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## Reproductive Performances of Holstein Cows with Different Milk Fat to Protein Ratio during Successive Stages of Lactation

Stoja Jotanović<sup>1</sup>, Ratko Mijatović<sup>2</sup>, Ivan Stančić<sup>3</sup>,  
Đorđe Savić<sup>1</sup>, Marinko Vekić<sup>1</sup>

<sup>1</sup>*Faculty of Agriculture, University of Banjaluka, Republic of Srpska, BiH*

<sup>2</sup>*Veterinary Station Banjaluka, Republic of Srpska, BiH*

<sup>3</sup>*Faculty of Agriculture, University of Novi Sad, Serbia*

### Abstract

Aim of study was to determine relationship between the energy status of cows, expressed through different milk fat to protein ratio values (FPR<1.0, 1.0-1.3 and >1.3), and their reproductive performances (interval from calving to first artificial insemination; calving to conception interval; insemination index; pregnancy duration; intercalving interval) during successive stages of lactation (day 15<sup>th</sup> to 45<sup>th</sup>; day 46<sup>th</sup>-75<sup>th</sup>; day 76<sup>th</sup>-105<sup>th</sup>; day 106<sup>th</sup> to 135<sup>th</sup> of lactation). The study included a total of 350 fresh calved Holstein cows (121 primiparous, 115 secundiparous and 114 cows which calved three or more times), kept and fed in usual farm conditions. Milk samples were taken during morning milking, and FPR value was calculated from concentrations of milk fat and protein for all milk samples. Reproductive parameters were calculated from farm data. Values of all reproductive parameters, except pregnancy duration, had generally increasing trend in all groups during successive stages of lactation. During successive stages of lactation, cows with optimal FPR values had generally better reproductive performances, compared to cows with increased or decreased FPR values, which indicates importance of FPR monitoring during early lactation for timely detection of cows predisposed for poor reproductive results.

*Key words:* cow, FPR, reproductive performances

## Introduction

During the last few decades, opposed to a significant increase in milk production, reproductive efficiency of cows decreased (Garcia-Ispuerto et al., 2007, Friggens et al., 2010). Decreasing of their reproductive efficiency is manifested through the delayed establishment of reproductive activity after calving, a frequent appears of irregular estrous cycles and reduced percentage of conception after mating or artificial insemination (Van Eerdenburg et al., 1996, Opsomer et al., 1998, Royal et al., 2002, Kerbrat and Disenhaus, 2004, Royal et al., 2008, Dobson et al., 2008). In addition to the increased incidence of reproductive disorders, it is considered that the decrease in reproductive efficiency is also a consequence of the negative energy balance (NEB), due to the inability of cows to adapt to the increased needs in energy and nutrients imposed by high milk production (Roche et al., 2000). Jorritsma et al. (2003) stated that the NEB usually lasts during the first 6-12 weeks of lactation, with a peak in the first three weeks. During this period there is a mobilization of body reserves of energy in the form of fat, and increasing of NEFA and ketone bodies, as well as decreasing of blood glucose concentration occurs (Chillard et al., 1998, Duffield, 2000), which all have influence of reproductive efficiency of cows.

Link between intense negative energy balance and reduced reproductive efficiency of cows lies in the activation of homeorhetic mechanisms to support lactation, which result in reduced frequency of LH pulses, decreased concentration of IGF-I and insulin, reduced production of estradiol in ovarian follicles exposed to NEB, and the harmful effects of metabolites (primarily NEFA and  $\beta$ -hydroxybutyrate) and reduced glycemia on survival of oocytes and early embryos (Leroy et al., 2006). Vitality of oocytes during early lactation decreases due to elevated concentrations of ketone bodies, urea and NEFA, and decreased glucose concentration in the follicular fluid (Leroy et al., 2008a,b). The sensitivity of the ovary to LH pulses during the period NEB is reduced (Butler, 2000), which leads to occurrence of irregular and anovulatory cycles. These findings are confirmed by Villa-Godoy et al. (1988) and Patton et al. (2007) who have found a positive correlation between energy status in early lactation and the duration of the period until the establishment of regular ovulatory estrous cycles. Similarly, Walsh et al. (2007) found negative correlation of ketonaemia intensity and duration to values of insemination index.

As a result of reduced food intake and mobilization of body reserves of energy during early lactation, changes of concentration of organic milk ingredients are occurring, which can be used as an indicator of the energy and nutritional status of the cow, and, potentially, for the prediction of their reproductive performances. Due to intensive lipomobilisation and increased

NEFA concentration in blood, concentration of milk fat increases, with the critical value of 45g /L (Šamanc et al., 2006, Savić et al., 2010, Kirovski et al., 2012). At the same time, as a result of reduced food intake and increased utilization of amino acids for gluconeogenesis, synthesis of microbial protein, main amino acids source for milk protein synthesis, is reduced, which leads to decrease of milk protein concentration (Broderick and Clayton, 1997, Jenkins and McGuire, 2006). Positive correlation between milk protein concentration and the energy status of cow is indicated by Magdus et al. (1988). In order to avoid the influence of various factors (breed, diet, age of animals) on milk fat and protein concentrations, milk fat to proteins ratio (FPR) has been used, as a relative indicator of cow's energy status. Optimal FPR value ranges from 1,0 to 1,3 (Čejna and Chládek, 2005, Kuterovac et al., 2005, Friggens et al., 2007). FPR values below 1.0 (inversion of milk fat and protein concentration) indicate subacute rumen acidosis and decrease in food intake, while those over 1.3 (but particularly over 1.5) indicate NEB and lipomobilisation with accompanying ketonaemia and ketoacidosis (Čejna and Chládek, 2005, Kuterovac et al., 2005, Friggens et al., 2007), which in each case would have a negative impact on health and reproductive parameters. Energy status of cows is gradually stabilizing and FPR value has declining trend with the progress of lactation, which is confirmed by our previous studies (Savić et al., 2012,2013).

Assuming all abovementioned, the aim of this study was to determine relationship between the energy status of cows, expressed through different FPR values, and their reproductive performances during successive stages of lactation.

## Material and Methods

The study included a total of 350 fresh calved Holstein cows (121 primiparous, 115 secundiparous and 114 cows that have calved three or more times), housed in a free system on the industrial type farm and fed standard rations for a given productive category and period of the year. Detection of estrus was done daily by using pedometer, as well as by visual inspection of cows during milking. Cows that showed signs of estrus were inseminated by trained inseminators within 12 hours of onset of signs of estrus. Diagnosis of pregnancy was performed by rectal examination 9-12 weeks after the last insemination. All cows with any health disorders were excluded from further research, in order to exclude the effect of any clinically manifested disease on reproductive parameters of cows. Cows with calving to conception interval longer than 135 days and those inseminated more than four times were considered as having reproductive disorders and excluded from study.

All surveyed cows, regardless of parity, were divided according to the stage of lactation (the first - day 15<sup>th</sup> to 45<sup>th</sup>; the second - day 46<sup>th</sup>-75<sup>th</sup>; the third - day 76<sup>th</sup>-105<sup>th</sup>; and the fourth - days 106<sup>th</sup> to 135<sup>th</sup> of lactation ), and within each of them formed subgroups in relation to the value of milk fat to protein ratio (FPR<1.0, 1.0-1.3 and >1.3). Following reproductive parameters were monitored: the interval from calving to first artificial insemination (days from calving date until the date of the first artificial insemination, CFI); calving to conception interval (days from the date of calving to date of a successful artificial insemination, CCI); insemination index (average number of artificial inseminations required for the establishment of pregnancy, II); duration of pregnancy (days from the date of successful artificial insemination to the date of the next calving, PD); intercalving interval (days from the date of one to the next calving date, ICI).

The milk samples for assessment of chemical composition were taken once a month, during the morning milking. The concentrations of milk fat and protein were determined by Milko-Scan FT 6000 (Foss Electric, Hillerød, Denmark). Based on the obtained values, milk fat to protein ratio was calculated, according to the formula:

$$FPR = \frac{\text{Concentration of milk fat (g/L)}}{\text{Concentration of proteins (g/L)}}$$

The results were analyzed by descriptive statistics and statistical significance of differences between the studied parameters was tested using ANOVA and with the help of Student's "t" -test. As statistically significant, differences at the level of P<0.05 and P<0.01 have been taken.

## Results and Discussion

Reproductive parameters of cows with different values of FPR in successive stages of lactation are shown in Table 1.

During the first stage of lactation, 57.55% of cows had optimal FPR values, vs. 13.67% cows with reduced and 28.78% cows with elevated FPR. Cows with optimal FPR values had significantly shorter calving to the first insemination interval and calving to conception interval (P<0.01, P<0.01), and lower insemination index compared to the cows with FPR values outside the optimal range, which resulted in the significantly shorter intercalving interval (P<0.01). The values of pregnancy duration showed no statistically significant differences between the surveyed groups of cows.

Tab. 1. Reproductive parameters of cows ( $M \pm SE$ ) with different values of FPR in successive stages of lactation  
*Reproduktivne parametari krava ( $M \pm SE$ ) sa različitim vrijednostima OMP tokom sukcesivnih stadijuma laktacije*

Stage of lactation Stadijum laktacije	Group Grupa	Parameter Parametar				
		CFI (days) PVO (dana)	CCI (days) SP (dana)	II IO	PD (days) G (dana)	ICI (days) MTI (dana)
First(days 15 <sup>th</sup> -45 <sup>th</sup> ) Prvi (15-45 dana)	FPR<1 OMP<1 (n=19)	83.11±6.69 <sup>A</sup>	102.26±5.42 <sup>A</sup>	1.63±0.22	278.47±1.14	380.74±5.92 <sup>A</sup>
	FPR 1-1.3 OMP 1-1.3 (n=80)	66.40±2.65 <sup>B</sup>	81.40±3.02 <sup>B</sup>	1.46±0.07	278.80±0.64	360.20±3.06 <sup>B</sup>
	FPR>1.3 OMP>1.3 (n=40)	67.83±2.68 <sup>C</sup>	82.70±4.20 <sup>C</sup>	1.50±0.11	278.48±0.94	361.18±4.05 <sup>C</sup>
Second(days 46 <sup>th</sup> -75 <sup>th</sup> ) Drugi (46-75 dana)	FPR<1 OMP<1 (n=17)	62.53±3.45	89.17±5.69	1.76±0.20	278.88±1.27	368.06±6.20
	FPR 1-1.3 / OMP 1-1.3 (n=64)	73.31±2.83	90.03±2.98	1.58±0.09	277.72±0.67	367.75±3.03
	FPR>1.3 OMP>1.3 (n=21)	76.48±6.22	89.76±6.06	1.48±0.16	279.00±0.93	368.76±6.50
Third(days 76 <sup>th</sup> -105 <sup>th</sup> ) Treći (76-105 dana)	FPR<1 OMP<1 (n=18)	82.11±5.27	106.00±3.36	1.83±0.19	278.06±1.18	384.06±3.34
	FPR 1-1.3 OMP 1-1.3 (n=32)	79.44±4.19	107.91±3.29	1.91±0.14	278.44±1.49	386.34±3.81
	FPR>1.3 OMP>1.3 (n=18)	67.11±3.64	98.17±3.57	2.22±0.15	278.78±1.12	376.94±3.96
Fourth (days 106 <sup>th</sup> -135 <sup>th</sup> ) Četvrti (106-135 dana)	FPR<1 OMP<1 (n=20)	73.00±3.05 <sup>A</sup>	125.30±1.33	2.85±0.15 <sup>a</sup>	278.10±1.66	403.40±2.14
	FPR 1-1.3 OMP 1-1.3 (n=21)	86.00±3.26 <sup>B</sup>	128.52±1.09	2.43±0.11 <sup>b</sup>	280.57±1.53	409.10±2.26

Legend: FPR - Fat to protein ratio; CFI - Calving to the first insemination interval; CCI - Calving to conception interval; II - Insemination index; PD - Pregnancy duration; ICI - Intercalving interval; Values with different superscript in same stage of lactation are statistically different (<sup>a, b, c</sup>  $p < 0.05$ , <sup>A, B, C</sup>  $p < 0.01$ )

Legenda: OMP - odnos mliječne masti: proteini; PVO - period od teljenja do prvog vještačkog osjemenjavanja; SP - servis period; IO - indeks osjemenjavanja; G - trajanje graviditeta; MTI - međutelidbeni interval; Vrijednosti sa različitim superskriptom unutar istog stadijuma laktacije se statistički značajno razlikuju (<sup>a, b, c</sup>  $p < 0.05$ , <sup>A, B, C</sup>  $p < 0.01$ )

In the second stage of lactation, the optimal values of FPR was found in 62.75% of the cows, vs. 16.67% cows with reduced and 20.59% cows with elevated FPR value. Calving to the first insemination interval was the shortest in cows with reduced FPR, which had the highest value of insemination index, even no statistically significant differences to the other groups of cows was found. Calving to conception interval and pregnancy duration had similar values in all groups of cows, which resulted in similar duration of intercalving interval.

During the first stage of lactation, 57.55% of cows had optimal FPR values, vs. 13.67% cows with reduced and 28.78% cows with elevated FPR. Cows with optimal FPR values had significantly shorter calving to the first insemination interval and calving to conception interval ( $P<0.01$ ,  $P<0.01$ ), and lower insemination index compared to the cows with FPR values outside the optimal range, which resulted in the significantly shorter intercalving interval ( $P<0.01$ ). The values of pregnancy duration showed no statistically significant differences between the surveyed groups of cows.

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During the third stage of lactation, the optimum value of FPR was found in 47.06% cows, vs. 26.47% cows with reduced and 26.47% cows with elevated FPR values. The interval from calving to the first artificial insemination, calving to conception interval and intercalving interval were the shortest in cows with elevated FPR values, although no statistically significant difference compared to other groups of cows was found. Value of insemination index was the lowest in cows with reduced FPR values, although no statistically significant difference compared to other groups of cows was found.

In the fourth stage of lactation, 51.22% cows had optimal value of FPR, vs. 48.78% of cows with reduced FPR values. Cows with optimal FPR values had significantly lower insemination index ( $P<0.05$ ) and longer calving to the first insemination interval ( $P<0.01$ ), but no statistically significant differences in the duration of calving to conception interval and intercalving interval.

Increased metabolic load for high milk production, intense NEB during early lactation and its extended duration are important reasons for decreasing of reproductive efficiency in high-yielding dairy cows. Opsomer et al. (2006) found that the increase in milk production by about 1000 liters per lactation resulted in prolongation of intercalving interval for eight days (from 399 to 407 days). This prolongation is, according to their interpretation, result of prolonged



calving to the first insemination interval, which is confirmed by their previous study (Opsomer et al., 2000), in which cows without oestrus occurrence in first 60 days after calving had longer calving to conception interval for 26 days (111 vs. 85 days) compared to control cows.

The duration of the interval from calving to the first artificial insemination in cows included in this study were in the majority group shorter compared to the results of Podpečan et al. (2013), found its values of 85 days in clinically healthy cows, 85 and 80 days in those with clinical ketosis and mastitis and 100 days in cows with reproductive disorders. Podpečan et al. (2007a) found that this interval was shorter in cows with better energy status (73.94 vs. 81.27 days), when cows were grouped on the basis of NEFA and  $\beta$ -hydroxybutyrate concentrations in day 14 after calving.

Podpečan et al. (2007b) found positive correlation between the FPR value during successive periods of early lactation and duration of calving to conception interval in Holstein and Brown Swiss cows ( $r=0.452$ ,  $r=0.358$  and  $r=0.415$  for successive periods of lactation, respectively). Similar results were reported by Paura et al. (2012), who found the extension of calving to conception interval for 20 days (140.4 vs. 119.9 days) in Latvian Brown and Holstein cows with FPR value outside physiological range (over 1.5 and below 1.1) compared to cows with FPR values inside that range. Podpečan et al. (2013) found the difference in the duration of the calving to conception interval of 35 days (87 vs. 122 days) with the division of clinically healthy cows to groups with FPR above and below the 1.37. In cows with health disorders these authors found differences of 14 (121 vs. 135) days in ketotic and 35 (136 vs. 171) days in cows with reproductive disorders. These authors found the strongest correlation between FPR and reproductive performance of cows during the second month of lactation, as opposed to their previous findings (Podpečan et al., 2008) that this correlation was strongest during the third month of lactation. Results of our study are consistent with the allegations of those authors, and better of them in almost all groups, which is probably caused by used experimental design and limiting the physiological calving to conception interval to 135 days. Also, in the interpretation of the obtained results, the number of cows in each group should be beared in mind, as in all periods of tests the largest number of cows had FPR value in the optimal range, which is reflected in the values of all surveyed parameters.

Loeffler et al. (1999) indicate that a cows with an increase in the value of the FPR in insemination time have a higher insemination index compared to cows in which the value of FPR remained the same or decreased within the physiological range. Values of insemination index, found in our study, were within acceptable interval of 1,5-2,0 (according to data given by Orešnik, 2009) in most of groups. Groups of cows with a higher insemination index

values (cows with FPR over 1.3 in the third and both groups of cows in the fourth stage of lactation) were previously unsuccessfully inseminated, which, in addition to the energy status, can be associated with inadequate detection of estrus and untimely insemination. The values of insemination index were positively related to the duration of the calving to conception interval and were generally lower in cows with the optimal values of the FPR.

Pregnancy duration in cows is genetically determined and does not show significant variations, which is confirmed in this study. The values established in different groups of cows in this study were almost identical, and similar to literature values (280 to 285 days, according to Ball and Peters, 2004). Intercalving interval duration in different groups of cows in our study ranged between 307 and 440 days, and was primarily influenced by the duration of the calving to conception interval. Our results are significantly better than those reported by Pocrnja et al. (2011), who found intercalving interval range from 323 to 696 days. Biderman et al. (2007) found that the increase of the FPR value for a single unit during the first 100 days of lactation resulted in a prolongation of calving interval for 66, 37 and 32 days in primiparous and 54, 38 and 42 days in multiparous cows (black and white, Simmental and brown breed, respectively). In support of the impact of FPR value on the length of calving interval, are results of Paura et al. (2012), who found its longer duration in cows with FPR values outside the range of 1.1 to 1.5 compared to cows with FPR value within this framework (425.4 vs. 402.6 days).

## Conclusion

Results of this study indicate a positive relation between energy status of cows, expressed through FPR value, and reproductive parameters. FPR values can be used as a good indicator of energy status, and for finding cows potentially susceptible to reproductive disorders, so diagnostic and therapeutic procedures for minimising of consequences of negative energy balance on reproductive performances can be undertaken.

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# Reproduktivne performanse krava holštajn rase sa različitim odnosom mliječna mast : proteini tokom sukcesivnih stadijuma laktacije

Stoja Jotanović<sup>1</sup>, Ratko Mijatović<sup>2</sup>, Ivan Stančić<sup>3</sup>,  
Đorđe Savić<sup>1</sup>, Marinko Vekić<sup>1</sup>

<sup>1</sup>Poljoprivredni fakultet, Univerzitet u Banjoj Luci, Republika Srpska, BiH

<sup>2</sup>Veterinarska stanica AD Banjaluka, Republika Srpska, BiH

<sup>3</sup>Univerzitet u Novom Sadu, Poljoprivredni fakultet, Srbija

## Sažetak

Cilj rada bio je da se ustanovi povezanost između energetskeg statusa krava, iskazanog kroz vrijednosti odnosa mliječna mast : proteini (OMP<1.0, 1.0-1.3 i >1.3) i njihovih reproduktivnih performansi (period od teljenja do prvog vještačkog osjemenjavanja, servis period, indeks osjemenjavanja, trajanje graviditeta, trajanje međutelidbenog intervala) tokom sukcesivnih stadijuma laktacije (15-45; 46-75; 76-105; 106-135 dana laktacije). Istraživanjem je obuhvaćeno ukupno 350 oteljenih krava Holštajn rase (121 prvotelka, 115 drugotelki i 114 krava koje su se telile tri i više puta), držanih i hranjenih u uobičajenim farmskim uslovima. Uzorci mlijeka uzimani su tokom jutarnje muže, a vrijednost OMP za sve uzete uzorke mlijeka izračunata je, na osnovu vrijednosti koncentracija mliječne masti i proteina. Vrijednosti reproduktivnih parametara izračunate su na osnovu podataka iz evidencije farme. Vrijednosti svih reproduktivnih parametara, sa izuzetkom trajanja graviditeta, imale su trend porasta u svim grupama krava tokom sukcesivnih stadijuma laktacije. Tokom sukcesivnih stadijuma laktacije, krave sa optimalnim vrijednostima OMP imale su generalno bolje reproduktivne performanse u odnosu na krave sa povišenim ili sniženim vrijednostima OMP, što ukazuje na značaj praćenja vrijednosti OMP tokom rane laktacije za pravovremeno otkrivanje krava predisponiranih za pojavu lošijih reproduktivnih parametara.

*Ključne riječi:* krava, OMP, reproduktivne performanse

Stoja Jotanović  
E-mail address: [stoja.jotanovic@agrofabl.org](mailto:stoja.jotanovic@agrofabl.org)

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