

BUTAPHOSPHAN, B12 AND C VITAMINS EFFECTS IN DAIRY COW METRITIS, MASTITIS PREVALENCE

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Cercetări Agronomice în Moldova
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B12 AND C VITAMINS ON METRITIS, MASTITIS
PREVALENCE AND REPRODUCTIVE PERFORMANCE
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ABSTRACT. Three groups of Holstein Friesian cows were used to test the effect of two intravenous solutions on metritis, mastitis prevalence and reproductive performance. The first solution containing 3000 mg Butaphosphan (100 mg/ml) and 1.5 mg B12 Vitamin (0.05 mg/ml) was administered to BB12 group in the first 3 days after parturition and the second solution containing 3000 mg Butaphosphan (100 mg/ml), 1.5 mg B12 Vitamin (0.05 mg/ml) and 7 g of C vitamin (0.1 g/ml) was administered to BB12C group in the same period. The control group (C) didn't receive any intravenous solution. The Tukey-Kramer multiple comparison tests were used to compare the results. The prevalence of clinical metritis, puerperal metritis and clinical mastitis was lower in the BB12C group ($p < 0.05$), compared to BB12 and C groups. Regarding the prevalence of metritis and mastitis no differences was observed in

the BB12 group, compared with the C group ($p > 0.05$). However, no difference was observed in the prevalence of the clinical endometritis ($p > 0.05$) for the all three groups of cows. Also, the BB12C group registered the best calving to first insemination interval and calving to conception interval ($p < 0.05$). In conclusion, the intravenous cocktail containing Butaphosphan, B12 and C Vitamin can reduce the prevalence of some uterus and udder infection in the first 7 days after parturition in dairy cows.

Key words: Dairy cow; Catosal; C Vitamin; Metritis; Mastitis.

REZUMAT. Efectul administrării intravenoase a butafosfanului, vitaminelor B12 și C asupra prevalenței mastitelor, metritelor și a performanțelor

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de reproducere la vacile pentru lapte. În acest studiu au fost folosite trei grupuri de vaci pentru lapte Holstein Friză pentru a testa efectul a două soluții cu administrare intravenoasă asupra prevalenței endometritelor, mastitelor și a performanțelor de reproducere. Prima soluție, compusă din 3000 mg butafosfan (100 mg/ml) și 1,5 mg vitamina B12 (0,05 mg/ml), a fost administrată grupului 1 de vaci (grupul BB12) în primele 3 zile după parturiție, iar a doua soluție, compusă din 3000 mg butafosfan (100 mg/ml), 1,5 mg vitamina B12 (0,05 mg/ml) și 7 g vitamina C (0,1 g/ml), a fost administrată grupului 2 (grupul BB12 C) în aceeași perioadă (în primele 3 zile după parturiție). Grupului 3 (grupul C), denumit și grup de control, nu i s-a administrat nicio soluție pe cale intravenoasă. Testul Tukey Kramer pentru comparații multiple a fost utilizat în scopul de a testa efectul soluțiilor utilizate. Prevalența metritelor clinice, metritelor puerperale și mastitelor clinice a fost mai mică la grupul BB12C ($p < 0.05$), în comparație cu grupurile BB12 și C. În ceea ce privește prevalența metritelor și mastitelor la grupul BB12, comparativ cu grupul C, nu au fost observate diferențe semnificative ($p > 0.05$). Prevalența endometritelor clinice a prezentat valori asemănătoare statistic ($p > 0.05$) la toate cele trei grupuri de vaci. Cele mai bune intervale fătare – prima inseminare și fătare – concepție au fost obținute la grupul BB12C ($p < 0.05$). În concluzie, administrarea intravenoasă în primele 3 zile post-partum a soluției formată din butafosfan, vitaminele B12 și C la vaci poate reduce prevalența anumitor infecții uterine și mamare în primele 7 zile post-partum.

Cuvinte cheie: vaci pentru lapte; Catosal; vitamina C; metrite; mastite.

INTRODUCTION

The transition from gestation to lactation is a period of great metabolic stress for dairy cows. This metabolic stress reduces the production and creates a poor reproduction. The estrus detection, breeding management and postpartum uterine diseases can influence the reproductive performance of dairy cattle. During postpartum period the uterus and udders of most cows is contaminated, but this doesn't mean that the cow most develop uterine diseases or mastitis. A prompt and adequate immune response plays a very important role in the control of excessive bacterial growth. In this context, the immune system of the cow is very important, but the other factors, such as age of animal, its metabolic status (negative energy balance, ketosis), mineral nutrition, periparturient stress and milk production level also affect the outcome of infection.

Several reports indicate a reduction in morbidity following administration of butaphosphan and cyanocobalamin (Sommer *et al.*, 1971; Flasshoff, 1974; Palmer, 1980; Schuh, 1994) and a recent report indicated that injection of butaphosphan and cyanocobalamin on the day of calving and 1 day later decreased the prevalence of subclinical ketosis during the week after calving in mature dairy cows (Rollin *et al.*, 2010). A few studies found that Catosal reduced postpartum diseases incidence and

improved conception risk in South African dairy cattle (Palmer, 1980).

Decreased concentration of ascorbic acid has been recorded from mastitis milk of cows (Naresh *et al.*, 2002). Many studies had been reported that its milk concentration significantly decreased in acute mastitis and SCM (subclinical mastitis), especially when the condition is accompanied by an increase in the levels of lipid hydroperoxide in erythrocytes (Weiss *et al.*, 2004; Kleczkowski *et al.*, 2005; Ranjan *et al.*, 2005). Subcutaneously injected, vitamin C may have therapeutic value for cows with mastitis (Naresh *et al.*, 2002; Ranjan *et al.*, 2005). In most cases, clinical mastitis is most likely to occur during the first month of lactation (Oviedo-Boyso *et al.*, 2007) and in many cases results from an infection established during the dry period or during early lactation (Goff and Horst, 1997).

In this study we hypothesized that intravenous injections of butaphosphan, B12 and C vitamin in the first 3 days after calving would reduce the prevalence of mastitis and metritis in dairy cows.

MATERIALS AND METHODS

Animals and treatments

The study was conducted in a commercial dairy farm herd from North-East of Romania with 325 Holstein cows with a milk yield 6603 kg/305 days. The cows were randomly divided into three groups (control C, BB12 and BB12C group) of 146 cows. Primiparous (n = 72) were paired according to the calving date,

and multiparous (n = 74) cows were paired by the calving date, parity and prior milk production. Prepartum transition, cows (almost 3 weeks prepartum) were maintained on dry lots and monitored for signs of calving by visual observation. After calving, the cows were sent for 7 days to the fresh lot where cows were daily monitored. The cows were housed in a tie stalls housing system and were fed the lactation diets two daily. In the first 3 days postpartum each cow from the BB12 group (n = 45) received the intravenous cocktail with 3000 mg (100 mg/ml) Butaphosphan, 1.5 mg (0.05 mg/ml) B12 Vitamin (Catosal, Bayer, Germany) and each cow from the BB12C group (n = 48) received the intravenous cocktail with 3000 mg (100 mg/ml) Butaphosphan, 1.5 mg (0.05 mg/ml) B12 Vitamin (Catosal, Bayer, Germany) plus 7 g (0.1 g/ml) of C vitamin (Vitamin C 10 %, Romvac, Romania). The control (C) group (n = 53) didn't receive any intravenous solution. After 7 days postpartum, the cows were kept to the tie stalls and fed to the lactation diets two daily. Diets for both pre and postpartum transition cows were formulated according to the requirements of lactating dairy cows established by the National Research Council (2001). The cows from the both groups were milked two times a day, starting with 4:30 a.m. and 4.30 p.m.

Diagnostic criteria

In this study metritis was defined as cow having watery, purulent or brown-colored, and fetid discharge (Chenault *et al.*, 2004) on days 5 to 7 after calving independently of their rectal temperature (Sheldon *et al.*, 2006). The clinical metritis was defined as cows having watery, purulent or brown-colored, and fetid vaginal discharge (Chenault *et al.*,

2004) and a rectal temperature $\leq 39.2^{\circ}\text{C}$ on days 5 to 7 after parturition. The difference between clinical metritis and puerperal metritis was represented by the rectal temperature which were $> 39.2^{\circ}\text{C}$ in the case of puerperal metritis (Smith *et al.*, 1998). Clinical endometritis was defined as cow having clear vaginal discharge with pus flecks or mucopurulent not fetid vaginal discharge or watery, purulent or brown colored and fetid vaginal discharge (Chenault *et al.*, 2004), between days 21 and 41 after parturition. The clinical mastitis was defined as a milking man observed abnormality in the milk and/or the udder.

The reproduction indices were calculated using the reproduction chart available on the farm and all data regarding cow production and reproduction were registered. Each cow from the experimental and control group had a voluntary waiting period of 45 days postpartum. Estrus detection was carried out x 3 daily and was based by mucous vaginal discharge. When a long string of clear, viscous mucus hangs from the vagina, the cow can be considered to be in estrus. The calving to first insemination interval (CFII) is defined as the interval from calving to the first insemination. The period from calving to conception interval (CCI) was the difference between the date of conception and the date of previous calving. The date of conception was estimated by subtracting 280 days from the calving date. Also, this study evaluates the pregnancy rate (percentage of cows that became pregnant) and conception rate (percentage of pregnancies as an effect of the first insemination).

The Tukey-Kramer multiple comparison tests were used to compare the results obtained for the C, BB12 and BB12C groups. Data are considered to be statistically reliable when $p < 0.05$.

RESULTS

From *Table 1* it can be observed that the highest prevalence was for the diagnosed clinical metritis and clinical mastitis. The highest prevalence of clinical metritis ($p < 0.05$) was observed for the cows from C and BB12 groups, compared with the BB12 C group. The same aspects were observed in the case of the puerperal metritis and clinical mastitis. No difference was observed in the prevalence of the clinical endometritis ($p > 0.05$) for the all three studied groups of cows (C, BB12 and BB12C).

Table 2 presented the studied fertility parameters for all three groups of cows (C, BB12, BB12C). Although the pregnancy rate and conception rate were similar for studied groups of cows the smallest ($p < 0.05$) calving to conception interval and calving to first insemination interval was observed for the cows which received the intravenous cocktail, formed from butaphosphan, B12 and C vitamin, compared with the cows which received butaphosphan and B12 vitamin and with the cows from control group.

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Table 1 - Comparison of metritis and mastitis prevalence of the cows from C, BB12 and BB12C groups

Groups	Parity	Clinical metritis	Puerperal metritis	Clinical endometritis	Clinical mastitis
C	<i>Primiparous</i>	43.7 ^a (14/32)	21.8 ^{a,b} (7/32)	15.6 (5/32)	37.5 ^a (12/32)
	<i>Multiparous</i>	40.9 ^{a,b} (18/44)	22.7 ^a (10/44)	13.6 (6/44)	31.8 ^a (14/44)
BB12	<i>Primiparous</i>	40.6 ^{a,b} (13/32)	18.7 ^{a,b} (6/32)	15.6 (5/32)	31.2 ^a (10/32)
	<i>Multiparous</i>	38.6 ^b (17/44)	20.4 ^{a,b} (9/44)	15.9 (7/44)	31.8 ^a (14/44)
BB12C	<i>Primiparous</i>	31.2 ^c (10/32)	9.3 ^c (3/32)	12.5 (4/32)	21.8 ^b (7/32)
	<i>Multiparous</i>	31.8 ^c (14/44)	13.6 ^{b,c} (6/44)	13.6 (6/44)	20.4 ^b (9/44)

The prevalence of clinical metritis, puerperal metritis, clinical endometritis and clinical mastitis of the studied groups are presented like % of cows (and proportion).

^{a,b,c} Values followed by different superscripts within columns differ ($p < 0.05$, $a > b > c$).

Table 2 - Comparison of fertility indicator for the cows from C, BB12 and BB12C groups

Fertility indicator	Groups		
	C	BB12	BB12C
Calving to first insemination interval (days)	78 ± 8.2 ^a	67 ± 6.3 ^{a,b}	54 ± 6.2 ^b
Calving to conception interval (days)	140 ± 22.1 ^a	139 ± 18.3 ^a	110.9 ± 14.3 ^b
Pregnancy rate (%)	87.5	82	80
Conception rate (%)	28	30	31

The calving to first insemination and calving to conception interval are presented like means ± standard deviation.

^{a,b,c} Values followed by different superscripts within rows differ ($p < 0.05$, $a > b > c$).

DISCUSSION

In this study we tested an intravenous drug cocktail containing two commercial products (Catosal and C vitamin), which was served to cows in the first 3 days after calving to improve the energy metabolism of cows by stimulating feed intake, improve immune function and help to reduce the prevalence of mastitis and metritis in dairy cows. High producing dairy cow are most prone to hypoglycemia during early lactation or at the peak of lactation

(Radostits *et al.*, 1994). Butaphosphan is an organic phosphoric acid (1-butylamino-1-methylethyl phosphonic acid), that has been used as a phosphorus source in cattle and that may support liver metabolism by donating P for ATP synthesis. Vitamin B12 is also involved to increase glucose availability and to decrease accumulation of hepatic lipids in periparturient dairy cattle (Preynat *et al.*, 2009). Cows had the ability to synthesis C vitamin from B-glucose or D-galactose through the glucuronic acid pathway (Basu and

Schorah, 1982). Even cows can synthesize vitamin C and vitamin C is not a required nutrient for dairy cows, cows with mastitis have lower concentrations of vitamin C in the plasma and milk (Weiss *et al.*, 2004; Kleczkowski *et al.*, 2005) and the severity of clinical signs is correlated with the magnitude of the decrease in concentrations (Weiss *et al.*, 2004). Chaiyotwittayakun *et al.* (2002) obtained in a previous study a reduction in the severity of some clinical signs of mastitis by injecting vitamin C, following an intramammary infusion of lipopolysaccharide.

Many risk factors were reported for metritis, like twins, dystocia, stillbirth, abortion, milk fever, negative energy balance, and deficiency in hygiene (Bartlett *et al.*, 1986; Correa *et al.*, 1993). However, the risk factor for metritis and mastitis can be summarized in two categories: factor increasing uterine bacterial contamination and factors reducing immune function. Taking into account that the uterine bacterial contamination is some limited the only way to reduce the prevalence of these diseases is to improve the immune function of the cow. Washko *et al.* (1993) found that intracellular concentration of ascorbic acid increase dramatically, when human neutrophils are activated, perhaps to protect the cells and surrounding tissues from damage caused by reactive oxygen species generate by the oxidative burst of the neutrophils. For example, TLR4 (toll-like

receptor) detects cell wall particles from Gram-negative bacteria, and after detection, cells of the endometrium (inner layer of the uterus) release a great variety of chemical messengers that activate the immune response (Singh *et al.*, 2008). Leukocytes, mainly neutrophils, respond to this activation by migration to the infection site, ingest and destroy bacteria leading to a reduction of bacterial load over time. It is unclear why most cows experience immunosuppression in the periparturient period, but it is apparent that metabolic challenges associated with the onset of lactation are factors capable of affecting immune function (Goff, 2006).

However, in our study the used of intravenous cocktail made from Catosal and vitamin C had a benefit effect by reducing the prevalence of uterus infection in the first 7 days after parturition, which was diagnosed by us like clinical metritis and puerperal metritis. Also, these intravenous cocktails reduce the prevalence of clinical mastitis in tested dairy cattle. The prevalence of clinical endometritis shows no differences in all three dairy cows groups (C, BB12, BB12C). Also, our study reveals that the administration of butaphosphan and B12 vitamin doesn't reduce the prevalence of the mastitis and metritis in dairy cattle.

CONCLUSIONS

This reducing in the prevalence of mastitis and metritis had a positive

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effect for some studied fertility indicators, like calving to first insemination interval and calving to conception interval and no effect for pregnancy rates and conception rates, which were similar for the all three studied groups.

This study demonstrates that the administration of the intravenous butaphosphan, B12 and C vitamin can be used like a prophylactic way to reduce the prevalence of some uterus and udder infection in the first 7 days after parturition.

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