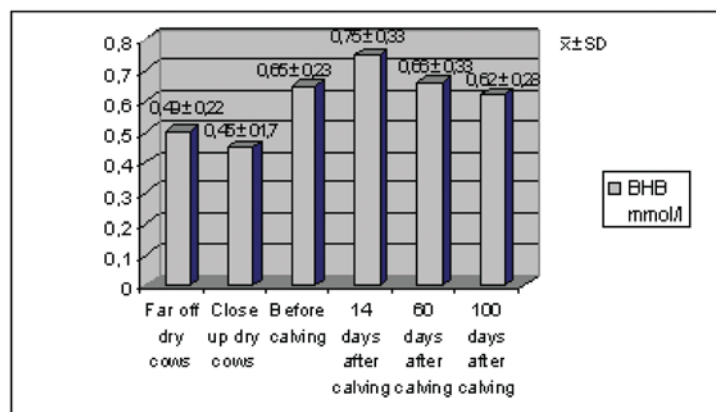
Histogram 1. Glucose concentrations (mmol/l) in dairy cows from different stages of lactation.



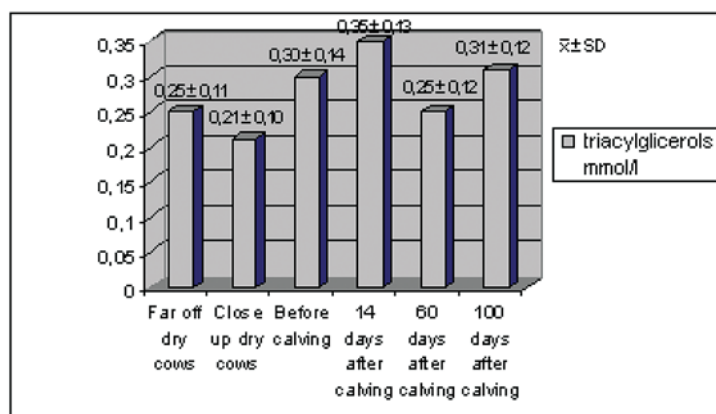
Histogram 2. NEFA concentrations (mmol/l) in dairy cows from different stages of lactation.



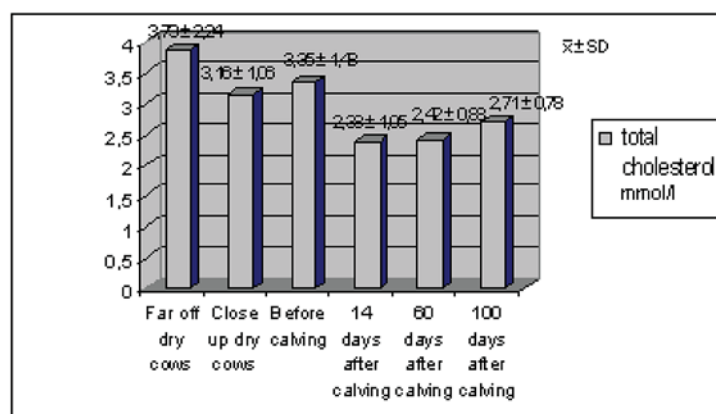
Histogram 3. BHB concentrations (mmol/l) in dairy cows from different stages of lactation.



Histogram 4. Triacylglycerols concentrations (mmol/l) in dairy cows from different stages of lactation.



Histogram 5. Total cholesterol concentrations (mmol/l) in dairy cows from different stages of lactation.



Correlations between biochemical parameters which were significant ($p < 0,05$) are shown on Table 1.

Table 1: Correlations between biochemical parameters

	r		r
"far of"		60 days after calvin	
total cholesterol : glucosis	0,29	triacylglycerols : NEFA	0,42
total cholesterol : triacylglycerols	0,38	NEFA : BHB	0,76
NEFA : BHB	0,54		
"close up"		100 days after calving	
NEFA : total cholesterol	-0,25	glucosis : triacylglycerols	-0,29
NEFA : BHB	0,39	total cholesterol : triacylglycerols	0,26
		glucosis : BHB	-0,28
		NEFA : B-HB	0,66
14 days after calving		middle lactation	
triacylglycerols : NEFA	0,30	triacylglycerols: BHB	0,43
triacylglycerols : BHB	0,32	total cholesterol : NEFA	-0,37
NEFA : BHB	0,74	NEFA : BHB	0,61

The modern method of breeding dairy cows and technology of storage and use are aimed at continuously increasing milk productivity. In high yielding dairy cows significant changes in the balance of power resulting in around calving. The last two months of pregnancy characterizes a positive balance of energy, why in this period cows imported more energy than the real needs, so excess energy is deposited in body reserves. If this positive energy balance take over the whole period of drying, significantly increasing the animal's body mass. Accumulated energy in the form of increased body fat storage and excessive and uncontrolled lipomobilisation is the main cause of subclinical and clinical disorders. At the whole animal level, the magnitude and duration of negative energy balance are influenced by feed intake and milk production (16). Many published results confirmed that the selection and measures to increase milk productivity significantly affect the increase in the intensity of metabolic processes in cows in periparturient period (3). In turn, negative energy balance is associated with elevated plasma NEFA and hepatic lipid concentrations (17). Ketosis is a disorder of carbohydrate and fat metabolism characterized by increased concentrations of ketone bodies in blood (ketonemia), urine (ketonuria), milk (ketolactia), and other body fluids (18). Subclinical ketosis is important common condition early lactation dairy

cattle. It is associated with losses in milk production and increased risk of periparturient disease (11).

Dairy cows included in our study were from three different dairy farms with similar mode of farming and approximately equal diet. Obtained results show great changes in drying period in groups "far from calving" and "close up calving" with great demand of energy for gravid uterus and udder prepare for galactogenesis. Dairy cows from "close up calving" group had increased turnover of lipids, which is evident in significantly increased concentration of NEFA ($p < 0,001$) and BHB ($p < 0,001$) compared with other groups, and decreased concentration of glucose, which is also significantly lower than the same values from other groups ($p < 0,001$). In this group, weak positive correlation occurs between total cholesterol and NEFA ($r = 0,25$) and a moderate positive correlation between NEFA and BHB ($r = 0,39$). This situation indicates that cows were exhausted even before calving as they were not adequately prepared toward dry period. Some authors refer that in the dry period there is positive correlation between metabolism of carbohydrates and lipids (18). Regulation of the metabolism between carbohydrates and lipids occurs between adipose tissue, liver, intestine and udder, and this is essential for the animal's adaptation to negative energy balance. Adaptation of the animals appeared before calving, as the physiological

process of incoming lactation. In dairy cows, the massive energy demand to support milk production is partly met through gluconeogenesis (9). Glucose concentration is under tight homeostatic control. Some dairy cows in early lactation cannot provide balanced relationship in the metabolic processes and regulatory mechanisms can induce pathological condition. Immediately after calving negative energy balance is more intensified, and that is evident with the values of NEFA, BHB and triacylglycerols, because their concentrations in group 14 days after calving are significantly elevated ($p < 0,001$) as a result of lipomobilization from the body fat storage. In this group weak positive correlation was found between BHB and triacylglycerols ($r = 0,30$), between triacylglycerols and NEFA ($r = 0,32$), and also an strong positive correlation between NEFA and BHB ($r = 0,74$). Increased glucose is provided by alimentary sources with glycogenoplastic compounds. All biochemical changes indicate activation of regulatory mechanisms in the adaptation of negative energy balance. There are large individual variations in dairy cows in the mobilization of fat. Decreased concentrations of NEFA of 0,35 mmol/l in dairy cows 60 days after calving, indicate that negative energy balance is gradually corrected, by reducing lipomobilization, as a direct indicator of negative energy balance as well as BHB of 0,66 mmol/l is indicator of ketogenesis. Providing glycogenoplastic precursors from alimentary sources, glucose increased, but this was not significant. Cows still remained in hypoglycemia because of increase milk production. Hypoglycemia was concomitant with decreased concentration of NEFA and ketogenesis was not intensified because of the lack of ketogenic substrate. Clinically apparent outbreak of "production disease" in group 60 days after calving did not appeared, which was evident with the positive correlation between NEFA and BHB ($r = 0,76$). Glucose concentration in dairy cows depends from gluconeogenesis in the liver, and glucose consumption in the udder for increase milk production. The positive correlation between triacylglycerols and NEFA ($r = 0,42$) in group 60 days after calving suggested a reduced ability of the liver synthesis of VLDL and reesterification of NEFA with glycerol with concomitant fatty liver infiltration. Development the fatty liver can lead to liver failure and drop milk production. Significant increase ($p < 0,001$) of glucose concentration of 2,41

mmol/l in group 100 days after calving, indicates regenerative liver function for gluconeogenesis. In this group indicators of lipid status triacylglycerols and NEFA were 0,31 mmol/l and 0,40 mmol/l, respectively, and this values were nonsignificantly higher compared with other groups. This is because in 100 days after calving high producing dairy cows reach peak of lactation and realise genetic potential for high milk production and increased anabolism of the udder. Correction of glucose concentration is enclose with reduced ketogenesis in the liver, which is shown in negative correlation between glucosis and BHB ($r = -0,28$). In middle lactation group organism is providing sufficient precursors for the galactogenesis, and an positive correlation between NEFA and BHB ($r = 0,66$) was found. Biochemical parameters in dairy cows in middle lactation are corrected in physiological ranges: glucose 2,97 mmol/l, NEFA 0,27 mmol/l, BHB 0,49 mmol/l, triacylglycerols 0,25 mmol/l, and total cholesterol 3,73 mmol/l. So, dairy cows came out from negative energy balance. There were sufficiently glycogenoplastic precursors as energy metabolites, for optimal relation of energy metabolism, for successfully getting out from negative energy balance.

CONCLUSION

High yielding dairy cows in the farms in our country have a high genetic potential for milk production. Homeoeretic mechanism prevails for adaptation to the new needs of energy redistribution of metabolism. Modern way of farming has to implement new measures in order to avoid the consequences that occur as a result of energy deficit. Biochemical parameters in blood as indicators of negative energy balance provide information on its early initiation and prolonged duration, but not with deep intensity and expressed, and thus high yielding dairy cows adapted towards stage of lactation, but they are not properly adapted to the genetic potential which possess. The results show a general conclusion that the dry cows occurs hypoglycemia and earlier lipomobilization (high concentration of NEFA and BHB). These results confirm that dairy cows have the ability for adaptation in different physiological stages of lactation, but the quality of nutrition and the system are not properly adequate to the genetic

potential they possess. Finally, the obvious health problems, which arise as a consequence of errors in the management of breeding and nutrition of dairy farms, can be defined as “production diseases” and constitute the most serious cause of reduced production effects.

REFERENCES

1. Leslie K. E., Duffield T., LeBlanc S. 2003. Monitoring and Managing Energy Balance in the Transition Dairy Cow, *J Dairy Sci* 86: 101-107
2. Van Saun R. J. 2002. Metabolic profiling and health risk in transition cows. *Proc Am Assoc Bov Pract.* 37: 212-213
3. Van Saun R. J. 2008. Metabolic Profiling of Transition Cows: Can We Predict Impending Problems? *Danish Bovine Practitioner Seminar, Middelfart, Denmark, January 24-25*
4. Waldron M. R., Overton T. R. 2006, Integration of metabolism and immunity in periparturient dairy cows, *J. Dairy Sci.* 87: 105–119
5. Quigley J. D., Caldwell L. A., Sinks G. D., Heitmann R. N. 1991. Changes in blood glucose, Nonesterified fatty Acids, and ketones in response to weaning and feed intake in young Calves. *J. Dairy Sci.* 74: 250-257
6. Duffield T. F. 2004. Monitoring strategies for metabolic disease in transition dairy cows, *23th world Buiatrics Congress, Québec, Canada.* July 11-16
7. Stengärde L., Tråvén M., Emanuelson U., Holtenius K., Hultgren J. Niskanen R. 2008. Metabolic profiles in five high-producing Swedish dairy herds with a history of abomasal displacement and ketosis. *Acta Veterinaria Scandinavica.* 50: 31
8. Gergác Z., Szücs, E. 2009. Critical points in the feeding of high yielding dairy cows in association with bcs and metabolic profile test, *Lucrări științifice Zootehnie și Biotehnologii.* 42(2): 568-580
9. LeBlanc S. 2006. Monitoring Programs For Transition Dairy Cows, *Word Buiatrics Congress, Nice, France*
10. Overton T. R. 2003. Managing the Metabolism of Transition Cows, *Proceedings of the 6th Western Dairy Management Conference, Reno, March 12-14*
11. Duffield T.F., 2000. Subclinical ketosis in Lacting Dairy Cattle, Metabolic disorders of ruminants. *The veterinary clinics of North America* 16(2): 231-253
12. Baird D. G. 1982. Primary Ketosis in the High-Producing Dairy Cow: Clinical and Subclinical Disorders, Treatment, Prevention, and Outlook, *J. of Dairy Sci.* 65: 1-10
13. Sato H., Matsumoto M., Hanasaka S. 1998. Relations between Plasma Acetate, 3-Hydroxybutyrate, FFA, Glucose Levels and Energy Nutrition in Lactating Dairy Cows. *J. Vet. Med. Sci.* 61(5): 447–451
14. Oetzel G. R. 2003. Herd-Based Biological Testing For Metabolic Disorders *Advances in Dairy Technology.* 15: 275-285
15. Carrier J., Stewart S., Godden S., Fetrow J., Rapnicki P. 2004. Evaluation and Use of Three Cowside Tests for Detection of Subclinical Ketosis in Early Postpartum Cows. *J. Dairy Sci.* 87: 3725–3735
16. Carrier J., Stewart S., Godden S., Fetrow J., Rapnicki P. 2004. Evaluation and Use of Three Cowside Tests for Detection of Subclinical Ketosis in Early Postpartum Cows. *J. Dairy Sci.* 87: 3725–3735
17. Colazo M. G., Hayirli A., Doepel L., Ambrose D. J. 2009. Reproductive performance of dairy cows is influenced by prepartum feed restriction and dietary fatty acid source. *J dairy Sci.* 92: 2562-2571
18. Geishauser T., Leslie K. E., Kelton D., Duffield T. 1998. Evaluation of Five Test for use with Milk to detect Subclinical Ketosis in Dairy Cows. *Journal of Dairy Science.* 83: 438-443

