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RESEARCH ARTICLE

Protective effect of nigella sativa and thymoquinone on relative liver weight increase caused by aflatoxin in broilers

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Çörek otu tohumu ve timokinonun broylerlerde aflatoksinin neden olduğu relatif karaciğer ağırlığı artışı üzerine koruyucu etkisi

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Öz

Amaç: Bu çalışmada çörek otu tohumu ve bunun farmasötik olarak etkin maddesi timokinonun, broylerlerde aflatoksikozisde şekillenen relatif karaciğer ağırlık artışını önleyici etkinliklerinin belirlenmesi amaçlanmıştır.

Gereç ve Yöntem: Çalışmada 120 adet 1 günlük broyler civciv kullanıldı. Bu civcivler 6 eşit gruba bölünerek 28 gün beslendi. Hayvanlara, gruplarına göre (Kontrol, AF, NS, TQ, AF+NS ve AF+TQ) 2 mg/kg total aflatoksin (AF; AFB1, AFB2, AFG1 ve AFG2), % 5 çörek otu tohumu (NS) ve 300 mg/kg timokinon (TQ) içeren yemler verildi. Deneme süresi sonunda gruplardaki 10'ar pilicin canlı ağırlıkları belirlendikten sonra dekapitasyon ile ötenazi uygulanarak sistemik nekropsileri yapıldı. Aflatoksin için hedef organ kabul edilen karaciğer uzaklaştırılarak relatif organ ağırlığının belirlenmesi için tartıldı. Karaciğerin relatif organ ağırlığı % olarak (karaciğer ağırlığı x 100 / canlı vücut ağırlığı) hesaplandı.

Bulgular: Aflatoksin uygulamasının relatif karaciğer ağırlığını önemli oranda artırdığı belirlendi (P<0,01). Timokinon grubu piliçlerin relatif karaciğer ağrılığı ile sağlıklı kontrol grubu arasındaki fark önemsizken, NS grubunda önemli derecede artış bulundu. Aflatoksinli diyete TQ ilavesinin relatif karaciğer ağırlığını önemli oranda normal değerlere düşürdüğü saptandı. Çörek otu tohumunun etkisinin ise TQ'a kıyasla daha kısmi olduğu gözlendi.

Öneri: Sonuç olarak, TQ ve NS'nin, AF'nin toksik etkisinden dolayı bozulmuş lipit metabolizmasını ve taşınmasını düzelterek, aflatoksikozisin önemli belirteçlerinden biri olan relatif karaciğer ağırlığı artışını önlediği değerlendirilmiştir.

Anahtar kelimeler: Aflatoksin, timokinon, çörek otu tohumu, karaciğer, relatif ağırlık.

Abstract

Aim: It is aimed to determine the preventive effect of black seed (Nigella sativa L; NS) and its pharmaceutically active substance volatile fatty acid thymoquinone on relative liver weight increase in broiler in aflatoxicosis..

Materials and Methods: One hundred twenty 1-d-old broiler chicks were used and divided into 6 equal groups and fed for 28 days. Animals were received feed with 2 mg/kg total aflatoxin (AF; AFB1, AFB2, AFG1 and AFG2), 5% black seed (NS) and 300 mg/kg thymoquinone (TQ), according to the groups; Control, AF, NS, TQ, AF+NS and AF+TQ. At the end of the experiment, euthanasia was performed by decapitation after determination of the live weight of ten broiler chickens in all groups, and systemic necropsies were performed. The liver, the main target organ for AF, was removed and weighed to determine relative organ weight. The relative liver weights were calculated as % (liver weight x 100 / live body weight).

Results: Aflatotoxin application significantly increased the relative weight of the liver (P<0,01). While the difference was not significant in the TQ group compared to the control group, a significant increase in liver weight was found in the NS group. Addition of TQ to feeds with AF significantly reduced the relative liver weight values to normal. NS effect was found more partial compared to TQ.

Conclusion: In conclusion, it was thought that TQ and NS prevented the relative liver weight increase, an important biomarker for aflatoxicosis, by probably ameliorating lipid metabolism and transport which is impaired by the toxic effect of AF.

Keywords: Aflatoxicosis, thymoquinone, nigella sativa, liver, relative weight.

Introduction

Aflatoxins (AF) are a serious threat to human and animal health due to their hepatotoxic, immunosuppressive, potential carcinogenic, teratogenic, and mutagenic effects (Bräse et al 2013, Ates et al 2017, Oguz et al 2018). Aflatoxins, which are found in four types (B1, B2, G1, and G2), are fungal secondary metabolites and that are commonly produced by Aspergillus (A.) flavus, A. parasiticus, A. nomius, A. tamarii, A. pseudotamarii, A. bombycis and A. ochraceoroseus (Cary et al 2005, Bräse et al 2013).

The presence of AF in poultry feed reduces hatching efficiency, hatching weight, growth ratio, feed conversion ratio, meat and egg production and quality. It also reduces immunization efficiency by suppressing the immune system, increasing susceptibility to disease and mortality (Fouad et al 2019). All these cause significant economic losses in the poultry industry, but no data were found to reveal these economic losses at the national level. In order to prevent aflatoxicosis, protection strategies that are applied meticulously at every stage from the production of food and feedstuffs to consumption are required. However, this is mostly not possible due to difficulties in implementation. For this reason, new trial substances with practical ease of use are needed to minimize damage and losses caused by AF (Oğuz et al 2011, Fouad et al 2019).

The bioactivation required for the conversion of aflatoxin B1 (AFB1) to toxic metabolites is mostly performed in the liver (Eaton and Gallagher 1994, Bedard and Massey 2006, Rawal et al 2010). The AFB1 in feeds is absorbed from the intestines, especially the duodenum, and reaches the liver (Gratz et al 2005). AFB1 bioactivation in the intestines is very limited (Guengerich et al 1996). AFB1, which is biotransformed by hepatic cytochrome p450 enzyme systems in the liver, is converted to reactive and electrophilic aflatoxin B1-8,9-exo-epoxide (AFBO), responsible for AF toxicity (Zhang et al 1997, Omar 2013, Ates 2019). As a biochemically highly unstable substance, AFBO binds to DNA, RNA and proteins and has a toxic effect on the liver, which is primarily responsible for AF metabolism (Eaton and Gallagher 1994, Gross-Steinmeyer and Eaton 2012).

Macroscopically, toxic effects of aflatoxins are characterized by an enlarged, discolored, easily degradable, and relatively weighted liver (Giambrone et al 1985, Huff et al 1986, Ortatatli and Oguz 2001, Yarru et al 2009, Monson et al 2015). Hepatic hydropic and fatty degeneration, bile duct proliferation, fibrosis are the most common histopathologic findings (Ortatatli and Oguz 2001, Karaman et al 2005, Ozen et al 2009, Karaman et al 2010, Yavuz et al 2017).
The combat against AF in poultry feed has great difficulties in all areas from field conditions to storage, and it does not seem possible to eliminate it completely. Researchers have searched for new and natural substances and methods because of the disadvantages of the substances used to prevent aflatoxicosis such as leaving residues at effective doses, inadequacy in inhibiting AF absorption, causing changes in the taste and odors of feeds, and expensive to use.

Recently, many studies have been carried out on the effects of medicinal plants and plant extracts on both human and animal health and their potential to protect or reduce cellular damage (Darakhshan et al 2015). For this purpose, one of the most promising medicinal plants studied intensely is the black seed (Nigella sativa; NS) (Ali and Blunden 2003). Nigella sativa is a traditional medicinal plant that has been widely used to solve various health problems since about 2000 years (Darakhshan et al 2015). Thymoquinone (TQ), which is considered to be a pharmaceutically active substance of NS, is the most important bioactive ingredient found in the essential oil of black seed in 27.8-57% (Ali and Blunden 2003).

Studies about the effects of hepatoprotective and antioxidant effectiveness of black seed and its components on relative liver weight and color changes are very limited. Liver weight increase and paleness are critical and almost solely the most prominent necropsy findings in aflatoxicosis. Therefore, the present study aimed to investigate the comparative protective effects of NS and TQ on relative liver weight increase, as a macroscopic biomarker for aflatoxicosis, in experimentally induced aflatoxicosis in broiler chickens.

Material and Methods

The material of this experimental study was obtained from a commercial hatchery and consisted of 120 unvaccinated Ross breed, 1-d-old male broiler chicks. Ethics Committee approval (No: 2017/58) was obtained by Selcuk University Veterinary Faculty Experimental Animals Production and Research Center Ethics Committee (SÜVDAMEK) regarding the appropriateness of the procedures to be performed on animals during the entire study process. After determining the live weight of these chicks, they were randomly divided into 6 equal groups; Control, AF, NS, TQ, AF+NS and AF+TQ. Animals received feed with 2 mg/kg total aflatoxin (AFB1, AFB2, AFG1 and AFG2), 5% NS and 300 mg/kg TQ, according to the groups. The chicks were fed with commercial broiler starter feed (1-10 days) and broiler grower feed (11-28 days) for 28 days at appropriate temperature and humidity conditions. Detailed trial design by groups is presented in Table 1.

At the end of the experiment, 10 animals were randomly selected from the groups and their live weights were determined. Then, they were euthanized by decapitation and systemic necropsies were performed. The liver, the main target organ for AF, was removed and weighed to determine relative organ weight and color changes were recorded.

Table 1. Detailed experimental design

	Groups	Experimental Design
I.	Group (Control)	Ad-libitum feeding with standard broiler starter / grower feed and fresh water for 28 days (normal diet).
II.	Group (AF)	Normal diet + 2 mg AF / kg feed
III.	Group (TQ)	Normal diet + 300 mg TQ / kg feed
IV.	Group (NS)	Normal diet + %5 (50 gr) NS / kg feed
V.	Group (AF+TQ)	Normal diet + 2 mg AF / kg feed + 300 mg TQ / kg feed
VI.	Group (AF+NS)	Normal diet + 2 mg AF / kg feed + 50 gr NS / kg feed

The relative organ weights of liver were calculated as % (liver weight x 100 / live body weight). The data obtained in these calculations were evaluated by the One-Way Anova post-Hoc Duncan test in Statistical Package for the Social Sciences (SPSS for Windows® version 25.0). P <0,05 was considered statistically significant.

Results

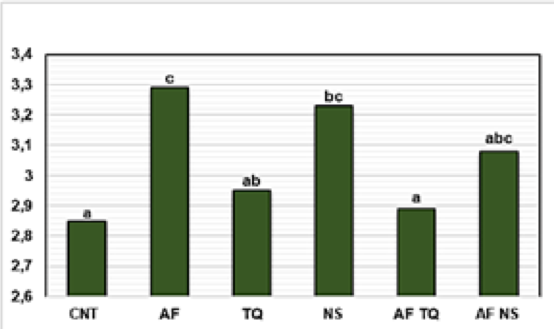
In the study, the relative organ weights of the liver were calculated according to the live weight of each chick in the groups. The relative organ weight averages and statistical results of the liver, which is the target organ for AF, are summarized in Table 2 and Figure 1 by groups. Accordingly, it was observed that AF application significantly increased the relative weight of the liver (p<0,01). In the TQ group, the difference was insignificant compared to the control group, while the weight increase was found in the NS group. Addition of TQ to feeds with AF (AF+TQ group) significantly reduced relative liver weight increase. Although there was no statistically significant difference in AF+NS group, a partial decrease in liver weight according to AF group was detected. In addition, macroscopically the livers of chickens in AF group showed color changes from pale or light yellow to dark yellow or light brown and were easily degradable. In the AF+TQ and AF+NS trial groups, severity of these findings were found to decrease compared to the AF group (Figure 2-3).

Table 2. Relative weight means of livers. *

Groups	Relative Liver Weights ($\bar{X} \pm S_{\bar{x}}$)
Control	$2,85 \pm 0,10^{a}$
AF	$3,29 \pm 0,11^{c}$
TQ	$2,95 \pm 0,04^{ab}$
NS	$3,23 \pm 0,13^{bc}$
AF+TQ	$2,89 \pm 0,07^{a}$
AF+NS	$3,08 \pm 0,11^{abc}$

P<0,01 (One-way ANOVA)

a-c According to one-way ANOVA and post-hoc Duncan test, the difference between non-common values in the column is significant (p <0.01).
* The values in the table represent the mean relative liver weight ± Standard Error ($\bar{X} \pm S\bar{x}$) of 10 broiler chickens in each group, and the relative organ weights of liver were calculated as % (liver weight x 100 / live body weight).



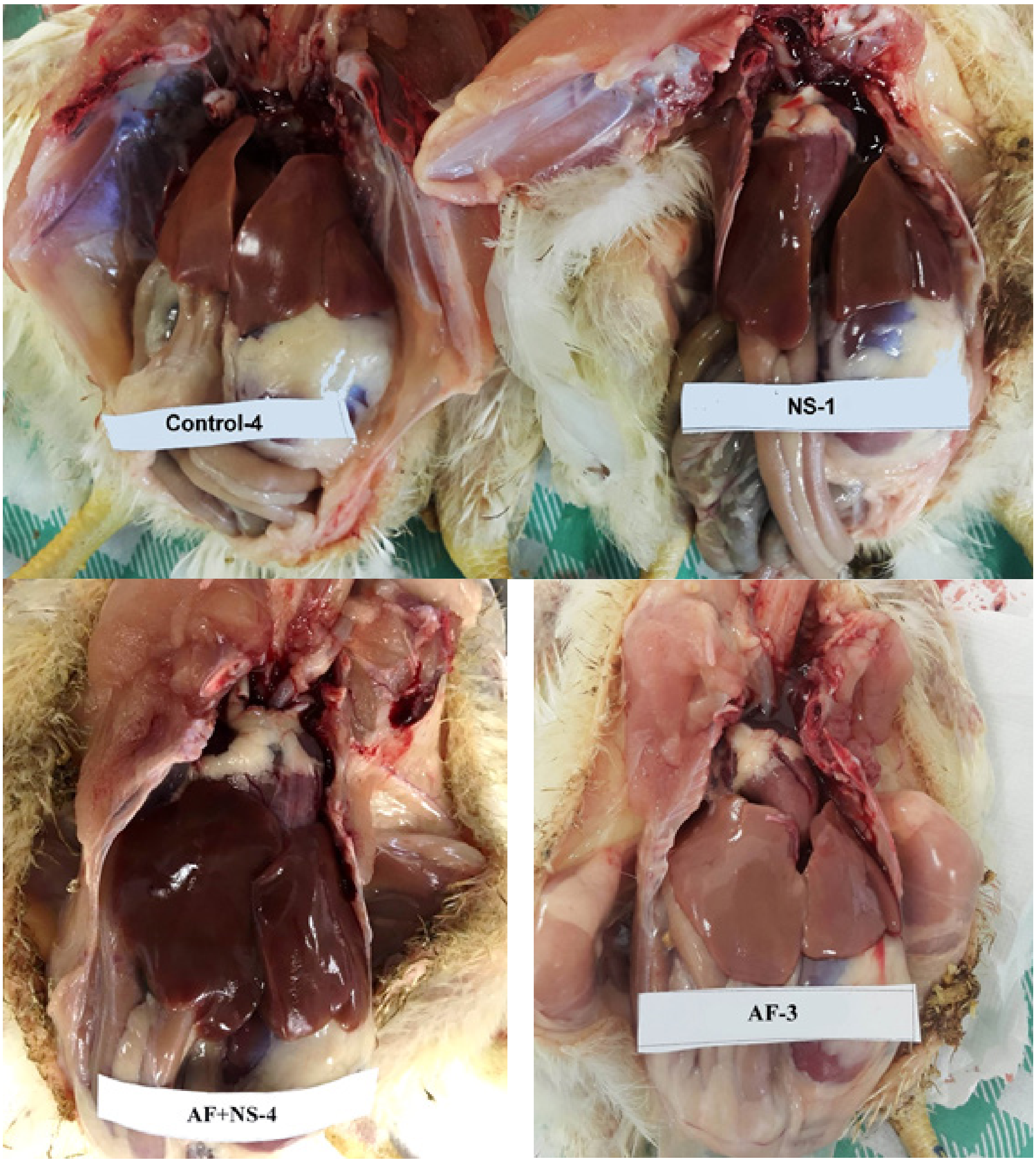


Figure 2. The effect of AF in terms of color on the livers of the experimental groups

Figure 3. Differences in color and size between livers. A: Control, B: TQ group, C: AF+TQ group, D: AF group. Note: Growth and paleness are seen in AF liver.

Discussion

Aflatoxicosis causes huge economic losses in poultry such as growth retardation, reduced feed conversion ratio, increased susceptibility to diseases and increased mortality. The liver is the main organ in which biotransformation and detoxification of AF takes place. Therefore, it is likely that morphological and histological damages in the liver, which are directly and indirectly affected by the toxic effects of AF, will result in dysfunction and pathomorphological changes. In addition to protecting the body from toxic effects, the liver has very important duties in the metabolism of lipid, carbohydrate, amino acid and some vitamins and their use. When these are considered, it can be understood to what extent a loss of function in the liver carries a vital risk. It has been reported that abnormal liver size due to degenerative changes may result in malfunction in aflatoxicosis (Fouad et al 2019). However, in determining the size of the liver, it is necessary to take into consideration the weight differences among the individuals that may arise from the weakness or fattened of the animals individually. Since the relative weight of the organs is calculated by proportional to the individual body weight of the trial animals, it provides very useful data by eliminating the disadvantages that may arise from the individual weakness or fattened of the chick as opposed to direct organ weight.
In the researches, it was reported that hydropic degeneration and fatty degeneration in hepatocytes were seen due to the toxic effect of AF in the liver, which is the organ where many xenobiotics, especially AF, accumulate and undergo biotransformation, lead to hepatomegaly (Ali Rajput et al 2017) and relative organ weight increase (Ortatatli and Oguz 2001, Valchev et al 2013, Liu et al 2018, Salem et al 2018). Also, Ortatatli et al (2005) emphasized the importance of relative organ weight by reporting that liver color and hepatomegaly are two important macroscopic indicators of aflatoxicosis. When the findings obtained in our study were examined, it was found that AF caused a significant increase in relative liver weight. This has been predicted to be due to the direct toxic effect of AF on hepatocytes, as well as damage to cells by reactive oxygen species and toxic metabolites produced during AF biotransformation. It was noted that in the other trial groups (AF + TQ and AF + NS), the relative liver weight was reduced according to AF group in varying degrees and TQ was more effective than NS in these activities. On the other hand, the color change of livers ranging from light yellow to dark yellow-brown in the AF group was thought to occur due to degeneration. It can be concluded that the amelioration of these color changes compared to the AF group in treatment groups (AF+TQ and AF+NS) is caused by the protective activities of the test substances used against the degenerative changes caused by AF. Another noteworthy situation, although the relative liver weight increase was seen in the NS group, the absence of paleness or color changes at the same time suggested that this may not have been due to degenerative changes (Figure 3). It was predicted that this may be due to other substances other than TQ in the composition of NS, or because of hypertrophy aimed at increasing the capacitive activity of the liver, which is shaped due to the increase in antioxidant activity in the liver.
In the literature review, there was no study of TQ and NS in aflatoxicosis in broilers, however, in some trials with different xenobiotics, TQ and NS have been reported to reduce relative liver weight increase (Abdel-Hamid et al 2013). From this it can be concluded that TQ and NS inhibit the growth of the liver, caused by fat and degenerative changes by correcting lipid metabolism and transport impaired by the toxic effect of AF. It was also evaluated that the antioxidant properties of these substances may resolve tissue damage by preventing AF-induced oxidative stress. Relative liver weight and color changes can be used as an important macroscopic criterion in cases of suspected aflatoxicosis and especially in field conditions where laboratory facilities are absent or limited.

Conclusion

Aflatoxins both adversely affect animal health and the livestock sector and can be transferred from poultry feeds to eggs, meat, and other eatable animal products, in this way threaten human health also as they are an important carcinogenic. In this study, it was concluded that the increase in relative liver weight and color changes, which are the important macroscopic findings of aflatoxicosis, were reduced by adding 300 mg/kg TQ or 50 g/kg NS to aflatoxin-containing feeds. However, it is thought that the evaluation of these data together with other parameters reflecting the effects of aflatoxicosis on the liver and microscopic findings will provide a more conclusive judgment.

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Conflict of Interest

The authors did not report any conflict of interest or financial support.

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