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Toxic Effects of Patulin on Sheep

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Abstract: The objective of this study was to evaluate the adverse effects of patulin on beef and dairy sheep. In the beef sheep trial, nine Corriedale lambs averaging 26±3 kg BW were used. The treatment group, n = 5, received 3.3 mg patulin/kg^{-1} BW twice daily (BID) on days 0 through 2; 4.5 mg kg^{-1} BW on days 3 through 5 and 5 mg kg^{-1} BW on day 6. Lambs received no patulin from day 7 through 13. The control lambs, n= 4, received an equal volume of water by stomach tube BID. The Dry Matter (DM) intake in dosed sheep was 22% less than that of control animals after three days of treatment with 3.3 mg patulin/kg^{-1} BW. This difference increased to 40% as the dose of patulin increased to 5 mg kg^{-1} BW. Differences in DM consumption remained significant through day 13 ($p<0.01$). After 6 days of increasing doses of patulin, the body weight of treated sheep was 89% of that of the control animals. By the end of the trial, body weight in the patulin group was 86% of that of the control group. The daily weight gain was lower in treated animals between days 0 to 7 and 0 to 13 ($p<0.05$). In the dairy sheep trial, thirteen crossbred F1 or F2 Milkschaw x Corriedale ewes averaging 56+6 kg BW were used. The treatment group, n = 7, received 3.3 mg patulin/kg^{-1} BW divided BID. The control group, n = 6, received an equal volume of water by stomach tube BID. Neither changes in body weight nor effects on feed intake were observed in the patulin treated group. Effects on milk production, serum biochemistry, electrolytes and hematology parameters were not detected. Contrary to what it was expected patulin treatment in almost equal doses shown to be effective for beef sheep failed to influence production and health in dairy sheep. Factors such as age, diet and composition of rumen microflora might have contributed to the difference in behavior.

Key words: Patulin, mycotoxins, dairy sheep, beef sheep

INTRODUCTION

Patulin, 4-hydroxy-4H furo (3,2C) pyran- 2 (6HO)-one, is a secondary metabolite of toxigenic strains of *Penicillium*, *Aspergillus* and *Byssochlamys* species[1]. These molds are common contaminats of fermented feeds. For example, *Penicillium* sp. molds were isolated from 82% of fermented feed samples collected in the Upper Midwest United States during a 2 year period[2].

Patulin is toxic to a wide range of organisms including microbes and animals[3,4]. Patulin adversely affects rumen microbial fermentation *In vitro*. Acetic acid production was reduced in a batch culture exposed to 100 g patulin mL^{-1}[5]. Also, *In vitro* Dry Matter (DM) and Organic Matter (OM) digestibility was reduced in wheat straw to which 10 *n*mol patulin had been added[6]. Concentrations of 10 to 90 ppm patulin adversely affected digestion of OM and fiber. These concentrations also adversely affected the production of bacterial end products, such as Volatile Fatty Acids (VFA) and microbial protein in continuous culture fermenters[7].

Patulin containing feeds can have a negative impact on ruminants. Moldy fermented feed containing patulin or patulin- producing molds have been associated with disease in cattle[8,10-12]. Sheep dosed with 15 to 50 mg kg^{-1} BW of patulin had GI tract damage, sero-fibrinous exudates and weight loss[13]. Reports of natural patulin intoxication of sheep have not been published to our knowledge.

The objective of this study was to evaluate the adverse effects of patulin on sheep. An understanding of the adverse effects of patulin in ruminants will advance knowledge of the risks associated with feeding aerobically deteriorated fermented feed to livestock. Two experiments were performed, a beef sheep study and a lactating sheep study.

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Table 1: Composition of diet fed to sheep

	A-beef sheep		B-dairy sheep		
	Corn	Alfalfa hay	Corn	Alfalfa pellets	Soybean meal
% DM	85.1	90.0	86.0	90.0	88.0
% CP	10.7	16.0	8.0	16.0	41.0
% NDF	11.8	58.0	12.4	28.0	7.0
% ADF	2.9	35.0	2.9	15.0	2.5

MATERIALS AND METHODS

A-beef sheep study: The objective of this study was to describe the effects of 3.3, 4.5 and 5.0 mg patulin/kg^{-1} BW on feed intake and rate of gain in beef sheep. A preliminary study had shown that these doses of patulin depressed DM intake and total VFA and acetate production in sheep.

Patulin source, sheep and feeding: Patulin was produced as previously reported[7]. Nine Corriedale lambs averaging 26±3 kg BW were housed in individual 4.5 m^2 pens in a covered barn. Lambs were adapted to the facilities and diet for 2 weeks before beginning the experiment. Sheep were fed at 7:00 AM and 7:00 PM. They received 400 g (340 g DM) of cracked corn, then were offered alfalfa hay *Ad libitum* daily (Table 1). The amount of corn was gradually increased from 50 g to 400 g/day during the two week acclimation period. *Ad libitum* hay intake was met by ensuring 10% orts. Orts were removed from the individual feeding bins and weighed daily just before the morning feeding. Daily orts were pooled and dried for DM analysis. The difference between the amount of DM offered and the amount of DM refused was used to calculate the daily feed intake for each lamb. Each ingredient of the diet was tested for mycotoxins and found to be negative for aflatoxins, T-2, ochratoxin, zearalenone[14] and patulin[15].

Treatment: After the adaptation period, the lambs were randomly divided into two groups. The treatment group, n= 5, received an aqueous solution of patulin by stomach tube every 12 hrs until the feed intake was less than 70% of the starting intake. Lambs received 3.3 mg patulin kg BW divided BID on days 0 through 2 (6 treatments); 4.5 mg kg^{-1} BW on days 3 through 5 (6 treatments) and 5 mg kg^{-1} BW on day 6 (2 treatments). Two sheep were only treated for the first three days because of their severe decrease in feed intake. Lambs received no patulin from day 7 through 13. The control group, n = 4, received an equal volume of water by stomach tube BID.

Measurements: All lambs were weighed at the beginning of the experiment, on day 7 and on day 13. The first weight was used to randomly assign them to treatment or control groups. Feed intake was recorded daily. Analysis of data was made on records from days 0, 3, 6, 7 and 13.

Statistical analysis: Data were analysed as repeated measures using ANOVA[16] with treatment included in the main plot and sampling day and its interaction with treatment in the subplot.

B-dairy sheep study: The objective of this experiment was to describe the effect of 3.3 mg patulin/kg BW on milk production, feed intake, milk composition and blood parameters in lactating ewes.

Sheep and feeding: Thirteen crossbred F1 or F2 Milkschaw x Corriedale ewes averaging 56+6 kg BW were used. Housing and acclimation period were as in the beef sheep study. All sheep were checked for mastitis before they were brought into the barn, then every three days during the experimental period. Ewes were fed and milked at 6:00 AM and 6:00 PM. They received 600 g (510 g DM) of concentrate plus 18 grams/day of a vitamin-mineral mix (Premin, Tandil, Argentina) and were offered alfalfa pellets *Ad libitum* (Table 1). *Ad libitum* intake of forage and DM intake were determined as in the beef sheep study. After each milking, teats were dipped with an iodine teat dip preparation.

Patulin treatment: Sheep were randomly divided into two groups after the adaptation period. The treatment group, n = 7, was dosed with an aqueous solution of patulin by stomach tube q 12 hrs for 7 days. Sheep received 3.3 mg patulin/kg BW divided BID. The control group, n=6, received an equal volume of water by stomach tube BID.

Sampling: Feed intake and milk production were recorded daily. Analysis of data is reported for days 0, 2, 5 and 7. Ewes were weighed at the beginning and end of the experiment.

Blood samples: Blood samples were obtained from each ewe just before the morning milking by jugular venipuncture. Samples for serum analysis were collected in tubes without any additive on days 0, 2, 5 and 7. Serum was removed after centrifugation (1,000 x g) and

Table 2.Performance of lambs treated with increasing[a] oral doses of patulin

	Group			
Item and day	Control(n=4)	Treated(n=5)	SEM	p-value
Daily DM intake, g				
0	1,148±55.6			
		1,103±59.5		
3	1,230±100.3	954±99.0	67.63	0.005
6	1,416±112.4	845±236.7	171.12	0.01
7	1,414±93.48	833±193.2	112.26	0.001
13	1,426±55.2	1,027±146.7	109.1	0.006
Body weight, kg				
0	25.7±1.93	25.8±1.11		
7	29.8±2.23	26.7±1.61	2.51	0.1
13	31.2±2.13	26.9±1.69	2.93	0.06
Daily weight gain, kg				
0-7	0.51±0.05	0.20±0.09	0.17	0.02
7-13	0.23±0.02	0.03±0.07	0.13	0.06
0-13	0.39±0.02	0.07±0.11	0.19	0.04

[a]Treated animals received,-3,3 mg patulin/kg b.w. divided BID from day 1 to 3.-4.5 mg patulin/kg b.w. divided BID from day 4 to 6.5 mg patulin/kg b.w. divided BID on day 7-Sheep did not receive any patulin treatment after day 8

Table 3: Daily dry matter intake, milk production and milk composition of lactating sheep dosed orally with 3.3 mg of patulin/kg body weight for 7 days

	Day of trial[a]			
Variable and group	0	2	5	7
Daily intake[c],g				
0	2,53±6288[b]	2,670±258	2,337±270	2,234±310
3.3	2,476±211	2,597±193	1,798±421	2,356±319
Milk production[c],mL				
0	1,139±146	1,080±166	1,150±169	988±148
3.3	1,151±51	1,224±91	1,169±105	1,010±171
Fat[c], (%)				
0	6.9±0.5	7.1±0.8	6.3±0.4	7.9±0.5
3.3	6.3±0.4	6.9±0.6	6.6±0.4	7.7±0.4
Protein[c], (%)				
0	6.2±0.1	6.3±0.3	5.9±0.1	6.2±0.3
3.3	5.7±0.1	5.6±0.1	5.5±0.1	5.6±0.1
Lactose[c], (%)				
0	5.4±0.1	5.4±0.1	5.4±0.1	5.3±0.1
3.3	5.3±0.0	5.3±0.1	5.4±0.1	5.2±0.1
Solids[c],(%)				
0	18.8±0.6	19.2±1.1	18.0±0.5	19.7±0.7
3.3	17.5±0.5	18.1±0.7	17.8±0.5	18.8±0.4

[a]Days relative to start of patulin dosing[b] Standard error[c] Significant differences were not observed ($p>0.05$)

stored at-20°C until analysis. Blood samples for hematological analysis were collected into EDTA tubes on days 0, 5 and 7.

Serum analysis: AST, ALT , LDH , GGT glucose, albumin, creatine and blood urea nitrogen were analysed with a clinical autoanalyzer (Quimica LISA 200 Hycel, France). Electrolytes were measured by an Easy Lyte Plus Na/K/Cl analyzer (Medica Corporation, MA, USA).

Hematology: Determination of red and white cell counts, platelet count, hemoglobin and mean corpuscular volume were made using an automated cell counter (Hemat 12 SEAC, Florence, Italy).

Milk analysis: Morning and evening milk samples were collected daily and pooled on days 0, 2, 5 and 7. Samples were collected into vials containing potassium dichromate preservative and kept at 4°C until analysis. Fat, lactose, protein and total solids were measured in milk using an infrared analyzer (MilkoScan, FOSS Electric, Denmark).

Statistical analysis: Data were analyzed as repeated measures using ANOVA[16] with treatment included in the main plot and sampling day and its interaction with treatment in the subplot.

RESULTS

A-beef sheep study: Patulin dosing was suspended in two lambs after the third day of treatment because of an excessive decrease in feed intake. One of them was depressed and reluctant to eat. Except for this lamb, other patulin dosed animals did not have clinical signs of disease or abnormal behavior.

Daily DM intake on day 0 was 4.2% of BW. Daily DM intake in the patulin dosed sheep decreased as the dose of patulin increased (Table 2). The DM intake in dosed sheep was 22% less than that of control animals after three days of treatment with 3.3 mg patulin/kg BW.

This difference increased to 40% as the dose of patulin increased to 5 mg kg^{-1} BW. Although daily DM intake increased from 833±193.2 to 1,027±146.7 in the treatment group by the 5th day after discontinuing patulin treatment (Day 13 of the study), it was still 28% less than what the control group was ingesting at that time. Reduced DM intake may have reduced daily weight gain in the beef sheep

Body weight tended to be lower ($p = 0.07$) in patulin treated lambs. All sheep in the beef study were weighed

Table 4: Serum chemistry of lactating sheep orally dosed with 3.3 mg of patulin/kg body weight for 7 days

Variable and group	Day of trial[a] 0	2	5	7
Glucose, mg/dl				
0	60.6±1.8	68.3±3.2	75.5±3.8	57.8±2.4
3.3	61.1±2.1	72.0±3.4	77.8±2.8	66.7±1.3
Albumin[c], g/dl				
0	2.2±0.1	2.1±0.1	2.3±0.1	2.4±0.1
3.3	2.0±0.1	2.1±0.1	3.0±0.9	2.2±0.1
Creatinine[c],mg/dl				
0	0.9±0.0	0.6±0.0	0.9±0.0	0.9±0.0
3.3	0.9±0.1	0.6±0.0	0.9±0.1	0.9±0.0
BUN[c], mg/dl				
0	63.6±4.7	48.3±3.9	64.3±4.0	58.0±2.6
3.3	71.5±4.5	51.9±1.4	54.5±3.1	51.7±1.9
AST[c], IU L^{-1}				
0	123.3±6.7	119.6±12.0	122.0±12.5	103.5±7.5
3.3	109.8±10.4	117.7±17.2	103.0±8.8	87.0±9.1
ALT[c], IU L^{-1}				
0	21.1±1.8	19.8±1.5	21.6±2.4	21.6±3.5
3.3	22.5±2.9	22.4±3.5	19.0±2.6	18.5±2.8
LDH[c], IU L^{-1}				
0	998.1±64.3	1030.1±129.1	988.8±152.6	1188.5±241.8
3.3	1039.4±58.1	862.1±43.4	867.2±58.2	638.1±58.1
GGT[c], IU L^{-1}				
0	59.8±4.9	62.3±3.8	62.8±4.5	57.0±4.2
3.3	63.2±4.7	56.5±4.1	68.0±3.2	63.2±3.6
Na[c], mEq L^{-1}				
0	144.1±0.5	145.8±0.4	147.7±1.1	144.4±0.6
3.3	143.5±0.6	146.5±0.7	146.1±0.9	144.5±0.6
K[c], mEq L^{-1}				
0	4.4±0.1	4.8±0.1	4.5±0.2	4.5±0.1
3.3	4.6±0.2	4.9±0.3	4.8±0.2	5.0±0.1
Cl[c], mEq L^{-1}				
0	107.2±0.9	109.1±0.7	109.3±1.0	106.8±1.1
3.3	107.8±0.9	109.1±0.9	110.0±0.6	106.1±0.4

[a]Days relative to start of patulin dosing ,[c] Significant differences were not observed (p>0.05)

on days 0, 7 and 13, but not on days 3 and 6. Consequently, daily weight gain is not calculated for each patulin dose, but rather for the treatment period collectively (Table 2). After 6 days of increasing doses of patulin, the body weight of treated sheep was 89% of that of the control animals. Specifically, the treatment group gained 0.20±0.09 kg/day for days 0 through 7, while the control group gained 0.51±0.05 kg/day in the same period. By the end of the trial, body weight in the patulin group was 86% of that of the control group. Similarly, the daily weight gain was lower in treated animals between days 0 to 7 and 0 to 13 ($p<0.05$) (Table 2).

B-dairy sheep study: Changes in body weight were not observed. Total DM intake averaged 4.4% of BW before initiating the experiment. Effects on feed intake were not observed in the patulin treated group (Table 3). One ewe had a decrease in feed intake and milk production on day 6. However, effects on milk production (Table 3), serum biochemistry, electrolytes and hematology parameters were not detected (Table 4 and 5).

DISCUSSION

The adverse effects observed in beef sheep may be due to systemic effects, lower feed intake, disruption of ruminal microbes, or a combination of the three. We propose that the adverse effects seen in the beef sheep are associated with disruption of rumen microflora activity. Doses of patulin associated with clinical effects in sheep have been reported at 10 to 50 mg kg^{-1} BW[13].

Acute intoxication characterized by loss of appetite, loss of rumination, elevation of Blood Urea Nitrogen (BUN) and low plasma protein levels have been produced

Table 5: Hemogram of lactating sheep dosed orally with 3.3 mg of patulin /kg body weight for 7 days

Item and group	Day of trial[a] 0	5	7
RBC[c], (x $10^{6)}$			
0	6.67±0.3	7.10±0.5	22±0.6
3.3	7.42±0.9	7.12±0.7	7.09±1.1
WBC[c], (x 10^3)			
0	10.04±1.5	9.15±0.5	9.85±1.7
3.3	9.48±1.5	12.37±1.8	15.42±3.4
Platelets[c],(x 10^3)			
0	1,466.2±249.6	1,044.0±183.9	1045.0±147.2
3.3	1,309.7±426.2	898.8±225.4	1,051.2±177.5
Hgb[c], g/dl			
0	9.1±0.5	9.6±1.0	10.2±0.7
3.3	10.1±1.3	9.2±0.8	10.0±1.2
MCV[c], fl			
0	37.8±1.1	36.1±1.1	40.1±1.9
3.3	39.1±2.0	38.1±1.7	42.2±2.8

[a]Days relative to start of patulin dosing [c]Significant differences were not observed (p>0.05)

in sheep by oral doses of patulin as high as 50 mg kg^{-1} BW[13]. Elevations in BUN or reductions of serum albumin were not observed in lactating sheep receiving 3.3 mg patulin/kg BW. So systemic adverse effects of patulin were not detected in these ewes. Reduced DM intake may be associated with disruption of rumen microbial activity. Fistulated sheep dosed with patulin in a preliminary study had a decrease in total VFA production and in the molar proportion of acetate. Such a reduction of total VFA's and molar acetate has been seen after *In vitro* patulin dosing at 10 mg patulin L of rumen fluid[7]. A decrease in the molar proportion of acetate and the degradation of fiber may indicate a decrease in activity of cellulolytic bacteria[7].

It is well known that rumen content and rate of rumen emptying have direct effects on the voluntary intake of hay[13,17]. Ingested fibrous forage must be broken down either by rumination or microbial fermentation in order to pass through the reticulo-omasal orifice[18]. Therefore, it is possible that in the beef trial, a decrease in cellulolytic activity of rumen bacteria led to a decreased rate and extent of fiber digestion which limited the clearance of digesta from the rumen causing a lower feed intake. This reduced feed intake may have resulted in a reduced daily gain. We propose that a dose of 3.3 mg patulin/kg BW sufficiently alters rumen VFA production and fiber digestion to reduce feed intake and rate of gain in 26 to 30 kg lambs. Whether this effect is the sole mechanism or acts in synergy with systemic effects is the topic of future study.

Contrary to what was expected, patulin treatment failed to influence production or health parameters in dairy sheep at a dose shown to cause adverse effects in beef sheep. Obvious distinctions in the studies are the age, weight and gender of the sheep. These factors don't seem directly related to the different outcomes, because the doses were based on the body weight of the sheep in the beef and lactating studies. For example, the lambs and ewes received 99 and 184.8 mg patulin per day on average for the 3.3 mg kg^{-1} BW dose, since the lambs averaged 30 and the ewes averaged 56 kg. Age may indirectly influence the rumen microflora but diet is more likely to be a more powerful influence.

Other distinctions include difference in the composition of rumen microflora, a difference in the relative contribution of ruminal cellulolytic activity, a difference in the nutritional parameters of the alfalfa, the interrelationship of these three factors, or other factors. Animals in the beef trial were fed a lower quality alfalfa hay that was coarsely chopped while dairy sheep were fed alfalfa pellets. The chemical composition of the diets were also different. The beef sheep ration had a higher fiber and lower crude protein content than the dairy diet. It can be speculated that the dairy sheep diet allowed a higher bacterial yield or induced a shift in the bacterial population towards microorganisms with a higher resistance to patulin.

CONCLUSION

Adverse effects observed in beef lambs were not observed in lactating ewes receiving the same dose of patulin. Beef lambs dosed with 3.3 mg patulin/kg BW divided BID experience reduced DM intake and reduced daily weight gain. However, adverse effects were not observed in 56 kg lactating ewes dosed with 3.3 mg patulin/kg BW for 7 consecutive days.

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