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Anti-inflammatory treatments in calving dairy cows: effects on haematological and metabolic profiles

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RIASSUNTO – Trattamento con anti-infiammatori nel post-parto in bovine da latte: effetti a livello del profilo ematologico e metabolico. *Con lo scopo di modulare la risposta infiammatoria nel post-parto in un allevamento caratterizzato da un'elevata presenza di problemi, 5 bovine hanno ricevuto per os acetilsalicilato (25 g/d x 5 giorni dopo il parto; ASS) e 5 sono servite da controllo (CTR). Tra -30 e +30 giorni dal parto sono stati monitorati la produzione di latte, il BCS, lo stato di salute ed il profilo ematologico-metabolico. Il trattamento con ASS ha determinato l'aumento della produzione di latte (-2 kg/capo/d), il minor aumento delle proteine infiammatorie (ceruloplasmina ed aptoglobina) ed una meno intensa lipomobilizzazione (NEFA e β -OH-butirato più bassi, minor calo di BCS). La somministrazione orale di acetilsalicilato ha fornito effetti analoghi al trattamento parenterale, attenuando le conseguenze negative dello stato infiammatorio nel post parto (aumento produttivo, minor ricorso alle riserve corporee, migliorate condizioni metaboliche).*

Key words: acetylsalicylate, acute phase proteins, transition period, dairy cows.

INTRODUCTION – High yielding dairy cows are particularly vulnerable during the transition period to any event able to stimulate immune system. In contrast, response to these events is easily controlled in other stages of lactation. Cytokines (i.e. IL-1, IL-6 and TNF α) released from monocyte-macrophage cells are the common mediators of these events (Elsasser *et al.*, 1997). These cytokines stimulate several organs and tissues and they exert a catabolic and pro-inflammatory effect in the following ways: activating a local inflammatory response, inducing the eicosanoids synthesis; increasing the body temperature; causing anorexia and lipomobilisation; diverting the protein synthesis in the liver by reducing the usual protein synthesis (i.e. apolipoproteins) and increasing acute phase protein synthesis (i.e. haptoglobin).

The changes of these indices of inflammatory status are usually observed in transition dairy cows (Cappa *et al.*, 1989) which show clear symptoms of diseases (Hirvonen *et al.*, 1999) or in those showing no symptoms (Trevisi *et al.*, 2002).

With the attempt to modulate eicosanoids and cytokines production we have treated cows with non-steroid anti-inflammatory drugs (NSAID) immediately after calving (Trevisi *et al.*, 2003). Results were encouraging, particularly using the acetylsalicylate. They showed significant increase of milk yield, improvement of fertility indices, changes in metabolic profile.

Therefore, there were two objectives for this study: to verify the anti-inflammatory effectiveness of oral administration of sodium acetylsalicylate at calving, and to investigate more deeply the effects of this treatment on haematological-metabolic level.

MATERIALS AND METHODS – Ten multiparous Italian Friesian dairy cows from a commercial herd of Po valley were used for this study. Cows were housed in a tie stall barn. The herd was characterized by high

frequency of metabolic troubles around calving (i.e. anorexia, retained placenta, displaced abomasum). Dry cows were housed in a very old and not comfortable barn (short stall, dark and poorly aerated, inadequate hygienic conditions). About 6 days before calving, cows were moved into the lactation group with stalls built to conform to more recent cow comfort recommendations. All cows were fed with a total mixed ration (TMR), administered once a day in the afternoon. Diets differed for dry and lactating cows, according to their requirements.

The cows close to calving were allocated in 2 groups according to predicted calving date: 5 cows (ASS) received orally 25 g/d of acetylsalicylate of Na for 5 consecutive mornings from the day after calving; 5 cows (CTR) did not receive any supplement.

Each cow was bled weekly, from 30 days before calving through 30 days in milk (DIM). Blood samples were withdrawn from jugular vein before TMR distribution, in 2 vacuum tubes with Li-heparin and K₃-EDTA as anticoagulant. The Li-heparin tube was used to determine PCV and Piacenza Metabolic Profile according to procedures previously described by Bertoni *et al.* (1998). The K₃-EDTA tube was stored at 4°C until haematological assay, using a multiparameter auto-analyzer (CELL-DYN 3500 SL, Abbott Lab., Illinois, USA). Concentration of red blood cells (RBC), leucocytes (WBC) and their fractions (neutrophils, lymphocytes, monocytes, eosinophils, and basophils), haemoglobin, mean corpuscular volume of RBC and platelet number was determined.

Body Condition Scores (BCS) were determined weekly after bleeding. Milk yield was recorded weekly through 30 DIM then monthly through the rest of lactation. The occurrence of any health problem, drug treatments and inseminations during experimental period were also recorded. The statistical evaluation was carried out by a repeated measure analysis of variance (Proc MIXED of SAS, version 8 TS M0). The model included treatment, DIM, cow nested within treatment and treatment-DIM interaction and effects.

RESULTS AND CONCLUSIONS – Milk yield was increased with ASS treatment (Table 1; ~2 kg/cow/day till 30 DIM; n.s.) confirming our previous results (Trevisi *et al.*, 2003). Nevertheless, the increase was lower than previously, and was likely due to the health status of this herd. Sixty percent of cows showed diseases immediately before calving (mainly fever associated to pneumonia problems and anorexia) and 30% at calving time (milk fever and retained placenta). These problems seem to be related to inadequate housing in dry period, with regard to hygiene and management. Treatments have not solved health problems because 60% of cows, in both groups, demonstrated anorexia. Moreover, at 21 DIM, 40% of ASS cows had fever associated with respiratory problems and were treated with antibiotic. The ASS group had more days open, but no cow was culled for infertility, while 40% of CTR cows did not conceive. In previous paper (Trevisi *et al.*, 2003) fertility indices were improved after NSAID treatment, but herds used did not have major health problems around calving. Since the pre-calving stage, BCS level was lower in ASS *vs.* CTR group, but the total reduction till 30 DIM was also lower (-0.46 *vs.* -0.59 points). Because many problems occurred before treatment and because many remained afterwards, the lower lipomobilization in ASS seems to suggest a reduced severity of diseases or a faster recovery of a satisfactory health condition.

Blood metabolic-haematological parameters during the trial are shown in table 1. Haptoglobin and ceruloplasmin, main indices of inflammation, had a lower increase in ASS cows *vs.* CTR. Immediately after calving, plasma Ca remained higher (n.s.) for treated cows (2.53 mmol/L) compared to CTR cows (2.36 mmol/L). In the same period, Zn was lower (P<0.05) in ASS (9.8 µmol/L) *vs.* CTR (13.3 µmol/L) while globulins were higher in ASS group, but both differences began before calving. Albumins were lower before calving in ASS (33.0 *vs.* 34.8 g/L; P<0.05), but they increased through 30 DIM nullifying difference *vs.* CTR. These variations could suggest an improvement of liver activity at the beginning of lactation in ASS group, that seems confirmed by lower (n.s.) post-calving increase of aspartate aminotransferase activity (ASS, 85 U/L *vs.* CTR, 98 U/L) and by the quicker reduction of total bilirubin. Interestingly, the increase of NEFA and β-OH-butyrate (BHB) after calving were significantly lower in ASS (Table 1). These changes agree with BCS variations (lowest losses in ASS) and confirm a lower adipose tissue mobilization. Because ASS cows also had higher milk yield, it can be supposed they had a higher feed intake, that higher levels of plasma urea (ASS, 4.9 mmol/L; CTR, 4.4 mmol/L; NS) could confirm.

At haematological level, there was an increase of WBC (both neutrophil and lymphocytes) immediately before calving, particularly in ASS cows. These remained higher for 2-3 weeks of lactation; however the start of inflammation before calving was confirmed.

Table 1. Effects of acetylsalicylate (ASS), administered for 5 days after calving on milk yield and on haematological metabolic parameters compared to controls (CTR).

Parameter	Milk Yield (kg/d)		Cucp (µmol/L)		Haptoglobin (g/L)		NEFA (mmol/L)		BHB (mmol/L)		WBC (K/µL)		LYM (K/µL)	
	(ASS)	(CTR)	(ASS)	(CTR)	(ASS)	(CTR)	(ASS)	(CTR)	(ASS)	(CTR)	(ASS)	(CTR)	(ASS)	(CTR)
Day -24	-	-	3.07	2.87	.29	.24	.183	.203	.42	.45	6.08	5.57	3.13	2.40
Day -17	-	-	3.25	2.78	.32	.23	.170	.281	.42	.48	5.49	5.79	2.78	2.48
Day -10	-	-	2.87	2.66	.27	.22	.255	.372	.42	.52	5.80	5.65	2.29	2.06
Day -3	-	-	2.96	2.84	.29	.23	.296	.414	.48	.58	8.06	6.95	3.80 ^a	2.49 ^a
Day 3	23.2	21.8	3.70 ^a	4.15 ^b	.48	.69	.426	.501	.56	.71	7.55	6.76	2.83 ^b	1.81 ^a
Day 10	32.5	30.2	3.81	4.32	.41	.45	.384 ^a	.566 ^b	.54 ^a	.87 ^b	8.16 ^b	5.61 ^a	3.22 ^b	1.78 ^a
Day 17	38.1	36.7	3.69 ^a	4.31 ^b	.41	.25	.251 ^a	.460 ^b	.57	.62	8.24 ^b	5.52 ^a	2.27	2.06
Day 24	39.6	37.9	3.76 ^a	4.30 ^b	.60	.42	.281 ^a	.490 ^b	.54	.64	6.44	5.94	2.10	2.33

Statistical differences between groups: A,B:P<0.05; a,b:P<0.1; Cucp: ceruloplasmin; LYM: Lymphocytes.

In conclusion, the oral administration of acetylsalicylate in post-calving cows offered analogous effects of parenteral administration, with an attenuation of inflammation consequences (higher milk yield with lower body losses and some metabolic improvement).

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