and curd in Sarda ewes with different milk production level

G. Battacone[1], M. Palomba[2], M.G. Usai[1], G. Pulina[1]

[1] Dipartimento di Scienze Zootecniche – Università di Sassari, Italy.
[2] Dipartimento Farmaco Chimico Tossicologico – Università di Sassari, Italy.

RIASSUNTO – Trasferimento delle aflatossine dalla razione al latte e alla cagliata in pecore Sarde con differente livello produttivo – *Scopo del lavoro è stato lo studio delle relazioni fra l'ingestione di aflatossina B1 (AFB1) e le concentrazioni di aflatossina M1 (AFM1) nel latte e nella cagliata di pecore Sarde con diversi livelli produttivi. Tre gruppi di cinque pecore, ciascuno con animali di due livelli produttivi, sono state alimentate per sette giorni consecutivi con una razione contaminata artificialmente con tre livelli di AFB1 (32, 64 e 128 μg/d per capo). Il livello produttivo degli animali non ha influenzato la concentrazione di AFM1 nel latte e nel siero, mentre la sua concentrazione nella cagliata ha mostrato una tendenza all'aumento al crescere del livello produttivo degli animali (P=0,089). La dose di AFB1 ha condizionato il contenuto di AFM1 nel latte, nella cagliata e nel siero, ma non il carry over che è quindi risultato influenzato dal livello produttivo.*

KEY WORDS: aflatoxin, sheep, milk, curd.

INTRODUCTION – Aflatoxin B1 (AFB1) is a toxin produced by some strains of *Aspergillus* growing in feedstuffs. Dairy animals fed with diet containing AFB1 excrete aflatoxin M1 (AFM1) into the milk. The carry over ratio (AFM1 excreted in milk/ AFB1 ingested) has been found lower in sheep (Battacone *et al.*, 2002a) than in cattle (Veldman *et al.*, 1992). Being AFM1 linked to milk proteins, its concentration in curd is higher than in milk. The AFM1 concentration in milk resulted not influenced by milk production level in cattle, therefore the total amount of AFM1 excreted in milk and, consequently, the carry-over ratio increased with milk yield (Munksgaard *et al.*, 1987; Veldman *et al.*, 1992). A previous study carried out on isoproductive dairy ewes showed an increase of the AFM1 concentration both in milk and in curd as the amount of AFB1 ingested increased (Battacone *et al.*, 2002b). Aim of the present work is to study the influence of AFB1 dose and milk production level on the transfer of AFB1 from feeds to milk and curd as AFM1 in dairy ewes.

MATERIAL AND METHODS – *Experimental design.* Three groups of five Sarda mature ewes, each consisting of 2 ewes with a low-medium (Lm, < 1250 g/d) and 3 with a medium-high (Hm, > 1250 g/d) milk yield, after an adaptation period of 15 days were fed with a diet containing different levels of AFB1 (32, 64 and 128 μg/ d per head, respectively) for 7 days. All the groups received the same diet (750 g/d of a concentrate mixture, hay and water *ad libitum*) and the aflatoxin was administered in two equal daily doses. Pure AFB1 was dissolved in methanol and known amounts of aflatoxin solution were included into a pellet of concentrate wich was manually administered to each ewe at milking time. At the 6th day of the experimental period, the milk yield at morning and evening milkings of each ewe was collected and mixed to determine the AFM1 concentration. In order to investigate the relationships between the amounts of AFB1 administered and the AFM1 concentration in curd and whey, 750 ml of each individual milk were processed into curd. Raw milk was heated at 36-37°C and added by standardised rennet solution. Curd and whey were collected and analysed for AFM1 concentration. The experiment was con-

ducted in accordance with the guidelines of the Council Directive of EC(86/609/EEC). *Analytical procedures*: AFM1 levels in milk, curd and whey were determined by an HPLC-MS method. AFM1 was extracted using immunoaffinity columns which were then eluted with methanol. The samples were analysed by HPLC under the following conditions: Merck Lichrocart 250-4 RP-18, 5 μm column, 50:50 methanol-water as eluent at 0.5 ml/min flow, 20 μl injected volume, ESI-MS spectrometer as detector set at SIM mode. Milk samples were analysed for fat with Gerber method, and for protein (Nx6.38) and casein using FIL-IDF methods. *Statistical analysis*: data were subjected to analysis of variance in order to evalue the effect of AFB1 intake and production level on AFM1 concentration in milk, curd, whey and on carry over.

RESULTS AND CONCLUSIONS – Fat, protein and casein contents of milk were not influenced by AFB1 intake or by milk production level (data not showed). The means of AFM1 concentration in milk, curd and whey (Table 1) were higher than those previously observed in dairy ewes in the same experimental conditions, but fed higer dose of concentrate (1500 g/d; Battacone *et al.*, 2002a). Ruminal detoxification and digestive adsorption, strongly related to diet composition, can explain the different passage of AFM1 in milk.

Table 1. Least square mean of AFM1 in milk, curd, whey and the carry over in ewes with different level of AFB1 intake and different level of milk yield.

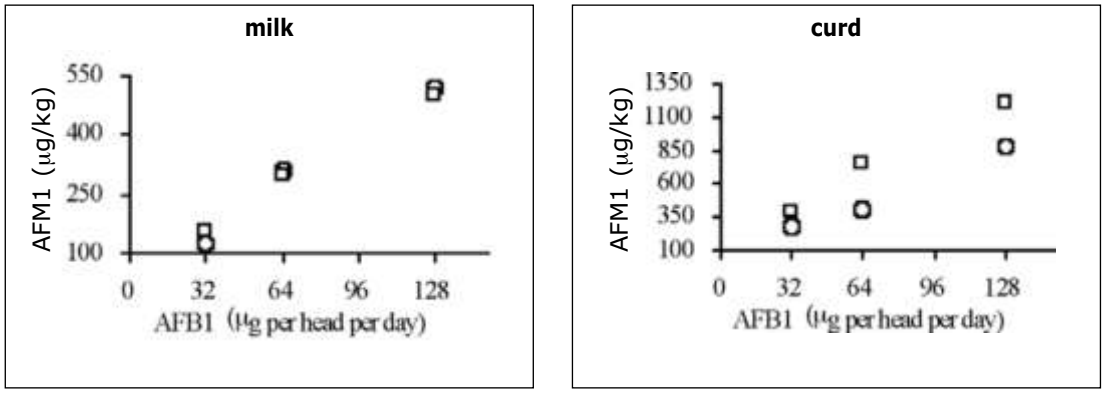
	AFB1 (μg/day)			PL			Contrast	
	32	64	128	Lm	Hm	RMSE	AFB1	PL
DMY (g/day)	1285	1199	1271	1071	1433		ns	**
AFM1(μg/kg)								
Milk	0.140	0.304	0.510	0.318	0.317	0.120	**	ns
Curd	0.337	0.580	1.042	0.518	0.789	0.270	**	†
Whey	0.170	0.275	0.474	0.311	0.302	0.067	**	ns
Carry over (%)	0.579	0.545	0.498	0.444	0.638	0.128	ns	*

*PL = production level: Lm < 1250g/d; Hm >1250g/d; DMY = daily milk yield; †P<0.10; *P < 0.05, **P < 0.01, and ns = not significant. AFB1xPL = ns.*

AFM1 concentrations in milk, curd and whey increased significantly (P < 0.01) with the level of AFB1 intake. This is in accordance with previous reports in sheep (Battacone *et al.*, 2002b) and cows (Munksgaard *et al.*, 1987; Veldman *et al.*, 1992). On the other hand, the carry over (AFM1/AFB1 ratio) was not significantly affected by the AFB1 intake, confirming previous result in sheep (Battacone *et al.*, 2002b) and cows (Veldman *et al.*, 1992).

Notwithstanding with no effect of production level among AFB1 treatements on AFM1 concentrations in milk (Figure 1) and whey were found. No effect of milk yield on AFM1 in whey was found, while a tendency to AFM1 in curd to increase for higher production levels was observed (P= 0.089). Actually, the mean AFM1 concentration in curd of all AFB1 groups resulted always higher in animals with higher production level (Figure 1). Since milk composition of animals with different production level was not different, this unexpected result need further investigations to clarify the relationship between milk yield and AFM1 concentration in curd.

Figure 1. Least square means of AFM1 concentrations in milk and curd of ewes with different milk production level treated with different doses of AFB1.



Lm = daily milk yield < 1250g (○); *Hm = daily milk yield > 1250g* (□)

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