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Protective effect of esterified glucomannan on aflatoxin-induced changes in testicular function, sperm quality, and seminal plasma biochemistry in rams

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ABSTRACT

The aim of this study was to determine the effect of aflatoxin (AF) on spermatologic, biochemical, and testis parameters in rams, and the protective efficiency of esterified glucomannan (EG) co-administered with AF. Thirty-two Merino rams (12–14 months old) were used. The experimental design consisted of four dietary treatments. The control group was fed commercial feed. The AF group was fed with commercial feed plus 250 μg/d of total AF. The EG group received commercial feed plus 2 g/d of EG. The AF + EG group was given commercial feed plus 250 μg/d of total AF and 2 g/d of EG. There were treatment, time, and treatment-by-time interaction effects on sperm motility, abnormal spermatozoa, damaged acrosome, and dead spermatozoa ($P < 0.01$). The percentage of motile sperm was lower and the percentages of abnormal sperm, sperm with damaged acrosomes, and dead sperm were greater in the AF group than in the control, AF+EG, and EG groups, as from week 3 until the end of week 12 ($P < 0.05$). As from week 3, hyaluronidase activity in the seminal plasma increased significantly in the AF group, compared with the control. The co-administration of AF+EG was found to be effective in preventing the increase in hyaluronidase activity. As week 4, malondialdehyde (MDA) levels were significantly higher in the AF group compared with the control. The combined administration of AF+EG was found to be effective in lowering the MDA levels, increased by AF, to the levels measured in the control ($P < 0.05$). Although glutathione (GSH) levels were determined to have significantly decreased in the AF group in comparison to the control, it was observed that, in the group co-administered with AF and EG, particularly after week 7, the GSH levels, which had decreased owing to AF, were largely ameliorated ($P < 0.05$). In conclusion, AF adversely affected spermatologic, biochemical, and testis parameters, and the combined administration of EG with AF reversibly eliminated these adverse effects in rams.

1. Introduction

Silage production is an increasing practice in many countries because it enables fodder to be used as a feed source over an extended period. Poor management of the silage procedure can result in excessive moisture or dryness, condensation, heating, and leakage of rainwater,

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leading to the growth of undesirable microaerobic acid fungi. Fungal growth in silage can give rise to the loss of nutritive compounds and contamination with aflatoxin (AF), which contains several molecules that are dangerous for livestock and humans [1,2]. In ruminants, AFs have a wide variety of effects, including weight loss, poor performance, decreased fertility, abortion, hepatotoxicosis, and immunosuppression [3–5]. Young sheep are more susceptible to aflatoxicosis than adult sheep [6]. Feed contaminated with these toxins causes severe economic problems in ruminant breeding. Aflatoxins also create potential public health risks by their transmission from livestock to humans through milk and meat consumption [7].

Aflatoxins are a group of metabolites produced by fungi belonging to the genus *Aspergillus*, and in particular by *A flavus* and *A parasiticus* [8]. The growth of *Aspergillus* species and the production of AFs require the presence of certain favorable environmental conditions. These conditions include, among others, the relative humidity of the feed material stored and the storage area, storage temperature, rate of oxygen in the environment, duration of storage, and quality of the feed stored [8–11]. Because AFs are fat soluble, they are readily absorbed from the gastrointestinal tract and transferred in blood to their site of biotransformation, which is also their main site of accumulation, namely the internal organs, primarily the liver and kidneys [8]. Similar to all mycotoxins, the general mode of action of AFs is based on the inhibition of DNA, RNA, and protein synthesis [12]. The toxicity of the most toxic type of AFs, AFB_1, is reported to arise not from the toxin itself, but from its metabolites generated as a result of the activity of certain enzymes, including cytochrome P-450 and aryl hydrocarbon hydroxylase [13,14]. Research has shown that AFB_1 metabolites react with the cell DNA, resulting in mutation [14,15].

The maximum residue limit of AFs allowed in food, as adopted in August 1966 by the Codex Alimentarius Commission, jointly formed by the Food and Agriculture Organization and the World Health Organization, is 30 ppb [16]. However, this limit has been reduced to 20 ppb by the US Food and Drug Administration. It has been reported that, when administered to rats at sublethal doses, AFB_1 causes the degeneration of the testes and the mortality of germinal cells, which in return results in decreased sperm production, concentration, and motility of spermatozoa [17,18]. Some researchers [19] indicated that AFs induced adverse effects on sperm count and morphology in male buffaloes. Research in poultry [20,21] has shown that exposure to AF decreases testes weight and plasma testosterone levels, and delays sexual maturity. To reduce the adverse effects of AFs, it is required that indigestible binders, which are inert in nature and have no nutritional value, be added into the ration. These substances are not absorbed from the gastrointestinal tract and bind AFs, thereby reducing the absorption of these mycotoxins from the gastrointestinal tract [22–27]. A different approach to biological detoxification is based on the use of *Saccharomyces cerevisiae* and its cell wall component (glucomannan) to ameliorate the adverse effects of AF [28,29]. Esterified glucomannan (EG) has a capacity of binding AF at very high levels (80%–97%) [26,28]. Glucomannan is reported to have immunoregulatory [30], anti-inflammatory, antitumoral, antidiabetic, cholesterol-reducing, antifibrotic, and hypoglycemic effects [31], which bear significance for animal health. It has been reported that, in previous studies in which it was administered as an adsorbent at varying doses, glucomannan eliminated the adverse effects of AF on biochemical [26] and hematologic parameters [32], performance [29], and immune response [5] either partly or completely [25,29,33]. The aim of this study was to determine the effect of AF on spermatologic, biochemical, and testis parameters, and the protective effect of EG co-administered with AF against the adverse effects of this mycotoxin.

2. Material and methods

2.1. Animals and diet

This study was approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine of Selçuk University (2008/061) and included thirty-two 12- to 14-months-old Merino rams. Animals were examined for general health. Ivermectin (Avromec-F, 1 mL/50 kg) and oxfendazole (Okzan-F, 1 tablet/50 kg) were administered for antiparasitic treatment. In addition, enterotoxemia (Pluritoxiven-8, 1 mL) and smallpox vaccinations were given. For the adaptation of the animals to the environment and the new feeding regimen, a 15-day acclimatization program was applied before the start of the study. Individually weighted rams were divided into four equal groups. The experimental feeding regimen was continued for ninety-two days. The duration of the treatment (92 days) was based on a possible cumulative toxicity as well as on the duration of spermatogenesis and spermiogenesis in rams. Water and alfalfa were given *ad libitum*. Each morning, before given feed the animals were administered with AF and EG, incorporated into 250 g of commercial feed.

2.2. Experimental design

The experimental design consisted of four dietary treatments. The control group was fed with commercial feed. The AF group received commercial feed plus 250 μg/d of total AF. The EG group was given commercial feed plus 2 g/d of EG (Mycosorb, Alltech, Victoria, Australia). The AF + EG group was fed with commercial feed plus 250 μg/d of total AF and 2 g/d of EG.

2.3. Aflatoxin

The AF used in this study was produced (on the premises of the Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Selçuk University, Konya, Turkey) from the *A parasiticus* NRLL 2999 culture (USDA, Agricultural Research Service, Peoria, IL) via the fermentation of rice using the method of Shotwell, et al. [34] with minor modifications by Demet, et al. [35]. Fermented rice was sterilized in an autoclave, dried at 70 °C, and ground to a fine powder. In compliance with the method described by Vicam [36], the extraction and purification of AF in fermented rice was performed by applying the rice to an immunoaffinity column (Down test; Vicam). The amount of