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The transfer of aflatoxin M1 in milk of ewes fed diet naturally contaminated by aflatoxins and effect of inclusion of dried yeast culture in the diet

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ABSTRACT

An experiment was carried out to investigate 1) the transfer of aflatoxin M1 (AFM1) into the milk of dairy ewes fed diets naturally contaminated with aflatoxin B1 (AFB1); 2) the effect of the addition of dried yeast culture in the diet on this transfer; and 3) the alteration of enzymatic activities in the liver of ewes fed diets contaminated with AFB1. Twenty-four Sarda dairy ewes were divided in 4 groups and fed a concentrate mix containing 4 amounts of wheat meal naturally contaminated with aflatoxins. The diet of the control group had no wheat meal, whereas that of treated groups had low, medium, or high amounts of contaminated wheat, which corresponded to 1.13, 2.30, and 5.03 μg of AFB1/kg of feed, respectively. The experiment lasted 14 d. On d 8 to 14 from the beginning of the trial, 12 g/d of a commercial dried yeast product (DYP) of *Kluyveromyces lactis* was added to the diet of each ewe. The AFM1 concentration in individual milk samples and the blood serum metabolites were measured periodically. The presence of AFM1 was first detected in milk on d 1 of administration, and then its concentration increased and approached a steady-state condition on d 3 simultaneously in all treated groups. The AFM1 in milk at the steady-state condition, which was linearly related to the AFB1 intake, was 39.72, 50.38, and 79.29 ng/L in the low-aflatoxin, medium-aflatoxin, and high-aflatoxin groups, respectively. The AFM1 concentration in milk of the high-aflatoxin group was approximately 1.5-fold greater than the European Commission maximum tolerance level (50 ng/kg). The addition of DYP to the diet did not affect the AFM1 concentration in milk. After the withdrawal of the contaminated concentrate mix, the AFM1 mean concentrations decreased quickly and were no longer detected after 3 d in all treated groups. Daily milk yield and composition did not differ because of aflatoxin treatment. Blood serum parameters (creatinine, glutamic oxalacetic transaminase, glutamic pyruvic transaminase, gamma glutamyl transferase, alkaline phosphatase, lactate dehydrogenase, cholesterol, protein, urea, calcium, and phosphorus) were not influenced by AFB1 intake. Therefore, the effect of DYP on certain blood parameters (gamma glutamyl transferase, urea, creatinine, and calcium) could not be attributed to amelioration of the aflatoxin-contaminated diet. In conclusion, diet contamination by AFB1 near the European Union tolerance level (0.005 mg/kg) in complete feed for dairy animals (e.g., high-aflatoxin group) can result in an AFM1 milk concentration higher than the European Commission maximum tolerance level. Transfer of aflatoxin from feed to milk was not affected by dietary addition of a commercial DYP.
Key words: sheep milk, aflatoxin, serum parameter, dried yeast

INTRODUCTION

Aflatoxins (**AF**) have been classified as carcinogenic to humans (group 1) by the International Agency for Research on Cancer (2002) of the World Health Organization. Ingested aflatoxin B1 (**AFB1**) is metabolized by the hepatic microsomal cytochrome P450 enzyme family to aflatoxin M1 (**AFM1**; Kuilman et al., 2000), which can be excreted in the milk of lactating animals. The presence of AFM1 in milk and dairy products is of great importance because of their high consumption by humans, especially children. In fact, the European Commission (2001) has established that maximum levels of AFM1 in liquid milk and dried or processed milk products should not exceed 50 ng/kg. The Commission Regulation (EC) N. 683/2004 amends Regulation 466/2001 setting the limit at 25 ng/kg for AFM1 in infant formulas and follow-on formulas, including milk (European Commission, 2004).

During the last 5 yr, the production of sheep milk in the world has remained stable at approximately 9 million tons, with approximately 30% being produced in the European Union (Food and Agriculture Organi-

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zation of the United Nations, 2008), where ovine milk is almost entirely used for cheese making. Some surveys on AFM1 contamination in raw ovine milk and dairy products have reported AFM1 concentrations in milk lower than the tolerance level of 25 ng/kg (Finoli and Vecchio, 2003; Kaniou-Grigoriadou et al., 2005). Roussi et al. (2002) demonstrated that 11% of samples of raw ovine milk produced in Greece contained AFM1 in the range of 21 to 50 ng/kg, and only 1 sample exceeded the legal limit of 50 ng/kg.

Studies on the addition of commercial pure AFB1 to the diet of dairy ewes showed that the concentration of AFM1 transferred to ovine milk was linearly related to the intake of AFB1 (Battacone et al., 2003, 2005) and that it linearly and positively influenced the AFM1 concentration in cheese (Battacone et al., 2005). However, Applebaum et al. (1982) found differences in the transfer of AFM1 in the milk between dairy cows fed equal amounts of pure or impure AFB1. Those authors hypothesized a higher toxic effect of impure AFB1 because of the combination of compounds in the impure extract.

Trials conducted on cows (Veldman et al., 1992) and ewes (Battacone et al., 2003, 2005) to quantify the transfer of AFB1 into milk as AFM1 have often considered intake levels of AFB1 higher than the maximum levels allowed by the European Commission (2003) in feed for dairy animals (0.005 mg/kg). Many surveys carried out on dairy farms have reported the occurrence of AFB1 in feedstuffs, even if the incidence and mean content of toxin have generally been low (Finoli and Vecchio, 2003; Pietri et al., 2004). For these reasons, more knowledge is required on the transfer of AFM1 to milk, even at low levels of AFB1 feed contamination, to develop better risk management strategies.

The effects of AF on liver function can vary with the amount and duration of intoxication. In dairy sheep, the ingestion of pure AFB1 did not alter liver enzymatic activity when the daily intake ranged between 32 and 128 μg/d for an exposure period of 1 wk (Battacone et al., 2005). In contrast, when sheep were fed 128 μg/d of AFB1 for a longer period of intoxication (2 wk), glutamic pyruvic transaminase (**GPT**) activity increased significantly (Battacone et al., 2003). In lambs fed 2.5 mg of AF/kg of diet for 35 to 67 d, serum contents of glutamic oxalacetic transaminase (**GOT**) and gamma glutamyl transferase (**GGT**) increased, indicating a transient alteration of liver enzymatic activities (Edrington et al., 1994). Some experiments showed that ruminants are more resistant to AF poisoning than monogastric animals (Pier, 1992). However, the data in the literature on the extent of ruminal degradation of AF and its effects on rumen microbial activity are not consistent (Jouany and Diaz, 2005).

Probiotic yeasts are widely used in animal feeding because of their positive effects on rumen microbial activity (Chaucheyras-Durand et al., 2008). In addition to the excellent nutritional value of the yeast, the glucomannans from yeast cell walls have been found to significantly reduce (approximately −60%) the concentration of AFM1 in the milk of cows consuming diets contaminated with 55 μg/kg of AFB1 (Diaz et al., 2004).

The objectives of the experiment were to investigate 1) the transfer of AFM1 into the milk of dairy ewes fed diets naturally contaminated with AFB1; 2) the effect of the addition of dried yeast culture in the diet on the transfer of AFM1 into ovine milk; and 3) the alteration of enzymatic activities in the liver of ewes fed diets contaminated with AFB1.

MATERIALS AND METHODS

Experimental Procedures

The experiment was designed following the European Commission Council Directive that regulates the use of animals for experimental and other scientific purposes in the European Union (European Communities, 1986).

Twenty-four Sarda dairy ewes in late lactation (approximately 180 DIM), with an average milk yield of 1,234 g/d per ewe at 5.48% of fat and 4.94% of protein, were used. After 10 d of adaptation to experimental conditions, the ewes were assigned to 4 groups balanced for milk production and were fed 1.4 kg/d of a concentrate mix (**CMix**) containing no (control) or a low (**L-AF**), medium (**M-AF**), or high (**H-AF**) percentage of wheat meal naturally contaminated with AF (Table 1). The ewes were housed together and the CMix was given individually, twice daily during the 2 milkings, at 0700 and 1900 h, whereas oat hay and water were offered ad libitum. A treatment (intoxication) period that lasted 14 d was used, considering the results of our previous experiments indicating that the AFM1 concentrations in sheep milk approached a steady-state condition 2 d after the first administration of AFB1 (Battacone et al., 2005). On d 8 to 14, a commercial dried yeast product (**DYP**) of *Kluyveromyces lactis* containing 10^9 cells/g, including the carrier on which it was grown, was added to the CMix of each ewe at 12 g/d. The carrier contained 30% CP, 10.9% crude fiber, 2.9% crude fat, and 11% ash (DM basis). At the end of the intoxication period, the contaminated meal in the diet was substituted by a grain mix, and the ewes were monitored for a 1-wk clearance period.

The ewes were mechanically milked using a portable-bucket milking machine. The milk yield of each ewe was

Table 1. Proportion of feed ingredients, chemical composition (% of DM, unless otherwise stated), and aflatoxin concentration of each concentrate mix (CMix) containing no (control) or a low (L-AF), medium (M-AF), or high (H-AF) percentage of wheat meal naturally contaminated with aflatoxins (AF)

	CMix			
Item	Control	L-AF	M-AF	H-AF
Ingredient				
Corn meal	14	14	14	14
Grain mix	86	68	50	21
Contaminated wheat meal	0	18	36	64
Chemical composition				
DM, g/kg	886.3	880.1	877.4	871.4
CP	18.68	18.69	18.70	18.73
Fat	2.70	2.98	3.25	3.65
NDF	27.44	26.91	26.39	25.34
ADF	10.01	9.30	8.59	7.37
Acid detergent lignin	0.91	0.99	1.08	1.20
Ash	10.72	10.47	10.23	9.74
NE_L,[1] Mcal/kg of DM	1.77	1.76	1.76	1.75
Aflatoxin concentration, μg/kg				
AFB1	0.38	1.13	2.30	5.03
AFB2	ND[2]	0.07	0.15	0.25
AFG1	ND	ND	0.14	0.26
AFG2	ND	ND	ND	ND

[1]Net energy for 3 times maintenance was calculated by using the summative equations of Weiss as reported by Van Soest (1994) and adapted to sheep by Cannas and Atzori (2005).

[2]ND = not detected.

recorded at each milking throughout the intoxication and clearance periods. Individual milk samples were collected during the morning milking on d 1, 3, 6, 8, 10, 12, 14, 15, 17, and 18 after the beginning of the experiment. Milk samples were stored at −18°C until analyses of AFM1 content. A sample of each CMix was obtained by collecting 3 subsamples from 3 different sites in the bin with a probe-type grain sampler (0.5 kg/subsample). The subsamples were then pooled and thoroughly mixed to obtain a single representative sample (0.5 kg) for analyses of AF and composition of each CMix.

Before and during the experimental period, the health of the animals was monitored continuously. To check the effects of treatments on liver function and hematological parameters, blood samples were collected by jugular venipuncture, before the morning administration of contaminated feed, on d 1, 7, and 14 and were immediately analyzed.

Analytical Procedures

The DM content of the CMix was determined by oven-drying at 105°C for 24 h. Dried CMix samples were analyzed for NDF, ADF, and ADL with the procedure of Van Soest et al. (1991) by using the filter bag equipment of Ankom (Ankom Technology Corp., Fairport, NY) and for ash (AOAC, 2000; method 942.05), CP (AOAC, 2000; method 988.05), and fat (AOAC, 2000; method 920.39). Net energy for 3 times maintenance was calculated by using the summative equations of Weiss, as reported by Van Soest (1994) and adapted to sheep by Cannas and Atzori (2005).

An immunoaffinity technique was used to extract the AFB1, AFB2, AFG1, and AFG2 from the feedstuffs and the AFM1 from the milk. The AFM1 and the other AF were separated on a Hewlett-Packard 1100 HPLC chromatograph (Hewlett-Packard, Palo Alto, CA) connected to a reversed-phase C_{18} column (Zorbax SB, 5 μm particle size, 150 × 4.6 mm i.d., Agilent Technologies, Palo Alto, CA) equipped with a Hewlett-Packard 1100 fluorescence detector with excitation at 365 nm and emission at 435 nm. The eluent was acetonitrile:water (25:75 vol/vol) + 1% acetic acid, using a flow rate of 1 mL/min. Standards of AFM1 and of the other AF (Sigma A-6428, Sigma-Chemical Co., St. Louis, MO) were dissolved in benzene:acetonitrile at a ratio of 9:1 to prepare a series of working solutions containing 0.001 to 0.5 ng/μL. Toxin-specific calibration curves were prepared by plotting the peak area for each standard against the quantity of toxin injected. Each equation of the calibration curve was used to compute the respective content of the samples.

The concentration of AFM1 was determined by HPLC as reported by Battacone et al. (2005). The carryover of AFM1 in milk was calculated as the ratio between the AFM1 excreted in milk and the intake of AFB1 at the time when the toxin output in milk reached a steady state (from d 3 to 14 of the intoxication period).

Milk was analyzed for protein, fat, and lactose with a MilkoScan 6000 instrument (Foss Electric, Hillerød, Denmark), and for SCC with a Fossomatic 360 in-

strument (Foss Electric). A clinical chemistry system (Dimension RXL, Dade Behring, Milano, Italy) was used to analyze the blood serum samples for creatinine, GOT, GGT, GPT, alkaline phosphatase, lactate dehydrogenase, cholesterol, protein, urea, calcium, and phosphorus.

Data Analysis

Data on AFM1, protein, fat concentration, and SCC in milk and blood parameters were analyzed, using the MIXED procedure of SAS (version 9.1, SAS Institute Inc., Cary, NC), with the following mixed linear model:

$$y_{ijk} = \mu + D_i + P_j + (D \times P)_{ij} + A_{(i)k} + \varepsilon_{ijk},$$

where y is the dependent variable; μ is the overall mean; D is the fixed effect of the diet; P is the fixed effect of the sampling period; D × P is the diet × period interaction; A is the random effect of animal nested within treatment; and ε is the random residual. Statistical significance of the treatment was tested against variance of animal nested within treatment according to repeated measures design theory (Macciotta et al., 2008). The SCC were divided by 1,000 and converted to the natural logarithm before statistical analysis. Hematological value of the blood serum samples taken before the first administration of contaminated feed on d 1 was added to the model as a covariate in the analysis of the hematological parameters.

RESULTS AND DISCUSSION

No AFM1 was found in the milk of control group ewes during the entire experimental period. This result was expected, considering the very low concentration (0.38 μg/kg) of AFB1 in the CMix administered (Table 1) and the negligible AFB1 concentration in the hay (0.08 μg/kg). Instead, the concentrations of AFB1 in the diets containing contaminated wheat meal were 1.13, 2.30, and 5.03 μg/kg for the L-AF, M-AF, and H-AF groups, respectively (Table 1). Results of the analysis carried out with the mixed linear model highlighted a significant effect of the diet ($P < 0.01$) and of the day of sampling ($P < 0.01$) on AFM1 milk concentration, whereas their interaction was not significant. The pattern of AFM1 concentration in milk of the 3 experimental groups fed the contaminated CMix is reported in Figure 1. The AFM1 was already detected in milk produced during d 1 of administration of the contaminated CMix. This confirms the fact that the AFB1 is readily absorbed within the gastrointestinal tract and is quickly transformed (Kuilman et al., 2000) and excreted in milk as AFM1, as previously reported in sheep (Battacone et al., 2003) and in cows (Frobish et al., 1986). The AFM1 concentration increased on d 3 and then reached a steady-state condition simultaneously in the L-AF, M-AF, and H-AF groups. This trend is in agreement with previous results in sheep (Battacone et al., 2005) and cows (Frobish et al., 1986) fed diets with different concentrations of AFB1. The concentration of AFM1 in milk at the steady-state condition was positively related to the AFB1 intake (Table 2), in accordance with previous studies carried out on dairy sheep (Battacone et al., 2003, 2005), goats (Nageswara Rao and Chopra, 2001), and cows (Frobish et al., 1986; Veldman et al., 1992).

The mean value of AFM1 in the milk of the M-AF group was very similar to the European Commission maximum tolerance level (50 ng/kg), whereas the AFM1 contamination in milk of the H-AF group was 1.5-fold greater than the European Commission maximum tolerance level, even though the AFB1 concentration in the CMix was quite similar to the European Commission maximum tolerance level (0.005 mg/kg) allowed in complete feeds for dairy animals.

The carryover of AFB1 from feed into AFM1 in milk was 2.90, 1.90, and 1.30% for the L-AF, M-AF, and H-AF diets, respectively (Table 2). These values are higher than those observed in a previous study on dairy sheep when using pure AFB1 at higher doses (Battacone et al., 2005). One reason for this discrepancy could be the fact that in the current experiment, AFB1 was present in the CMix as naturally contaminated meal, which was also contaminated by other AF (e.g., AFB2, AFG1, and AFG2), even if at very low levels (Table 1). This explanation is supported by the higher carryover values observed previously in cows fed impure AF compared with those fed pure AFB1 (Applebaum et al., 1982).

The carryover of AFB1 into AFM1 decreased significantly as the AFB1 intake increased (Table 2). This relationship is in agreement with that observed in our previous experiment in lactating ewes, in which the carryover values of AFB1 from feed into AFM1 in milk tended ($P = 0.11$) to decrease (from 0.33 to 0.26%) as AFB1 increased (from 32 to 128 μg of AFB1/head per day; Battacone et al., 2005). Similarly, Frobish et al. (1986) showed that the carryover in milk from high-producing cows was 2.33, 2.13, and 1.94% with intakes of 492, 1,144, and 2491 μg/d of AFB1, respectively. Even though Polan et al. (1974) reported that the carryover of AFM1 in milk did not differ significantly in cows fed diets contaminated with approximately 86 or 466 μg/kg of AFB1, the carryover values after d 4 and 8 of AFB1 feeding were numerically lower in cows fed the diet with the highest contamination. The inverse

Table 2. Aflatoxin B1 (AFB1) daily intake and least squares means of concentration of aflatoxin M1 (AFM1) and carryover in milk of the experimental groups that received concentrate mix containing no (control) or a low (L-AF), medium (M-AF), or high (H-AF) percentage of wheat meal naturally contaminated with aflatoxins

Item	L-AF	M-AF	H-AF	SEM	*P*-value
AFB1 intake, μg/d	1.58	3.22	7.04		
AFM1, ng/L	39.72^{A}	50.38^{B}	79.29^{C}	6.91	<0.001
Carryover,[1] %	2.90^{C}	1.90^{B}	1.30^{A}	0.35	<0.001

$^{A–C}$Means within a row with different superscripts differ significantly ($P < 0.001$).

[1]Carryover = percentage of AFB1 that was converted to AFM1 and excreted in milk.

relationship between carryover and AFB1 intake could be related to the biotransformation processes of this mycotoxin in animal tissues. In fact, in cultured bovine hepatocytes incubated with AFB1, the increase in AFM1 concentration was less than proportional to the increase in AFB1 concentration, and the formation of AFM1 was time dependent (Kuilman et al., 2000). Furthermore, in bovine mammary epithelial cells (in vitro), the biotransformation of AFB1 into AFM1 was directly correlated with the substrate concentration and time of exposure, whereas the percentage of AFM1 to AFB1 values decreased as the AFB1 concentration increased (Caruso et al., 2008). In contrast to the studies described above, Price et al. (1985) reported a linear positive relationship between the amount of AFM1 excreted in cow's milk and the AFB1 intake. Veldman et al. (1992) reported that the carryover of AFM1 in lactating cows was not affected by the intake of AFB1, even though it was positively related to milk yield. Frobish et al. (1986) and Masoero et al. (2007) reported a positive effect of milk yield on carryover of AFM1 as well. These contradictory results regarding the carryover of AFB1 would be expected because its gastrointestinal absorption and subsequent excretion as AFM1 in milk can vary among animals because of the influence of nutritional and physiological factors, feeding regimens, feed digestion, animal health, hepatic biotransformation, and milk yield (Van Egmond, 1989; Jouany and Diaz, 2005; Fink-Gremmels, 2008).

The AFM1 concentration in milk was not affected by the addition of the DYP in any experimental CMix. Therefore, the DYP fed at 12 g/d per ewe, which is commercialized as a probiotic feed supplement for high-producing dairy ruminants, did not affect absorption of the AFB1 in the gastrointestinal tract of dairy ewes. This lack of effect of DYP administration on the trans-

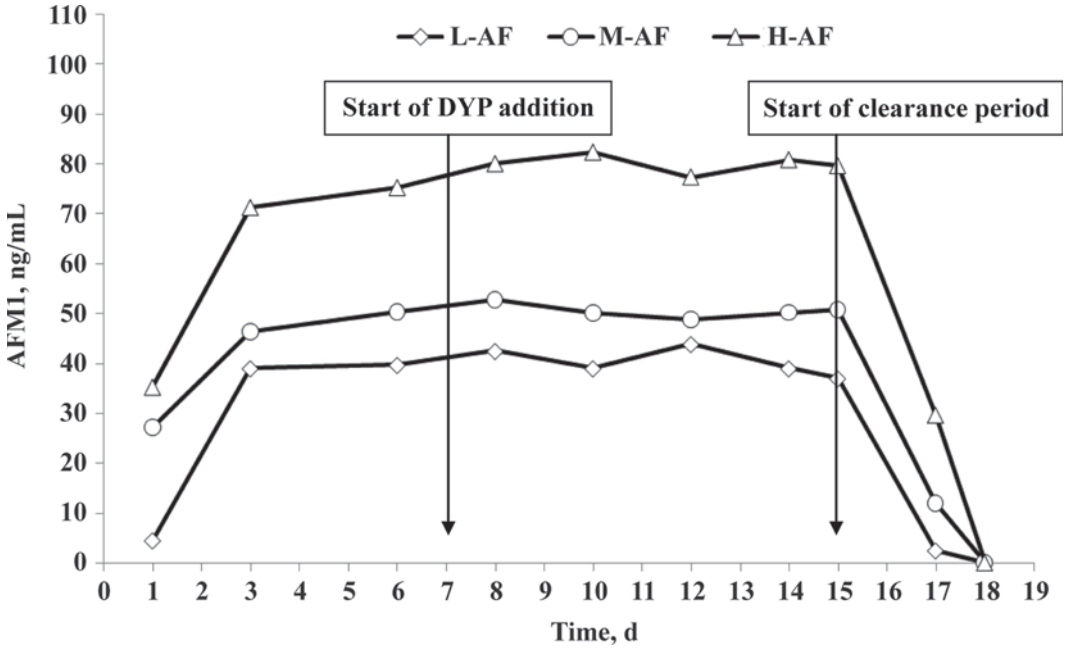

Figure 1. Aflatoxin M1 (AFM1) concentrations in the milk of ewes fed concentrate mix (CMix) containing a low (L-AF), medium (M-AF), or high (H-AF) percentage of wheat meal naturally contaminated with aflatoxins, without and with the addition of a commercial dried yeast product (DYP), followed by a clearance period during which the CMix did not contain naturally contaminated wheat meal or DYP.

Table 3. Least squares means of daily milk yield and composition of experimental groups that received concentrate mix containing no (control) or a low (L-AF), medium (M-AF), or high (H-AF) percentage of wheat meal naturally contaminated with aflatoxins without (first week) and with (second week) the addition of a commercial dried yeast product

	First week				Second week				*P*-value[1]		
Item	Control	L-AF	M-AF	H-AF	Control	L-AF	M-AF	H-AF	D	P	D × P
Milk yield, g/d	1,214	1,217	1,230	1,168	1,107	1,130	1,148	1,093	0.839	0.067	0.996
Fat, %	5.38	5.99	5.45	5.26	5.20	5.89	5.42	5.31	0.113	0.666	0.959
Protein, %	5.08	5.21	5.12	5.17	5.00	5.12	5.02	5.07	0.796	0.341	0.999
SCC, log cells/μL	1.76	2.13	2.10	1.77	1.72	2.33	2.10	1.87	0.064	0.659	0.924

[1]D = diet; P = period (week); D × P = diet × period interaction.

fer of AFM1 in milk is in agreement with the results reported in several studies (Stroud et al., 2006; Kutz et al., 2008; Waltman et al., 2008) in which the addition of different kinds of nondigestible yeast oligosaccharides was not effective in reducing the AFM1 concentrations in cow's milk. It is important to highlight that the DYP used in our experiment was not a specific mycotoxin-sequestering agent. Actually, the manufacturing process of specific yeast wall-derived cells to be used as mycotoxin-sequestering agents includes physical and chemical treatments that enhance the ability of the esterified glucomannan polymer to adsorb onto and reduce the bioavailability of toxins in the digestive tract (Diaz and Smith, 2005).

As the contaminated CMix was withdrawn (d 15), the AFM1 concentration decreased quickly and was no longer detected after 3 d in all treated groups. The time of disappearance of the AFM1 was lower than that reported in other experiments testing higher intakes of AFB1 (Battacone et al. 2003, 2005). However, the observed pattern of AFM1 clearly demonstrated that the time at which AFM1 was no longer detectable in milk after removal of the contaminated diet was not related to AFB1 concentration in feed, as already observed in sheep (Battacone et al., 2003) and cows (Frobish et al., 1986).

The milk yield and composition values for treated and control ewes are summarized in Table 3. The average daily milk yield did not differ among treatments, in agreement with previous experiments on dairy sheep (Battacone et al., 2005) and dairy cows (Polan et al., 1974; Frobish et al., 1986).

Means for serum constituents in blood samples collected at the end of the first week (i.e., before addition of the DYP to the CMix) and at the end of the second week (i.e., when contaminated feed was withdrawn) are reported in Table 4. The diet × period interaction was not significant for any of the variables analyzed. The covariate was significant for alkaline phosphatase, GGT, GPT, urea, creatinine, protein, and calcium. During the entire experimental period, the values of all serum constituents were within the physiological range when compared with the reference intervals reported by Dimauro et al. (2008) in healthy Sarda dairy sheep. Only the serum concentrations of urea and phosphorus were significantly affected by the AF content of the diet, even though a clear trend could not be observed. Serum enzyme activities were not significantly influenced by AF intake. In animals fed diets contaminated with toxicants, the serum levels of these enzymes increased after liver damage because of increased membrane permeability or because of liver cell necrosis and cytosol leakage into the serum (Ozer et al., 2008). Liver cell damage caused by AF was detected by measuring the activity of liver-specific enzymes in the serum of lambs receiving 2.5 mg of AF/kg of feed for 35 d (Edrington et al., 1994). In contrast, in a previous experiment in which dairy ewes were fed pure AFB1 at doses of between 0 and 128 μg/d, a significant association between the AFB1 intake and serum and hematological parameters was not found (Battacone et al., 2005).

The addition of DYP to the diet significantly affected the blood serum concentrations of GGT, urea, creatinine, and calcium (Table 4), without a significant interaction between period (DYP) and AF for all variables. The lower GGT activity observed in the last week of the experiment in all groups could be explained by a positive effect of the DYP on liver function. Increased hepatocyte production of GGT is considered a liver-specific indicator of hepatobiliary disorders and cholestasis in ruminants (Russell and Roussel, 2007). Instead, the serum concentrations of urea and creatinine, as indicators of kidney activity, were significantly higher in the second week than in the previous one. However, our data did not permit us to attribute certain kidney-adverse effects to the DYP intake.

CONCLUSIONS

The continuous administration for 14 d of CMix contaminated with AFB1 to lactating dairy ewes showed that the milk AFM1 concentration increased as the AFB1 intake increased. Based on the relationship between the AFB1 concentration in the CMix and the

Table 4. Least squares means of hematological parameters of experimental groups that received concentrate mix containing no (control) or a low (L-AF), medium (M-AF), or high (H-AF) percentage of wheat meal naturally contaminated with aflatoxins without (first week) and with (second week) the addition of a commercial dried yeast product

	First week				Second week				*P*-value[2]		
Item[1]	Control	L-AF	M-AF	H-AF	Control	L-AF	M-AF	H-AF	D	P	D × P
ALP, IU/L	182.8	139.5	241.4	156.8	237.7	147.0	163.2	151.9	0.218	0.835	0.317
GGT, IU/L	67.7	68.1	65.1	64.2	57.4	58.2	55.8	56.2	0.436	0.001	0.844
GPT, IU/L	45.3	47.9	50.2	48.4	48.3	50.9	50.5	48.7	0.229	0.182	0.749
GOT, IU/L	147.0	165.2	159.7	180.9	167.0	173.2	174.4	154.9	0.738	0.633	0.259
LDH, IU/L	447.9	450.7	439.9	513.2	457.7	489.8	491.4	459.9	0.617	0.515	0.193
Cholesterol, mg/dL	63.1	64.9	66.5	66.9	67.8	69.4	72.5	59.5	0.507	0.521	0.351
Urea, mg/dL	41.3^{bc}	37.2^{c}	42.1^{b}	37.3^{c}	53.9^{a}	44.8^{b}	49.4^{b}	44.8^{b}	0.022	0.001	0.488
Creatinine, mg/dL	0.54	0.54	0.54	0.51	0.62	0.57	0.59	0.56	0.457	0.009	0.820
Total proteins, g/dL	8.01	8.25	8.32	8.33	8.39	8.39	8.32	8.31	0.918	0.394	0.744
Calcium, mg/dL	9.95	9.40	9.78	9.66	10.25	9.88	10.16	10.24	0.307	0.007	0.909
Phosphorus, mg/dL	4.75^{ab}	3.44^{c}	4.49^{ab}	4.43^{ab}	4.88^{a}	4.39^{ab}	4.09^{bc}	3.95^{bc}	0.033	0.802	0.165

[a–c]Means within a row with different superscripts differ ($P < 0.05$).

[1]ALP = alkaline phosphatase; GGT = gamma glutamyl transferase; GPT = glutamic pyruvic transaminase; GOT = glutamic oxalacetic transaminase; LDH = lactate dehydrogenase.

[2]D = diet; P = period (week); D × P = diet × period interaction.

relative concentration of AFM1 in milk observed in this experiment, it can be concluded that even when the CMix contamination by AFB1 is near the European Union tolerance level (0.005 mg/kg) in complete feed for dairy animals, there is no guarantee that the AFM1 concentration of AFM1 reaching the milk will be below the European Commission maximum tolerance level. Moreover, the addition of a yeast that was not specifically manufactured as a mycotoxin-sequestering agent did not reduce the transfer of AFM1 from feed into milk.

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