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IMPACT OF NEGATIVE ENERGY BALANCE ON PRODUCTION AND FERTILITY IN SLOVENIAN BROWN-BREED DAIRY COWS

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The aim of this study was to investigate the production and reproduction ability of Slovenian brown-breed dairy cows according to their exposure to negative energy balance (NEB). Body condition score (BCS), concentrations of metabolites such as non esterified fatty acids (NEFA), beta hydroxy butyrate (BHB), cholesterol and urea were measured on day 14 postpartum and progesterone profile was determined for each animal. The postpartum period was closely monitored from day 14 to conception by ultrasound examination in 10 day intervals. Reproductive parameters were followed up, also. Cows were divided in two groups according to NEFA and BHB concentrations with a threshold of 0.5 and 1.4 mmol/L, respectively. Cows with elevated NEFA and BHB levels (Group 2) in comparison to Group 1 expressed a higher decrease in BCS 30 days postpartum (0.64 vs 0.33 point; P<0.01), more abnormal progesteron patterns (P<0.001) and had a significantly longer interval to first progesteron rise (49.54 vs 33.82 days; P<0.05). Milk protein concentrations 30 days postpartum were significantly lower in Group 2 comparing to Group 1 (3.08 % vs 3.36 %, P<0.001) and protein ratio (FPR) 30 days postpartum was significantly higher in Group 2 (1.37 vs 1.21; P<0.05) comparing to Group 1. Measurement of NEFA and BHB concentrations, body condition scoring and milk data recording are found to be good criteria for the recognition of NEB in dairy cows in the early postpartum period.

Key words: dairy cows, negative energy balance, body condition score, reproduction

INTRODUCTION

Metabolism in high yielding dairy cows is under pressure in the early *postpartum* period, mainly because of milk production. Most of dairy cows in that period are unable to consume adequate quantities of food needed for milk production. Therefore, they enter a period of negative energy balance (NEB), which leads to mobilization of body reserves, mainly fat, to balance the deficit between food energy intake and production requirements. NEB delays the time of first ovulation through inhibition of LH pulse frequency and low levels of blood glucose, insuline and insuline-like growth factor-I that collectively restrain estrogen production by the dominant follicle (DF) (Butler, 2000). Butler and Smith (1989) reported that NEB usually reaches its maximum during the first two weeks *postpartum* and the rate of body reserves mobilization is directly related to *postpartum* interval to the first ovulation and lower conception rate. According to Duffield (2004), negative energy balance, fat mobilization and subsequent elevations in ketone body concentrations play a contributing role in the expression of fatty liver syndrome, clinical ketosis and abomasal displacement. During this period some authors payed special attention to the rise of some metabolites such as non esterified fatty acids (NEFA) or beta hydroxy butyrate (BHB) (Schillo, 1992; Opsomer, 1999; van Knegsel *et al*., 2005; Vanholder *et al*., 2005b) and the decrease of glucose and cholesterol (Opsomer *et al*., 1999; Klinkon *et al*., 2001, van Knegsel *et al*., 2005). Duffield (2004) suggested NEFA levels above 0.5 U/L one week prior calving as a risk for subsequent development of displaced abomasum and BHB levels above 1400 μmol/L as a definition of subclinical ketosis in the first two weeks postcalving.

Changes in body condition score (BCS) are used to indicate body tissue mobilization. Some methods have been described to define body condition score using a 1 to 5 points scale (Wildman *et al*., 1982; Edmonson *et al*., 1989). Several studies indicate that cows with excessive loss in BCS in the first two months *postpartum* are significantly at a higher risk to reproduction failure (Ferguson, 2000; Loeffler *et al*., 1999a, Ferreira *et al*., 2005).

Increased fat mobilization in a period of negative energy balance is shown by lower BSC, higher milk fat concentration and decrease in voluntary dry matter and energy intake, which results also in low milk protein concentration (Eicher, 2004). Thus, changes in fat – protein ratio (FPR) in milk could be an indicator of the ability of a cow to adapt to the demands of the early *postpartum* period with sufficient concentrate feed intake (Loeffler *et al*., 1999b).

The appearance of normal early cyclic ovarian activity after calving is of great importance in dairy cows. Among some methods such as oestrus observation or rectal palpation, resumption of ovarian activity can be measured also by the appearance of the first significant progesteron rise (Fagan in Roche, 1986; Opsomer *et al.*, 1998; Petersson *et al*. 2006). Ovarian activity can be shown as an alternation in milk progesteron concentration in longer *postpartum* periods.

The aim of the present study was to investigate production and reproduction ability of Slovenian domestic brown breed cows according to their exposure to NEB and to define criteria for practical recognition of NEB in dairy herds.

MATERIAL IN METHODS

Animals

The study was performed in a herd of 50 dairy cows of Slovenian domestic brown breed, with average milk production of 7000 kg in 305 days. Cows were milked twice a day, kept in a free-stall barn system and fed total mixed rations.

Basic ration was composed of hay, grass and maize silage. According to production and milk yield, protein concentrate was supplemented (19 % digestible raw protein), as well as roughly crushed maize grains and a vitamin-mineral mixture. All cows could access food and water *ad libitum* during the year in the stall. Culling rate of the herd was 25.3 %, dry period of cows lasted approximately 60 days. Thirty randomly selected cows were included in the study. Cows were inseminanted by a professional inseminator when standing heat was observed. Voluntary waiting period at the farm was 60 days. The study took place from December 2003 until February 2005.

Body condition score and ultrasound examination

Body condition score was based on a method developed for freely moving animals (Edmonson *et al*., 1989). A score on scale of 1 to 5 points with 0.25 increments was assigned to each cow at calving, day 30 and day 60 post calving. Changes in BCS during two months were recorded. Ultrasound imaging of reproductive organs, using Aloka SSD 500 with 5 MHz linear transrectal probe, was performed in all animals every ten days from day 14 *postpartum* until day 30 of pregnancy or culling. Diameter of the dominant follicle (DF) was measured at the time of artificial insemination (AI) of each cow. Cystic ovarian disease (COD) and endometritis (EM) were also diagnosed using ultrasonography. COD were defined as hipoechogenic sturctures more then 2.5 cm in diameter which were present on ovaria for more then 10 days. EM was defined as hiperaemic endometrium with intrauterine fluid, with or without visible vaginal dicharge and with a present active *corpus luteum*.

Blood and milk samples

Blood samples were collected from the tail vein on day 14 *postpartum*, clotted and centrifuged at 3000 rpm. Serum was separated and stored at -20°C until assayed.

Milk samples were collected twice weekly, starting on day 14 *postpartum* and continuing until confirmation of a new pregnancy by ultrasound examination or culling. Samples were taken as strippings before milking. Special atention was paid to take samples only from healthy quarters. Samples were kept frozen (-20°C) until assayed.

Metabolic blood parameters

For the determination of metabolic parameters of cows, concentrations of NEFA, BHB, cholesterol and urea were measured. Animals revealing increased NEFA levels above 0.5 mmol/L or BHB levels above 1.4 mmol/L were classified in group 2. Those with levels below those were atributed to group 1.

Table 1. Methods used for the determinination of cholesterol, urea, BHB and NEFA concentrations using a biochemical analyser (COBAS MIRA)

Parameter	Method	Producer
Cholesterol	Enzyme calorimetric method (CHOD/PAP)	Roche Diagnostics
Urea	Enzymatic UV test with urease and GIDH	Roche Diagnostics
BHB	Williamson modify enzyme method	RANDOX
NEFA	Enzyme calorimetric method (ASC-ACOD)	WAKO Chemicals

Milk progesteron profiles

Milk progesterone concentrations were measured by a commercial kit Ovucheck Milk (Biovet Canada, No. VB-C006). Progesterone concentrations above 5 ng/mL in whole milk were considered as an indicator of luteal activity (Fagan in Roche, 1986). Progesterone profiles were defined according to Opsomer *et al*. (1998) as follows: normal profile, delayed cyclicity, cessation of cyclicity, prolonged luteal phase, short luteal phase and irregular profile.

Statistical analysis

Statistical comparison of the results obtained between the groups was performed with Mann-Whitney U-test using SigmaStat 2.1 program.

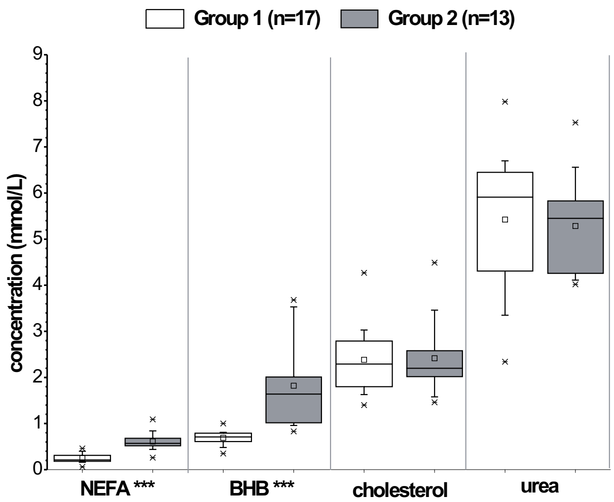
RESULTS

All animals included in the present study were divided in two groups according to cut off levels of NEFA and BHB concentrations set as 0.5 and 1.4 mmol/L, respectively. Graph 1 shows the level of blood serum metabolites in both groups. There is a significant difference in the mean concentrations of NEFA and BHB between Group 1 and Group 2 (0.25 mmol/L, 0.69 mmol/l and 0.61 mmol/L, 1.82 mmol/L; $P<0.001$ respectively), whereas the levels of cholesterol and urea did not differ significantly between the groups.

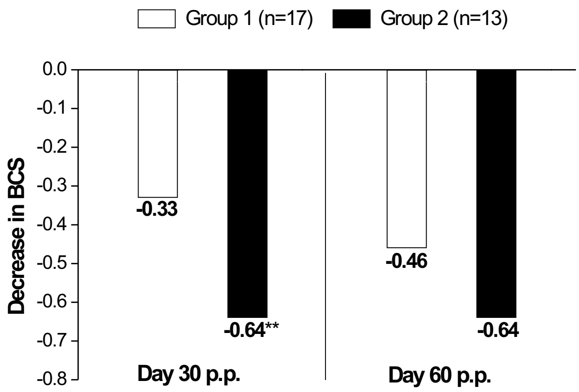
Graph 2 shows the average decrease in BCS from the day of parturition until the day 30 and 60 *postpartum*. Cows in Group 2 lost significantly more body tissue in 30 days *postpartum* comparing to Group 1 (0.64 vs. 0.33 BCS point). In 60 days *postpartum* the difference in decreasing BCS is not significant, although in Group 2 the average decrease was 0.18 BCS point higher than in Group 1.

Analysis of progesterone profiles (Table 2) shows that a significantly higher percentage of abnormal progesterone profiles (92.3%) were found in Group 2 comparing to Group 1 (23.5%). Delayed cyclicity represents 50.0% of all abnormal progesterone profiles in Group 2 and 43.7% of all abnormal profiles in the study.

Graph 3 shows the average decrease in BCS from the day of parturition until the day 30 and 60 *postpartum* in groups of cows with normal and abnormal progesterone profiles. Cows with an abnormal progesterone profile show higher decrease in BCS than cows with a normal progesterone profile (0.56 and 0.64 point at 30 and 60 day *postpartum*, respectively).



Graph 1. The level of metabolites in blood serum of dairy cows on day 14 *postpartum*. Box plot shows the median, upper and lower quartiles and range of the values
***P<0.001



Graph 2. Decreasing of body condition score in dairy cows from parturition until day 30 and 60 p. p.
**P<0.01

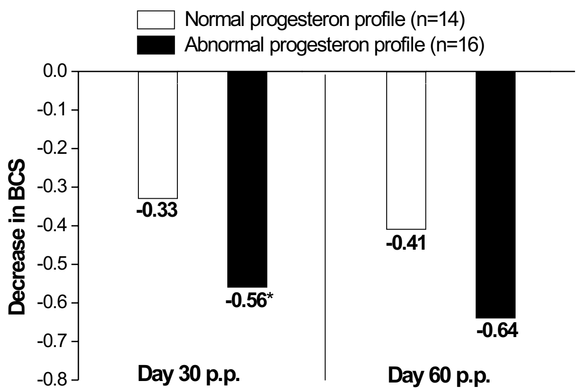
Reproductive and productive parametrs of both groups are presented in Tabele 3. Group 2 showed impaired reproductive efficiency in longer intervals from parturition to 1st AI and to conception comparing to Group 1 (81.3 and 106.8 days vs 73.9 and 89 days) and also a significanty longer period for the first progesterone rise was observed in Group 1 than in Group 2 (49.5 days vs 33.8

days). Appearance of COD is significantly higher in Group 2 comparing to Group 1 (23.1 % vs 5.9 %).

Table 2. Distribution of progesterone profiles in both groups

Type of Progesterone Profiles	Group 1 (n=17)	Group 2 (n=13)
Normal Profile (No of animals)	13	1
Abnormal Profiles (No of animals)		
Delayed Cyclicity	1	6
Prolonged Luteal Phase	1	3
Short Luteal Phase	1	3
Cessation of Cyclicity	1	0
Irregular Profile	0	0
Abnormal Profiles (%)	23.5	92.3***

*** P<0.001



Graph 3. Decreasing of body condition score in cows with normal and abnormal progesterone profiles
**P<0.05

Although between groups, there was no significant difference in milk yield during 100 days of lactation, cows in Group 2 showed significantly lower milk protein concentrations 30 and 60 days *postpartum* and higher Fat Protein Ratio 30 days *postpartum*.

Table 3. Comparison of reproductive and productive parameters in both groups

Parameters	Group 1 (n=17)		Group 2 (n=13)	
	Mean	SD	Mean	SD
Interval from parturition to 1st AI	73.94	23.52	81.27	13.43
Interval from parturition to conception	89.00	28.42	106.82	73.96
First observed estrus (day p.p.)	52	35.11	66	32.95
First progesterone rise (days p.p.)	33.82	12.99	49.54*	24.73
Diameter of DF at AI	1.58	0.37	1.54	0.24
Endometritis (%)	11.8		23.1	
COD (%)	5.9		23.1*	
Milk in 100 days (kg)	2479	359.24	2400	563.19
Milk fat (%) day 30 p.p.	4.17	1.05	4.23	0.53
Milk fat (%) day 60 p.p.	3.99	0.65	3.98	0.34
Milk protein (%) day 30 p.p.	3.36	0.14	3.08***	0.13
Milk protein (%) day 60 p.p.	3.47	0.15	3.16***	0.16
FPR day 30 p.p.	1.21	0.24	1.37*	0.14
FPR day 60 p.p.	1.15	0.20	1.23	0.10

*P<0.05; ***P<0.001;
DF – Dominant Follicle; COD – Cystic Ovarian Disease; FPR – Fat to Protein Ratio

DISCUSSION

The present study is based on a hypothesis that Slovenian domestic brown breed cows with increased concentration of NEFA and BHB above 0.5 and 1.4 mmol/L respectively in early *postpartum* period intensively mobilize body reserves and have impaired productive and reproductive parameters. 46.3 % of animals in the study were above those criteria. Several studies indicate decreasing in NEFA concentrations during the first few weeks *postpartum* and increasing BHB concentrations, which reach a maximum around day 14 *postpartum* (Opsomer, 1999; Duffield, 2004; Vanholder *et al*., 2005b). Butler and Smith (1989) and Nebel and McGillard (1993) also report that NEB usually reaches its nadir 14 days *postpartum*.

Cows were divided in two groups according to the cut off levels for NEFA and BHB set as 0.5 and 1.4 mmol/L, respectively. The significant decrease in BCS in Group 2 compared to Group 1 on 30 and 60 days *postpartum* confirmed the selection of cut off values for NEFA and BHB concentrations. However, cows in Group 1 have lost their weight more consistently during two months *postpartum* than cows in Group 2 (0.33 vs 0.41 BCS point), in the meantime BCS in Group 2 reached its nadir in the first 30 days postpartum and in the second month BCS in this group did not decrease anymore (0.64 BCS point). Animals which lost significantly more body weight in 30 and 60 days postpartum (0.56 and 0.64 BCS point respectively) were unable to cycle normally. Most authors agree that cows losing more then 0.5 points BCS during 30 days postpartum and more then 0.75 points during 60 days *postpartum* are significantly more at risk of reproduction failure (Loeffler *et al*., 1999a; Ferguson, 2000; Ferreira *et al*., 2005). On the other hand Opsomer *et al*. (2000) report that cows with normal progesterone profiles lost on average 0.26 BCS points in 30 days and 0.26 points in 60 day *postpartum*, while cows suffered from delayed ovarian function lost 0.39 and 0.49 points on days 30 and 60 postpartum, respectively.

The results in our study demonstrate a high occurrence of postpartal ovarial dysfunction. More then half of the progesterone profiles (53.3 %) showed an abnormal pattern. Delayed cyclicity represents 43.7 % of abnormal progesterone profiles and occured in 23.3% of cows included in the study. Our results are in agreement with the study of Opsomer *et al*. (1998) who found 47% of abnormal progesteron profiles and among them delayed cyclicity occured in 20.5% of cows. Incidence of abnormal progesterone profiles in our study is significantly higher in Group 2.

Cows in Group 1 started to cycle significantly earlier when compared to Group 2 which was, confirmed by the first progesteron rise on a day 34 and 50, recpectively. Results of many studies show a prolongation of the interval from parturition to first ovulation, indicated by first progesterone rise from days 25 to 37 postpartum (Fagan in Roche, 1986; Opsomer *et al.*, 1998; Petersson *et al*., 2006). Lucy (2001) found an interval to first ovulation of 29 days postpartum for a control line maintained as it was in year 1964 and an interval of 43 days for moderen high yielding dairy cows.

High percentage of COD in Group 2 (23.1%) is hardly explained just on the basis of NEB or high yield. Result concerning the correlation between COD and high milk yield are contradictory (Day, 1991). Recent studies indicate negative effects of NEFA on bovine granulosa cell function (Vanholder *et al.*, 2005a). However, they could not find significant correlations between NEFA and BHB concentrations and COD (Vanholder *et al*. 2005b).

Milk yield during 100 days of lactation was comparable between groups. Milk fat concentrations 30 days postpartum were higher in Group 2 wich can be explained by increased fat mobilization in a period of NEB. Significantly lower protein concentrations on days 30 and 60 postpartum in Group 1 than Group 2 (3.36 and 3.47% vs 3.08 and 3.16%, respectively) and higher fat-protein ratio in 30 days postpartum (1.21 vs. 1.37) in Group 2 than Group 1 could also indicate lower energy intake and/or decreased dry matter intake in the early postpartum period. Duffield *et al*. (1997) defined FPR 1.33 as a high margin; a value greater than 1.5 is considered a risk factor for metabolic disorders such a ketosis. Heuer *et al*. (1999) showed that large FPR in the early postpartum period, lower first service conception and increased number services per conception are associated. Loeffler *et al*. (1999b) found out that cows with extreme changes in FPR in a positive direction between test days had the lowest predicted chance of pregnancy.

According to results presented in our study we could conclude that cows with increased NEFA and BHB concentrations, and a higher decrease in body weight have impaired reproductive and productive parameters. They were exposed to NEB more then cows with lower levels of NEFA and BHB.

The aim of the present study was to examine how Slovenian brown-breed cows with stressed metabolism suffer from decreased fertility. We believe NEFA and BHB concentrations, body condition scoring, milk fat, protein and progesterone measurement are good criteria to practitioners for the recognition of the severity of NEB in the postpatum period. A timely recognition of NEB enables practitioners to react and to improve health status of animals, as well as their reproductive and productive efficiency.

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REFERENCES

1. *Butler WR*, 2000, Nutritional interactions with reproductive performance in dairy cattle, *Anim Reprod Sci*, 60-61, 449-57.
2. *Butler WR, Smith RD*, 1989, Interrelationships between energy balance and post partum reproductive function in dairy cattle, *J Dairy Sci*, 72, 767-83.
3. *Day N*, 1991, The diagnosis, differentiation and pathogenesis of cystic ovarian disease, *Vet Med*, 86, 753-76.
4. *Duffield TF, Kelton DF, Leslie KE, Lissemore K, Lumsden JH*, 1997, Use of test day milk fat and milk protein to predict subclinical ketosis in Ontario dairy cattle, *Can Vet J*, 38, 713-8.
5. *Duffield TF*, 2004, Monitoring strategies for metabolic disease in transition dairy cows, *Med Vet Quebec*, 34, 1, 34-5.
6. *Edmunson AJ, Lean IJ, Weaver LD, Farver T, Webster E,* 1989, A body condition scoring chart for Holstein dairy cows, *J Dairy Sci*, 72, 68-78.
7. *Eichner R,* 2004, Evaluation of the metabolic and nutritional situation in dairy herds: diagnistic use of milk components, *Med Vet Quebec*, 34, 1, 36-8.
8. *Fagan JG, Roche JF*, 1986, Reproductive activity in post partum dairy cows based on progesteron concentrations in milk or rectal examination, *Irish Vet J,* 40, 124-31.
9. *Ferguson JD*, 2000, Nutrition and reproduction in dairy herds, University of Pannsylvania – School of Veterinary Medicine, 65-82.
10. *Ferriera AM, Sa WF, Viana JHM, Camargo LSA, Pereira PAC et al,* 2005, Feed intake restriction, conception rate and parturition to conception interval in crossbred Gir-Holstein cows, Anim Reprod, 2, 2, 135-8.
11. *Huer C, Schukken YH, Dobbelaar P*, 1999, Postpartum body condition score and results from the first test milk as predictors of disease, fertility, yield and culling in commercial dairy herds, *J Dairy Sci,* 82, 295-304

12. *Klinkon M, Zadnik T, Nemec M, Mesaric M*, 2001, Evaluation of some blood parameters significant for estimation of energy and protein status of dairy cows during periparturient period, Proc 10th International Symposium Vet Lab Diagnostics, Salsomaggiore-Parma, 219-20.
13. *van Knegsel ATM, van den Brand H, Dijkstra J, Tamminga S, Kemp B,* 2005, Effect of dietary energy source and energy balance, production, metabolic disorders and reproduction in lactating dairy cattle, *Reprod Nutr Dev*, 45, 665-88.
14. *Loeffler SH, de Vries MJ, Schukken YH, de Zeeuw AC, Dijkhuizen AA et al,* 1999a, Use of AI technician scores for body condition, uterine tone and uterine discharge in a model with disease and milk production parameters to predict pregnancy risk at first AI in Holstein dairy cows, *Theriogenol*, 51, 1267-84.
15. *Loeffler SH, de Vries MJ, Schukken YH*, 1999b, The effects of time of disease occurrence, milk yield and body condition on fertility of dairy cows, J Dairy Sci, 82, 2589-600.
16. *Lucy MC,* 2001, Reproductive loss in high-producing dairy cattle: Where will it end?, *J Dairy Sci*, 84, 1277-93.
17. *Nebel RL, McGilliard ML*, 1993, Interactions of high milk yield and reproductive performance in dairy cows, *J Dairy Sci*, 76, 3257-68.
18. *Opsomer G, Coryn M, Deluyker H, de Kruif A*, 1998, An analysis of ovarian dysfunction in high yielding dairy cows after calving based on progesterone profiles, *Reprod Dom Anim*, 33, 193-204.
19. *Opsomer G*, 1999, Anoestrus postpartum in high yielding dairy cows: a field study, Gent, Faculteit Diergeneeskunde, PhD thesis, 121-47.
20. *Opsomer G, Grohn YT, Hertl J, Leavens H, Coryn M, de Kruif A,* 1999, Protein metabolism and resumption of ovarian cyclicity post partum in high yielding dairy cows, Annual ESDAR Conference, 54-7.
21. *Opsomer G, Grohn YT, Hertl J, Coryn M, Deluyker H, de Kruif A*, 2000, Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: a field study, *Theriogeno*l, 53, 841-57.
22. *Petersson KJ, Strandberg E, Gustafsson H, Berglund B*, 2006, Enviromental effects on progesteron profile measures of dairy cow fertility, *Anim Reprod Sci*, 91, 201-4.
23. *Schillo KK*, 1992, Effect of dietary energy on control of luteinizing hormonesecretion in cattle and sheep, *J Anim Sci*, 70, 1271-82.
24. *Vanholder T, Leroy J, Van Soom A, Opsomer G, Maes D et al*, 2005a, Effect of non-esterified fatty acids on bovine granulosa cell steroidogenesis and proliferation in vitro, Anim Reprod Sci, 87, 33-44.
25. *Vanholder T, Leroy J, Dewulf J, Duchateau L, Coryn M et a*l, 2005b, Hormonal and metabolic profiles of high yielding dairy cows prior to ovarian cyst formation on first ovulation post partum, *Repr Dom Anims*, 40, 46-9.
26. *Wildman EE, Jones GM, Wagner PE, Boman RL, Troutt HF et al,* 1982, A dairy cow body condition scoring system and its relationship to selected production characteristics, *J Dairy Sci*, 65, 495-501.

UTICAJ NEGATIVNOG ENERGETSKOG BALANSA NA PRODUKTIVNOST I PLODNOST SLOVENAČKOG MRKOG GOVEČETA

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SADRŽAJ

Cilj ovih ispitivanja je bio praćenje proizvodnih i reproduktivnih osobina Slovenačkih smeđih mlečnih krava u uslovima negativnog energetskog bilansa (NEB). Određivana je njihova telesna kondicija (BCS), koncentracija pojedinih metabolita kao što su neesterifikovane masne kiseline (NEFA), beta hidroksi butirat (BHB), holesterol i urea počevši od 14 dana *postpartum*. U istim intervalima je merena i koncentracija progesterona. Postpartalni period je praćen I ultrazvučnim pregledima svakog 10. dana, a registrovani su i reproduktivni parametri. Krave su podeljene u dve grupe u zavisnosti od koncentracije NEFA i BHB u serumu a kao granične vrednosti su uzete 0.5 i 1.4 mmol/L respektivno. Krave sa povećanom koncentracijom NEFA i BHB (grupa 2) u poređenju sa grupom 1, imale su veći pad u BCS 30 dana postpartum (0.64 vs 0.33 $P<0.01$), veća odstupanja u progesteronskom profilu ($P<0.001$) i znatno duži interval do prvog skoka u koncentraciji progesterona (49.54 vs 33.82 dana; $P<0.05$). Koncentracija proteina u mleku bila je 30 dana *postpartum* zanačajno manja u grupi 2 (3.08 % vs 3.36 %, $P<0.001$), dok je odnos mast/protein (FPR) 30. dana postpartum bio u ovoj grupi značajno veći (1.37 vs 1.21; $P<0.05$). Autori zaključuju da su merenje koncentracije NEFA i BHB, ocenjivanje telesne kondicije i rezultati analize mleka dobri kriterijumi za prepoznavanje negativnog energetskog bilansa kod mlečnih krava u ranom postpartalnom periodu.