Effect of the addition of modified glucomannan (Mycosorb ®) in diets containing Zearalenon on reproductive and metabolic parameters of sheep

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ABSTRACT

The mycotoxin, among them the zearalenon, causing injury to the productivity of ruminants, through changes reproductive and metabolic disorders. One of the means of control of mycotoxins is the addition of adsorbents substances in the diet, as modified glucomannan (Mycosorb ®). The objective of this study was to determine the effects of Mycosorb ® on reproductive and metabolic parameters of sheep submitted to diets containing zearalenone. The zearalenon caused a decrease in serum levels of glucose, for the CONTROL ($p < 0.05$), while the levels of cholesterol and BHBA showed no difference between groups ($p > 0.05$). In groups ZEA and ZEA + ADS levels of urea were higher than CONTROL ($p < 0.05$), indicating a higher protein metabolism in animals subjected to diet containing zearalenone. The albumin was not influenced by the addition of mycotoxin or sorbent to the diet. Serum levels of calcium, phosphorus and magnesium were not influenced by the presence of mycotoxin or Mycosorb ® in diet. The zearalenon increased levels of AST and GGT compared to group CONTROL ($p < 0.05$), indicating that this mycotoxin caused some degree of liver injury. Still, all groups showed physiological values of AST and GGT levels above the reference values for the sheep. As for reproductive parameters assessed (expression of estrus, diameter and colour vulvar), zearalenone presented no effect in this experiment. It is concluded that the Mycosorb ® was effective in decreased the hepatic aggression caused by this mycotoxin, shown by the decrease in the levels of AST.

INTRODUCTION

With the growing increase in the levels of ruminant production, the addition of concentrated in the diet of these animals became of great importance. But the components of this diet concentrated forms of conservation and provide an appropriate environment for the development of fungi, which produce toxic substances called mycotoxins, which have adverse effects on productive rates of ruminants (JOUANY & DIAZ, 2005).

Among the mycotoxins that cause damage to the ruminant performance, zearalenone, produced by several species of Fusarium, stands out to cause reproductive changes, acting through its similarity in structure with stradiol, (D'Mello et al., 1999). Furthermore, the zearalenone can be converted to zeranol through hydrogenation in the rumen, which is a hormone strogenic that acts as promoter of growth (KENNEDY et al., 1998), may cause metabolic disturbances in ruminants.

An important way to control the mycotoxins is the addition of substances adsorbents to diet, avoiding that mycotoxins are absorbed by the intestinal epithelium. The effect of sorbent modified glucomannan (Mycosorb ®) retrieved from the wall of yeast, mainly the *Saccharomyces cerevisiae*, has been widely studied in recent decades, especially in poultry and pigs (ARAVIND et al., 2003). However, the results are still very few known in ruminants, because of the small economic impact that had on mycotoxins creations extensive. But with the intensification of production this situation is changing, becoming a significant problem, when you desire to get an adequate productive performance of cattle and sheep confined.

The purpose of this study was to determine the influence of the addition of Mycosorb ® on reproductive and metabolic parameters of sheep submitted to diets containing zearalenone.

MATERIALS AND METHODS

This experiment was conducted in dependencies of the Veterinary Clinics Hospital in Universidade Federal de Pelotas (UFPel), Pelotas/RS, Brazil, at the Center for Experimentation in Sheep (CEO) using 34 female sheep, obtained from the crossing of races Corriedale and Texel, presenting 1.5 years.

The sheep were kept confined, receiving the basic diet of alfalfa hay (90.5% dry matter, 19.2% crude protein, 1.4% calcium, 0.2% phosphorus), tifton hay (85.8% dry matter, 5.8% crude protein, 4.8% ash, 0.7% ether extract, 25.7% crude fiber) and concentrated (15% crude protein, 12% ash, 1.5% calcium, 0, 9% phosphorus, 2% ether extract, 13% crude fiber), which is in quantities equivalent to 1.5% of body weight in a relationship roughage 65/35 concentrated. This relationship was based on the average weight of females at the start of the experiment, which was 47.5 kg.

Females were separated from four groups, and distributed evenly according to their body condition (scale of 1 to 5; RUSSEL et al., 1969) and weight. Table 1 presents the division of groups used in the experiment, according to the presence of zearalenone and / or Mycosorb ® in the diet of females.

Ewes used were released for use in experiments, by the Ethics Committee on Animal Experiments of UFPel, on March 27, 2007.

The zearalenone was produced by LAMIC - Laboratory Analyses Mycotoxins - UFSM, being obtained by the method of extraction, clarification and fully automated derivation and analysis by the High Efficiency Liquid Chromatography (HPLC), has submitted a concentration of 7118.26 mg/kg.

Table 1. Levels of zearalenone and Mycosorb ® (ADS) in the diet of each experimental group.

Group	**n**	**Zearalenon** [a]	**Mycosorb®** [a]
ZEA	6	1,0 mg/kg	-
ZEA+ADS	6	1,0 mg/kg	2 kg/ton
CONTROL+ADS	5	-	2 kg/ton
CONTROL	5	-	-

[a] Level of inclusion in relation to the concentrated fraction of the diet.

Females have been adapted to the diet for a period of 21 days before the inclusion of mycotoxin and Mycosorb ®, is considered the Day 0 of the trial on the start of the supply of these substances.

From Day 0, collections were made from blood and weighing of the females, every 7 days, for a period of 42 days, totaling 6 collections. Also reproductive evaluations

were performed, every 10 days, being rated the colouring of the vaginal mucosa, sorted in rosea or congest and diameter of vulva, measured through caliper.

For biochemical evaluation were collected samples of serum, plasma (EDTA 10%) in plasma with the addition of fluoride, potassium (12%), for determination of glucose, triacylglycerol (TAG), cholesterol, β-hydroxy butyrate (BHBA), albumin, urea, calcium, phosphorus, magnesium, gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST). The samples were centrifuged to 3000 RPM, for 15 minutes, and kept cooled or frozen, according to the analysis to be performed. The biochemical analyses were made through photocolorimetry, using visible light spectrophotometer (FEMTO 435 ®). The analysis of BHBA was held only in the last of blood collection.

On Day 21 began the synchronization of estrus females, with the placement of intravaginal device impregnated with 50 mg of medroxyprogesterone acetate (MPA). After 12 days (Day 33) were removed intravaginal devices and held application of 250 IU equine chorionic gonadotropin (Novormon 5000 ®, Tecnopec, Brazil) and 2 mL of prostaglandin (Prostaglandin Tortuga, Tortuga, Brazil). The monitoring of the expression of estrus was performed twice daily using a ram with misuse of penis. Twelve hours after the identification of estrus females were subjected to controlled mounts, with a Corriedale ram of the breed. The expression of estrus was evaluated for 5 days (MORAES et al., 2001).

Statistical analyses were performed using the SAS program (1986), using analysis of variance with comparison between medium according to the test Tukey HSD ($P < 0.05$).

RESULTS AND DISCUSSION

As the assessment of the weight of the females, there was no difference between groups ($p > 0.05$), with average weights of each group: ZEA: 49.80 kg; ZEA + ADS: 47.91 kg; CONTROL + ADS: 47.06 kg; CONTROL: 50.73 kg.

The coloration of vulvar mucosa was evaluated with the purpose of diagnosing signs of estrus in females, which would be consistent with a higher estrogen activity. There was a 85% rate of congestion of mucous in the CONTROL group ($p < 0.05$), the groups differed ZEA and ZEA + ADS (58.3% 54.2%, respectively - $p > 0.05$). Already the group CONTROL + ADS (60.0%) did not differ from group CONTROL. With the same purpose

of evaluating the estrus expression these females was estimated the diameter vulvar, with no difference between groups (ZEA: 1.75 cm, ZEA + ADS: 1.85 cm, CONTROL + ADS: 1.75 cm; CONTROL: 1.97 cm) ($p > 0.05$). After the estrus synchronization, his expression was 66.7%, 50% and 100%, for groups ZEA, ZEA + ADS and CONTROL, respectively, but with no difference between groups ($p > 0.05$). Thus, there was no effect of zearalenone on the estrus expression these females.

Table 2 is shown serum levels of markers energy, protein, minerals and enzyme evaluated in this experiment.

The addition of zearalenone in the diet of females decreased serum levels of glucose, for the CONTROL ($p < 0.05$), demonstrating that these animals had a higher expenditure of energy in relation to other groups. This is due, probably, to the effect of metabolites of the anabolic zearalenone, as zeranol (KENNEDY et al., 1998). Already with the addition of Mycosorb ®, there was no difference with the CONTROL. For physiological values of glucose, only the CONTROL + ADS (control with the addition of Mycosorb ®) remained within the physiological range of 50-80 mg / dL (GONZÁLEZ & SILVA, 2003).

Serum levels of TAG did not differ between groups ($p > 0.05$), indicating that they received diets with the same levels of fat (GONZÁLEZ & SILVA, 2003).

The levels of cholesterol and BHBA showed no difference between groups ($p > 0.05$). Also, the levels of cholesterol remained within the physiological values (52-76 mg / dL; GONZÁLEZ & SILVA, 2003), whereas the values of BHBA were higher in the groups ZEA + ADS and CONTROL (6-10 mg / dL; GONZÁLEZ & SILVA, 2003).

In groups ZEA and ZEA + ADS levels of urea were higher than CONTROL ($p < 0.05$), indicating a higher protein metabolism in animals subjected to diet containing zearalenone, but with all groups within the physiological patterns (4-10 mmol / L = 24-60 mg / dL; GONZÁLEZ & SILVA, 2003).

The serum albumin was not influenced by the addition of mycotoxin or sorbent to the diet, which is due to the fact this marker protein to be more stable than urea, answering only the changes for long periods in the levels of protein in the diet (CALDEIRA et al. , 2005), with no time to experiment in this kind of amendment.

Serum levels of calcium, phosphorus and magnesium were not influenced by the presence of mycotoxin or Mycosorb ® in the diet, which indicates that the presence of modified glucomannan did not interfere in the intestinal absorption of these minerals. Still, all the minerals analyzed showed their values within the physiological patterns (Calcium: 7,4-13 mg / dL; Phosphorus: 2,0-9,6 mg / dL; Magnesium: 1,8-3,0 mg / dL ; GONZÁLEZ, 2002).

The zearalenon significantly increased the levels of AST and GGT compared to group CONTROL ($p < 0.05$), indicating that this mycotoxin caused some degree of liver injury. This result shows that the damage reproductive zearalenon that question can not be only due to its direct action on the hormonal profile, but having an indirect effect by changing the liver function. In literature are not cited metabolic alterations caused by this mycotoxin, is a given and a major new assessment in subsequent experiments. Thus, further studies would be needed, with a more detailed assessment of the effect of different doses of zeralenona activity in the liver of ruminants, to confirm this hypothesis. Still, all groups showed physiological values of AST and GGT levels above the reference values for the sheep (AST: 0-90UI / L; GGT: 20-52 IU / L; CONTRERAS et al., 2000).

Table 2. Average values of parameters of metabolic lambs submitted to diets containing zearalenon, with or without addition of modified glucomannan (Mycosorb ®).

Metabolite	**ZEA**	**ZEA +ADS**	**CONTROL +ADS**	**CONTROL**
Glucose (mg/dl)	41,98[c]	46,10[bc]	51,86[a]	49,89[ab]
TAG (mg/dl)	33,09	32,96	32,02	30,28
Cholesterol (mg/dl)	69,40	64,25	54,35	63,89
BHBA (mg/dl)	10,57	12,73	10,51	13,11
Urea (mg/dl)	51,38[a]	51,70[a]	48,40[ab]	46,28[b]
Albumin (g/dl)	2,48	2,45	2,42	2,47
Calcium (mg/dl)	9,42	8,41	8,39	8,85
Fhosfhorus (mg/dl)	9,02	8,74	8,26	9,53
Magnesium (mg/dl)	2.59	2.49	2.48	2.34
AST (UI/l)	82,49[a]	68,24[b]	70,98[b]	73,21[b]
GGT (UI/l)	114,42[a]	83,50[ab]	76,24[b]	77,28[b]

Values with different letters on the same line differ statistically ($p < 0.05$)

From the results of this experiment we can conclude that zearalenone causes a metabolic imbalance in sheep due to the aggression liver caused by this mycotoxin,

characterized by high serum levels of AST and urea, as well as by reduced levels of glucose observed in these females.

Still, by comparison between groups CONTROL and CONTROL + ADS, it was concluded that administration of Mycosorb ® in the diet of ruminants presents no deleterious effect on the absorption of nutrients, since no statistical differences were observed (p> 0, 05) the parameters analyzed.

CONCLUSION

As to the effect of modified glucomannan on the metabolic alterations, we can conclude that the Mycosorb ® was effective in decreased the hepatic aggression caused by this mycotoxin, shown by the decrease in the levels of AST. Also, it might be observed that the sorbent had no effect on serum levels of minerals.

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