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GEREŠ, D., R. TURK, D. ŽUBČIĆ, B. VULIĆ, N. STAKLAREVIĆ, B. ŽEVRNJA: Influence of nutrition in the postparturient period on the fertility of dairy cows. Vet. arhiv 79, 119-130, 2009.

ABSTRACT

Essentially, nutrition affects the fertility of dairy cows. The incidence of functional forms of subfertility is constantly increasing, especially due to disturbances in the balance of degradable proteins and energy in forage. This research was undertaken on one hundred primiparous Frisian dairy cows, divided into two groups (group A, n=50; group B, n=50) in the early breeding period, 44th to 52nd day postpartum. Average lactation in both groups was 32 litres of milk. Gynaecological findings in group A were: proestrus 5, oestrus 3, dioestrus 6, cystic ovarian disease (COD) 6, anoestrus 24, endometritis 4 and pyometra 2. Findings in group B: proestrus 6, oestrus 5, dioestrus 6, COD 5, anoestrus 21, endometritis 4, pyometra 3. All cows with clinically diagnosed with endometritis, pyometra and COD were treated twice or three times with synthetic prostaglandin F2alfa analogues. Over three weeks the cows from group A were fed extensively, ad libitum, and the feed was composed of corn silage (50% less than in the feed for group B) lucerne haylage, lucerne hay and 10% concentrated type food without soybean, maize and sunflower cake, with barley and cca 70-80% bran. Cows from group B were fed with standard farm concentrated mixture for dairy cows (meal of 34.5 kg of food was composed of corn silage, lucerne haylage, lucerne hay and c 19% concentrated type food, with 20.03 kg of dry matter and 16.3% proteins. The concentrated type food was composed of soybean (6%), maize (20%), sunflower cake (1%), TMR Camisan (5%), bran (50%) and barley (18%). Three weeks later the gynaecological findings in groups A: B were: proestrus 19:7, oestrus 7:6, dioestrus 20:6, COD 0:7, anoestrus 3:20, endometritis 1:3, pyometra 0:1. Within one hundred days from calving 29 cows from group A (58%) conceived, with average service time of 94 days, and 14 cows (28%) from group B conceived, with average service time of 96 days. Up to the 150th day of lactation 47 cows from group A (94%), and 36 (72%) from the control group conceived. Average lactation

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during the three weeks of research was 30 litres in group A, and 31 litres of milk in group B. In the beginning of the research, the body condition score of the cows was between 1.5 and 2.5 points. On the 100th day after calving the cows from group B were unequal in condition (BCS from 1.5 to 3.25, average 2.03 points), and cows from group A were equal in condition with an average of BCS 2.93. On the 150th day after calving the average BCS of cows from group A was 3.04, and cows from group B 2.69. Cows . fed with corrected nutrition with natural high rumen-degradable proteins showed good reactions to the procedures of introduction to breeding. The goal of this study was to compare the effects of two different regimes of nutrition on the beginning of reproduction and on reproductive efficiency, i.e. to prove the better effect of nutrition rich in rumen-degradable proteins compared to standard farm mixture for nutrition of diary cows in lactation.

Key words: dairy cow, subfertility, postparturient period

Introduction

Dairy farming has only one aspiration, higher production, with the consequence of decreased fertility. Average conception rate with the first insemination is lower than 40% (BUTLER, 2000; BOUCHARD and TREMBLAY, 2003). Inadequate nutrition during gestation, lactation and dry period, and insufficient conversion of energy in early lactation, have resulted in prolongation of the period of negative energy balance (NEB) with the consecutive delay of the beginning of reproductive functions with fertility reduction (BUTLER, 2001). Almost 50% of cows in modern farming suffer from ovarian dysfunctions in the preservice postpartum period. The most important disorders are delayed cyclic resumption, anovulation and the prolonged luteal phase, and they represent almost 90% of all abnormalities (OPSOMER et al., 1998).

Nutrition affects the neuroendocrine regulation of reproduction. Cows in high lactation are in a negative energy balance which negatively affects the normal development of follicles. The decrease in reproductive efficiency in early lactation is probably a consequence of disorders in metabolic adjustment of cows to conditions of NEB, a dynamic process to which they adapt more or less successfully. Metabolic status is characterised by decreased levels of insulin, IGF, leptin, nonesterified fatty acids (NEFA) in plasma (WEBB et al., 1999; JORRITSMA et al., 2003; BOUSQUET et al., 2004) and progesterone in the first three cycles (VILLA-GODOY et al., 1988).

NEB, because of the decreased levels of glucose and insulin, with an elevated NEFA level delays the beginning of pulsating level of gonadotrophins LH and FSH, which are indispensable for folliculogenesis stimulation. A decreased level of insulin leads to reduction of IGF-1 production in the liver, with consecutive ovarian refractibility to gonadotrophins. Reduced secretion of progesterone after calving slows down uterine involution and ovarian activity. Sustaining a balanced regime of nutrition is essential because it is not possible to compensate for an insufficient intake of energy.

Severe negative energy balance causes a considerable decrease in body condition (BC) for the first thirty days after calving and a consecutive delay of the first ovulation; as a result 50% of cows have anovulatory cycles until the 50^{th} day of lactation, which

proves the reversed correlation between conception rates and BC loss (STAPLES et al., 1990; STEVENSON, 2001). There is a great possibility that cows with anovulatory cycles prolonged to more than 50 days will not conceive. Energy status in the first 20 days of lactation is in inversed correlation with physiological ovulation. In cows with severe NEB, ovarian function rises 10 days after the most serious disbalance, at that time normalisation and production of periovulatory follicles begins. Usually it is a question of cows which have lost a lot of weight in the peripartal period and early lactation (BUTLER et al., 1981). Improvement of body condition, as a result of the decline of NEB, is the main modulator in the resumption of cyclic activity.

The goal of the study was to research the effects of nutrition on the incidence of functional forms of subfertility in dairy cows in farm conditions. Also, the goal was to investigate the positive effects on reproductive efficiency of nutrition with high degradable (HD) proteins, without any intake of excessive energy.

Materials and methods

All of 100 cows were primiparous Frisian dairy cows, bought in the Netherlands and seven months gravid, on average 26 months old. Cows were used in the research from 44th to 52nd day after calving. They all came from the same surroundings and were kept extensively in half-open buildings. Until the beginning of research, all the cows were fed with standard farm mixture with 16% protein (*ad libitum*, the meal was composed of corn silage, lucerne haylage, lucerne hay and c 19% of concentrated type of food, with 20.03 kg of dry matter and 16.3% proteins. Concentrated type was composed of 6% soybean, 20% maize, 1% sunflower cake, 5% TMR Camisan, 50% bran and 18% barley. Cows were picked out randomly (odd and even), and among the chosen there were no cows with a history of dystocia, retained placenta, milk fever or toxichemical metritis. Also, cows with lameness and clinically manifested endometritis were eliminated. Two groups (A and B) were formed, including 50 cows each. Group A was experimental, and B the control group.

At the beginning of research cows were examined gynaecologically and using ultrasound (Aloka device with 5 MHZ rectal linear probe). Cows from group A were fed for three weeks with feed *ad libitum*, composed of corn silage (50% less than in the feed for group B), lucerne haylage, lucerne hay and 10% concentrated type food without soybean, maize and sunflower cake, with barley and c 70-80% of bran. Cows from group B were fed the usual concentrated mixture for dairy cows with average production of 35 litres with feed of 34.5 kg of food (corn silage, lucerne haylage, lucerne hay and c 19% concentrated type food, with 20.03 kg of dry matter and 16.3% proteins. The concentrated food was composed of 6% soybean, 20% maize, 1% sunflower cake, 5% TMR Camisan, 50% bran and 18% barley. During the three weeks cows were regularly

controlled gynaecologically. After 21 days, before the beginning of breeding, they were examined gynaecologically again.

Cows with endometritis, pyometra and cystic ovarian disease (COD) were treated repeatedly with synthetic analogues PGF2alfa (2-3 protocols). Some of the cows underwent oestrus synchronisation (GPG) and induction of ovulation procedures, and afterwards they were artificially inseminated.

The final artificial insemination (AI) was performed on the 100th day after calving, and afterwards all the cows that did not conceive were inseminated until conception. The first gynaecological and ultrasound diagnosis of early pregnancy was performed on the 21st day after AI, the second on the 30th and the third control on the 40th day after AI. Standard feeding procedures were restored for the cows in group A, whilst the non-gravid cows were treated until conception.

Statistical differences between the investigated groups were found, after testing the data for normality and equal variance, by either Mann-Whitney rank sum-test or Student's t test. The values P<0.05 were considered significant using SigmaStat 3.0 for Windows (Systat Software Inc., Richmond, California, USA).

Results

In group A, before the beginning of research proestrus was diagnosed in 5 cows (10%), oestrus in 3 (6%), dioestrus in 6 (12%), COD in 6 (12%), anoestrus in 24 (48%), endometritis in 4 (8%) and pyometra in 2 cows (4%). After three weeks of changed nutritional treatment proestrus was diagnosed in 19 cows (38%), oestrus in 7 (14%), dioestrus in 20 (40%), anoestrus in 3 (6%), endometritis in 1 (2%) cow, and there were no diagnosed COD or pyometra (Fig. 1).



Fig. 1. Incidence of ovarian activity and reproductive disorders in cows in group A before and three weeks after nutritional treatment

In group B, before the beginning of the research, proestrus was diagnosed in 6 cows (12%), oestrus in 5 (10%), dioestrus in 6 (12%), COD in 5 (10%), anoestrus in 21 (42%), endometritis in 4 (8%), and pyometra in 3 cows (6%). Three weeks after the beginning of research proestrus was diagnosed in 7 cows (14%), oestrus in 6 (12%), dioestrus in 6 (12%), COD in 7 (14%), anoestrus in 20 (40%), endometritis in 3 (6%), and pyometra in 1 cow (2%), (Fig. 2).



Before the nutritional treatment
Three weeks after the nutritional treatment

Cows from group A, in comparison with their status before nutritional treatment, showed an increased incidence of cyclic ovarian activity (proestrus, oestrus) and ovulatory cycles, and a decreased incidence of anoestrus and COD. In cows from group B cyclic activity was hardly elevated, but incidence of COD was increased (4%).

Table 1. Body condition score (BCS) in cows in group A and B before the nutritional treatment as well as 100th and 150th day postpartum

	Mean ± SD	
	Group A	Group B
44 - 52 days postpartum (before the nutritional treatment) (1)	1.79 ± 0.29	1.79 ± 0.29
100 th day postpartum (2)	$2.93 \pm 0.42^{*1,2}$	$2.03 \pm 0.48^{*1,2}$
150 th day postpartum (3)	$3.04 \pm 0.35*$	$2.69 \pm 0.61^{*2,3}$

^{*}statistical significant difference between groups A and B; ^{1,2} statistical significant difference between 44-52 days postpartum and 100th day postpartum in each group; ^{2,3} sstatistical significant difference between 100th and 150th day postpartum in each group

Fig. 2. Incidence of ovarian activity and reproductive disorders in cows in group B before and three weeks after the nutritional treatment

On the 100th day of lactation 29 (58%) cows from group A and 14 (28%) cows from group B conceived. Average service time of cows from group A was 94 days, and cows from group B 96 days. On the 150th day of lactation 47 (94%) cows from group A and 36 (72%) cows from group B conceived (Fig. 3)



Fig. 3. Conception rate (%) in cows in group A and group B until 100th and 150th day postpartum

Before the beginning of the research BCS was not significantly different between cows from group A and cows from group B, and its value was between 1.5 and 2.5. On 100th day after calving the statistical values of BCS were significantly higher in cows from group A (P<0.001). Also, on the 150th day of lactation cows from group A had significantly higher BCS than cows from group B (P=0.005).

On the 100th day of lactation, cows from group A reached the desirable BCS, which was significantly higher than before the beginning of the research (P<0.001) and it did not significantly change between the 100th and 150th days of lactation.

The statistically significant differences of the values before the beginning of the research and the 100^{th} day of lactation (P=0.020) and of the values between the 100^{th} and 150^{th} day of lactation (P<0.001) show that the BCS in cows from group B gradually increased. On the 150^{th} day of lactation cows from group B reached a BCS equal to the BCS that cows from group A had on the 100^{th} day of lactation (Table 1).

Discussion

Breeders frequently ignore or do not know normal bovine physiology. Cows in their dry period and in late pregnancy are not fed properly because they are not producing, which is wrong, because the consequences of low-quality nutrition will not show themselves until 40 days later (ELROD and BUTLER, 1993; BALL et al., 1995). In intensive dairy farming subfertility is most commonly a consequence of COD, luteal persistency,

irregular oestrus intervals, incorrect timing of artificial insemination and anoestrus (OPSOMER et al., 1996). Some authors state that COD is the most common endocrine disorder in dairy cows, which occurs 30 to 60 days after parturition in 1-30% of cows, especially in Holsteins (LOPEZ-DIAZ and BOTSU, 1992; JEFFCOATE and AYLIFFE, 1995). The average incidence of COD in both groups at the beginning of the research was 11% (12% in group A and 10% in group B). At the end of the research, the incidence in group A was 0% and 14% in group B, which confirmed the positive effect of nutrition rich in high-degradable proteins. OPSOMER (1999) states that the problem of COD is even more complex because of its close relationship with insulin metabolism. Obstinacy and late appearance of cysts after the 50th day of the service period is a consequence of the nonoccurrence of ovulation and increased luteinisation, most probably due to the intake of large amounts of estrogenic substances (percentage of soy is 60%), so this corresponds to OPSOMER et al. (1996). The incidence of anovulatory cycles with regression of the early stages of follicles in cows from group B is the most common consequence of feeding with too high energy value feed, corresponding to the statements by MORGAN and WILLIAMS (1989). A reduced level of LH causes mature follicles to keep producing estradiol, which in turn causes failure of ovulation and growth of intraovarian follicular cysts (ROYAL et al., 2000). FORTUNE (1993) thinks that occurrences in oestrus are limiting factors for conception because adjustment to changes caused by disorders is not possible. Results of the research correspond to what is stated above because the incidence of cysts in cows from group B increased in the mid lactation period.

Anoestrous cycles are caused by factors like retained foetal membranes, (WRIGHT et al., 1992; OPSOMER et al., 1996), but a more common case is poorly manifested oestrus in suboestric cows, which appears every 5-7 days (minimally two FSH surges in the period of one oestrus cycle). ARTHUR and BEE (1996) state that the main causes of problems are to be found in management, especially nutrition. RISCO et al. (1995) sees the way out of the problem in quality postpartum management, especially nutrition, which is usually poor in degradable proteins, rich in undegradable proteins and energy, and it has low degradability which makes it impossible for the liver to metabolize nutrients properly. The consequences are lipid mobilisation syndrome and fatty liver. Excessive feeding in the late lactation period and the dry period does not cause reproduction problems but it does cause puerperal problems (De KRUIF and MIJTEN, 1992). Cows from group B were fed with feed too rich in energy (the system of nutrition was adjusted to cows producing 35 litres, average production of 31 litres). A bran and hay diet was enough to maintain average production of 30 litres. Three weeks of the modified nutrition regime resulted in a significant increase of ovarian activity, while in group B the incidence of anoestrus did not change.

The beginning of ovulation may be delayed for a few days. SENGER (1994), FORTUNE (1993), NEBEL et al. (1994) emphasize the importance of oestrus detection and consequences due to the incorrect timing of artificial insemination. Highly productive cows may ovulate within four weeks postpartum but oestruses will, most probably, not be detected (MACMILLAN et al., 1996). LAMMING and DARWASH (1995) state that ovulation induction procedure is necessary for these cows. The incidence of ovulatory cycles after three weeks did not increase in group B, but in group A it tripled (6:20).

Body condition loss is an important factor of reproductive efficiency. Optimal BCS at calving is 3 to 3.75; 2.25 to 2.75 at high yielding; 3 to 3.5 from the 150^{th} -200th day of lactation and 3.0 to 3.75 in dry cows. In cows that were overfed during the dry period (BCS >=4) there is a 2.5 times higher risk of illness (dystocia, retained placenta, metritis, abortus) in the next lactation, in comparison to cows with BCS 3 to 3.5 (GEARHART et al., 1990). In cows that lose 10% body mass in a short period of time there is a great possibility of decreased fertility (BUTLER and SMITH, 1989), because the decrease in condition in early lactation should not be lower than 0.5 units, so that the negative effects on fertility would be minimized. Cows may gain weight in late lactation, but should not gain weight in the dry period (ZAAIJER and NOORDHUIZEN, 2003). Cows from group A achieved the desirable condition faster and more equally after three weeks (BCS A 2.93: B 2.03), and cows from group B did not achieve this even by the 150th day of lactation (BCS A 3.04:B 2.69).

Subfertility is a consequence of high production, fatty liver and metabolic diseases with an increased predisposition to infection. Highly productive cows have great needs, so there is a deficit of energy in the first weeks of lactation (NEB), due to milking capacity and overfeeding in the dry period. KRUIP et al. (1998) conducted research on two groups of cows. One group was given high energy value feed (119MJ/d), and the other lower energy value (49 MJ/d). In both groups severe NEB occurred in early lactation, with loss of body mass over 100 kg, increased levels of NEFA and decreased levels of insulin. In the group given high energy value feed the beginning of the first ovulation was delayed, the total number of ovulations within 100 days was lower and the incidence of metabolic disorders was higher. Ovarian inactivity was explained as a consequence of severe loss of body mass and decreased levels of leptin and insulin, which consequently results in the blockage of GnRH and LH. In our research, cows from group B that were fed too high energy value meals for their recent production, had half the percentage of conception (58% : 28%) within the service period of 100 days, and a significantly lower percentage of conception after the 150th day of lactation (94% : 72%), in correlation with cows from group A. These results correspond to the research of that proved that the lower conception rate on the 150th day of lactation was a consequence of nutrition with higher levels of NEFA.

With modified nutrition in 50 cows from group A, we achieved a faster end of the negative energy balance period, and improvement of reproductive status. The best proof of

this is the significant decrease of anovulatory cycles in group A. This represents evidence of the beneficial effect of degradable proteins. Also, there is a significant increase in luteinised follicles, oestrus and dioestrus (BALL et al., 1995). Ovaries are «set at ease», production of primary follicles is slowed down, lutein domination is increased and the number of suboestric animals reduced, and there is a significantly higher percentage of spontaneous ovulations. As a result of the increased intake and conversion of degradable proteins, LH due to negative feedback, strengthens luteinisation and ovulation effects (ELROD and BUTLER, 1993).

This explains the high conception rate in cows from group A. Cows that were treated with luteolitic substance (WHITE and DOBSON, 1990) reacted with accentuated oestrus, ovulation, and conception (GORDON, 1996). The same reactions were noticed in cows that were submitted to Ovsynch treatment. They had accentuated oestruses after the first injection of GnRH and after application of PGF2alfa.

The most significant proof of the beneficial effects of natural degradable proteins is the fourfold increase in ovulatory cycles in group A, and the cows had spontaneous ovulations. This shows the high efficiency of the balanced LH hormone, a glycoprotein composed from the carbohydrate group and peptide chain. When the nutrition has a sufficient amount of protein, the protein components will be balanced. For this reason the active isomer of the LH hormone will be the one with accentuated LH activity (MORGAN and WILLIAMS, 1989). Too high energy value feed prevented the interruption of the negative energy balance period.

In the management and production of cows from group A, the intake of natural degradable proteins was not excessive, nor harmful. It caused milk yield lower by one litre, and at the same time as the lower price of meal, expressed in savings in the value of four litres of milk.

Conclusions

This research has proved the harmfulness of nutrition with too high energy value forage, especially if it is not adjusted to lactation. This is shown in the lower conception rate and higher incidence of reproductive aberrations. Better results were achieved in the group fed with balanced meals of voluminous forage with degradable proteins. In these animals condition recovery and conception began earlier, with economical profit.

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> Received: 6 June 2006 Accepted: 21 December 2008

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SAŽETAK

Hranidba bitno utječe na plodnost mliječnih krava. Incidencija funkcionalnih oblika smanjene plodnosti u stalnom je porastu, posebice zbog neujednačenoga odnosa razgradivih proteina i energije u krmi. Istraživanje je načinjeno na stotinu prvorotkinja frizijke pasmine, podijeljenih u dvije skupine (skupina A, n=50; skupina B, n=50) u fazi ranog rasplođivanja, u razdoblju od 44. do 52. dana poslije porođaja. Prosječna laktacija u obje skupine bila je 32 litre mlijeka. Ginekološki nalaz u skupine A bio je: proestrus 5, estrus 3, diestrus 6, cistična ovarijska bolest 6, anestrija 24, endometritis 4, piometra 2. Nalaz u skupine B: proestrus 6, estrus 5, diestrus 6, COD 5, anestrus 21, endometritis 4, piometra 3. Sve krave s dijagnosticiranim endometritisom, piometrom i COD obrađene su dvokratno ili trokratno sintetskim analozima prostaglandina F2alfa. Tijekom tri tjedna, krave iz skupine A hranjene su ekstenzivno ad libitum obrokom koji se sastojao od kukuruzne silaže (njezin je udio bio za 50% manji u odnosu na obrok kojim su hranjenje krave iz skupine B), sjenaže lucerke, sijena lucerke i 10% koncentrata bez soje, kukuruza i suncokretove pogače, s ječmom i 70 do 80% stočnoga brašna. Krave iz skupine B bile su hranjene standardnom farmskom mješavinom za mliječne krave. Obrok od 34,5 kg se sastojao od kukuruzne silaže, sjenaže lucerke, sijena lucerke i oko 19% koncentrata, s 20,03 kg suhe tvari i 16,3% proteina. Koncentrat se sastojao od 6% soje, 20% kukuruza, 1% suncokretove pogače, 5% TMR Camisan, 50% stočnog brašna i 18% ječma. Tri tjedna kasnije ginekološki nalaz u skupinama A:B bio je: proestrus 19:7, estrus 7:6, diestrus 20:6, COD 0:7, anestrus 3:20, endometritis 1:3, piometra 0:1. Unutar stotinu dana od teljenja koncipiralo je 29 krava iz skupine A (58%), uz prosječni servis period 94 dana. Iz druge skupine, unutar stotinu dana koncipiralo je 14 krava (28%), uz prosječni servis period 96 dana. Do 150. dana laktacije, koncipiralo je 47 krava iz skupine A (94%) i 36 (72%) krava iz kontrolne skupine. Prosječna laktacija u tijeku tri tjedna pokusa u skupini A bila je 30 litara, a u skupini B 31 litar mlijeka. Početkom pokusa kondicijski indeks krava kretao se od 1,5 do 2,5 bodova. Dana 100. poslije teljenja krave iz skupine B bile su neujednačene kondicije (BCS od 1,5 do 3,25, prosječno 2,03 bodova), a krave iz skupine A ujednačenog prosječnog BCS 2,93. Dana 150. nakon teljenja prosječni BCS krava iz skupine A bio je 3,04, a krava iz skupine B 2,69. Plotkinje hranjene prirodnim razgradivim bjelančevinama, dobro su reagirale na postupke uvođenja u rasplođivanje. Cilj istraživanja bio je usporediti učinke dvaju različitih načina hranjenja na početak rasplođivanja i reprodukcijsku učinkovitost, odnosno dokazati bolje učinke hranjenja hranom bogatom razgradivim prirodnim bjelančevinama u odnosu na standardnu farmsku mješavinu za hranjenje mliječnih krava u laktaciji.

Ključne riječi: mliječna krava, smanjena plodnost, poslijeporođajno razdoblje