Effect of different dietary levels of corn naturally contaminated with DON and its derivates 3+15 Ac-DON and DON-3-glucoside on the performance of broilers

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ABSTRACT

In the field of mycotoxin research, there is an increasing requirement to understand the effect of these toxins at realistic contamination levels, and as mixtures, on animal health and performance. Although there are recommendations of maximum levels of some mycotoxins in feed, it is known from practice that concentrations below the maximum recommended levels already negatively affect livestock production. In the present study, we exposed broilers to three different levels of naturally contaminated diets containing deoxynivalenol (DON) and its derivates 3 + 15 Acetyl-DON (3 + 15 Ac-DON) and DON-3-glucoside (DON-3-G) to evaluate their effect on birds performance. 630 day-old Ross 308 broilers were housed in 30 pens (21 birds per pen) and fed diets containing increasing levels of DON (Low: 1,650–1,890 μg/kg; Moderate: 2,500–2,880 μg/kg; DON; and High: 3,220–3,900 μg/kg), 3 + 15 Acetyl-DON (Low: 25.6–39.4 μg/kg; Moderate: 42.3–49.1 μg/kg; and High: 58.4–71.1 μg/kg), and DON-3-G (Low: 356–362 μg/kg; Moderate: 405–637 μg/kg; and High: 625–787 μg/kg). Each diet had 10 replicate pens. During the grower period (D13-28) broilers fed diets containing moderate and high contamination levels presented a significantly increased feed intake but accompanied by significant impairment in FCR when the broilers were fed the highest contamination level. Based on this, it can be concluded that broiler production is affected when feed is contaminated with a mixture of DON and its derivates, even at levels below the EU maximum recommendation of 5,000 μg/kg. Furthermore, extra attention should be given to multi-mycotoxins contamination in diets for broilers up to 28 days old.

1. Introduction

The economic losses caused by mycotoxins are mostly related to the extra costs employed to reduce or eliminate mycotoxin contamination in the diet, and the financial losses due to suboptimal animal production (Magnoli et al., 2019). Among the mycotoxins affecting livestock production, the Fusarium mycotoxin deoxynivalenol (DON) appears as one of the most important ones. The knowledge regarding the effects of DON on broilers is mostly based on studies using artificially contaminated diets. Besides, many studies showing the negative impact of DON in broiler chickens are still performed with DON levels far above the recommended one by the EU guidelines, i.e. 5,000 μg/kg (Commission Recommendation 2006/576/EC). For example, the effect of this mycotoxin on intestinal morphology was tested by exposing broilers to a crude extract of DON at a final concentration of 10,000 μg/kg (Wu et al., 2018), or even 19,300 μg/kg in the diet (De Souza et al., 2020). Also, gut leakage was observed in broiler chickens fed a naturally contaminated diet containing 7,500 μg/kg DON levels combined with one of its derivates (3-Ac-DON – 1,481 μg/kg) (Osselaere et al., 2013). Lucke et al. (2017) observed a decrease in the body weight of broiler chickens fed a diet artificially contaminated with 5,000 μg/kg DON. More recently, however, Keçi et al. (2019) showed that dietary contamination with 2,500 μg/kg DON is enough to impair bone mineralization in chickens.

In a longitudinal study, Kolawole et al. (2020) showed that more than 50% of diets used to feed broilers contain the DON derivates 3-Acetyl-DON (3-Ac-DON) and DON-3-glucoside (DON-3-G), at mean concentrations of 42.1 and 46.5 μg/kg, respectively. Both 3 + 15 Ac-DON and DON-3-G are often found at high levels in feedstuffs contaminated with high DON levels because 3 + 15 Ac-DON is produced by the same Fusarium producing DON (Payros et al., 2016), while DON-3-G is produced as a response of the infected plant, where the host UDP-glucosyltransferases conjugate a portion...
of DON with glucose (Karlovsky, 2011). No maximum recommendations for 3 + 15 Ac-DON and DON-3-G are given yet and there is still some information lacking on their effect on broiler health and performance. Likewise, although there are no maximum recommended levels for NIV in animal feed, this mycotoxin is more toxic than DON, where gizzard erosion already occurs when broilers are orally exposed to 3,000 μg/kg NIV (EFSA CONTAM Panel, 2013). However, such a high dietary level is not observed in practice. The maximum acceptable levels of DON in cereals and cereal products used for feed production is 8,000 μg/kg, while for maize by-products fodder materials is 12,000 μg/kg (Commission Recommendation 2006/576/EC). The inclusion levels of maize distiller’s dried grains with solubles (DDGS) in broilers’ diet range from 6 to 16% depending on the birds age (Abudabos et al., 2017; Fries-Craft and Bobeck, 2019; Damasceno et al., 2020). Therefore, under realistic conditions, a maize-based diet prepared with a high inclusion level of such a contaminated grain will have a final DON level of approximately 4,000 μg/kg or less. Therefore, to properly determine risks of losses and action points, it is necessary to evaluate the effects of naturally contaminated diets containing DON levels close to those observed in practice. Furthermore, when the grain is contaminated with DON, most probably its derivates will be also present in the final diet.

Based on the continuous detection of multi-mycotoxin contamination in broiler diets in practice, this study evaluated the effect of a corn-based diet containing different levels of DON and its derivates 3 + 15 Ac-DON and DON-3-G on broiler performance.

2. Materials and methods

The experiment was conducted at Schothorst Feed Research (52.45°N, 5.46°E) in April 2020 according to the guidelines of the Animal and Human Welfare Codes/Laboratory practice codes in the Netherlands. The protocol was approved by the Ethics Review Committee (AVD246002016450). The ambient temperature was gradually decreased from 34.5 °C at arrival to 17.6 °C at 37 days of age. The light was continuously on for the first 24 h to allow birds to readily find feed and water. After that, the light schedule was 2D:22L during one day, and then changed to 4D:10L:2D:8L during the remaining experimental period, complying with the EU legislation of a minimum of 6 h of darkness with at least one period of 4 h uninterrupted darkness (Council Directive 2007/43/EC).

A contaminated corn batch from Canada was used as a natural source of DON (~8,000 μg/kg) together with its derivates 3 + 15-Ac-DON (186 μg/kg) and DON-3-G (1,660 μg/kg). This corn batch had normal nutrient contents (12.8% crude protein, 3.7% fat, and 2.2% crude fiber), and moisture of 11.6%. Experimental diets were produced based on the expected final DON level by preparing diets with highly and marginally contaminated corn to achieve increasing contamination levels. All diets met the broilers’ nutritional requirements (NRC, 1994) and are shown in Table 1. Mycotoxins’ dietary levels were

Table 1. Composition of the experimental diets.

| Ingredients (%) | Starter (D0-13) | Grower (D13-28) | Finisher (D28-37) |
|-----------------|----------------|-----------------|-------------------|
| Corn            | 45.00          | 45.00           | 45.00             |
| Soybean meal    | 34.91          | 30.73           | 26.39             |
| Wheat           | 13.79          | 17.39           | 21.19             |
| Soybean oil     | 0.00           | 0.76            | 0.00              |
| Poultry fat     | 2.79           | 3.00            | 4.00              |
| Palm kernel fatty acids | -   | -               | 0.50              |
| Salt            | 0.33           | 0.24            | 0.23              |
| Limestone       | 0.83           | 0.83            | 0.79              |
| Monocalcium Phosphate | 1.26       | 0.89            | 0.68              |
| Sodium Bicarbonate | 0.00       | 0.10            | 0.11              |
| Lysine HCl      | 0.23           | 0.22            | 0.20              |
| DL-Methionine   | 0.30           | 0.27            | 0.21              |
| Threonine       | 0.06           | 0.07            | 0.06              |
| Valine          | 0.01           | -               | -                 |
| NSP enzyme (Glu-Xyl) | -        | -               | 0.25              |
| Vitamin & Mineral premix | 0.50 | 0.50            | 0.40              |
| AMEn, kcal/kg   | 2,900          | 3,000           | 3,075             |
| DM, g/kg        | 878            | 878             | 878               |
| Ash, g/kg       | 54             | 48              | 43                |
| Crude protein, g/kg | 222     | 206             | 188               |
| Crude fat, g/kg | 58             | 67              | 74                |
| Crude fibre, g/kg | 22         | 21              | 21                |
| Starch, g/kg    | 386            | 407             | 430               |
| Ca, g/kg        | 6.46           | 5.72            | 5.07              |
| P, g/kg         | 6.46           | 5.47            | 4.83              |
| Mg, g/kg        | 1.61           | 1.52            | 1.43              |
| K, g/kg         | 9.69           | 8.92            | 8.13              |
| Na, g/kg        | 1.40           | 1.30            | 1.30              |
| Cl, g/kg        | 3.00           | 2.43            | 2.31              |
| dEB, meq        | 225            | 217             | 200               |
measured by an independent and accredited (BELAC 057-TES-T/ISO17025) laboratory (Primoris Holding, Gent, Belgium) via LC-MSMS.

A total of 630 male Ross 308 broilers were housed in 30-floor pens (21 broilers per pen) with wood shavings as bedding material in the broiler facilities of Schothorst Feed Research, Lelystad, the Netherlands. Each pen had a surface area of 2.2 m² and contained one feeder and two or three drinking nipples. The experiment comprised three dietary treatments and ten replicates. Treatments (Table 2) were randomly allocated per block to the pens. Birds had unrestricted access to feed and drinking water.

All birds were weighed per pen at 0, 13, 28, and 37d. Body weight gain (BWG) was calculated per pen for the separate experimental periods and the entire period, based on average body weight at the beginning of the experimental period minus average body weight at the end of the experimental period. Feed intake was calculated for the periods 0–13, 13–28, 28–37, and 0-37d. Weight of the empty feeders was recorded. Feed that was added per feeder was weighed and recorded. At feed transition on 13 and 28d, the residual feed including the feeder was weighed (weighing feeders with feed) and recorded. The same procedure was performed at 37d but without feed transition.

Feed intake (FI) was calculated per pen and expressed as g/bird. Feed intake was calculated for the periods 0–13, 13–28, 28–37, and 0–37d. Feed intake was corrected for mortality. Feed conversion ratio (FCR) was calculated as FI per kg BWG for the periods 0–13, 13–28, 28–37, and 0–37d. FCR was also corrected for mortality.

Litter quality was visually scored at 13, 28, and 37d, on a scale of 1–10: 1 (low quality (wet)) and 10 (high quality (dry and friable)). Statistical analysis was conducted with the GenStat statistical software (GenStat for Windows 20th Edition, VSN International, Hemel Hempstead, UK; https://www.vsni.co.uk/downloads/genstat/). The null hypothesis was that there was no treatment effect on the response parameter. Treatment means were compared according to Fisher’s posthoc LSD (for a two-sided test and P ≤ 0.05) after a significant treatment effect was confirmed by ANOVA. Effects with P ≤ 0.05 were considered to be statistically significant. Each pen was an experimental unit. The statistical model used to analyze the data was:

\[ Y = \mu + \text{block}_i + \text{treatment}_j + e_{ij} \]

In which:
- \( Y \) = Response parameter
- \( \mu \) = General mean
- \( \text{block}_i \) = Effect of replicate (i = 1…10)
- \( \text{treatment}_j \) = Effect of Treatment (j = 1, 2, 3)
- \( e_{ij} \) = Error term

Table 2. Mycotoxins levels in the starter, grower, and finisher diets.

| Diets (contamination levels in μg/kg) | Low   | Moderate | High  |
|---------------------------------------|-------|----------|-------|
| **Starter (D0-13)**                   |       |          |       |
| DON                                   | 1,890 | 2,740    | 3,900 |
| 3 + 15 Ac-DON                         | 39.4  | 46.0     | 58.4  |
| DON-3-G                               | 362   | 637      | 703   |
| Nivalenol                             | 70.8  |  -       |  -    |
| Ochratoxin A                          | -     |  -       |  -    |
| Fumonisin B1+B2                       | -     |  30.2    |  37.0 |
| Zearalenone                           | 385   | 456      | 588.0 |
| Alternariol                           | -     |  -       |  -    |
| Beauvericin                           | 9.8   |  -       |  -    |
| **Grower (D13-28)**                   |       |          |       |
| DON                                   | 1,650 | 2,880    | 3,440 |
| 3 + 15 Ac-DON                         | 25.6  | 42.3     | 71.1  |
| DON-3-G                               | 358   | 442      | 787   |
| Nivalenol                             | -     |  -       |  -    |
| Ochratoxin A                          | -     |  -       |  -    |
| Fumonisin B1+B2                       | 58.2  | 39.4     | 101   |
| Zearalenone                           | 290   | 602      | 542   |
| Alternariol                           | -     |  -       |  -    |
| Beauvericin                           | -     |  -       |  -    |
| **Finisher (D28-37)**                 |       |          |       |
| DON                                   | 1,740 | 2,500    | 3,220 |
| 3 + 15 Ac-DON                         | 38.6  | 49.1     | 59.5  |
| DON-3-G                               | 356   | 405      | 625   |
| Nivalenol                             | -     |  -       |  -    |
| Ochratoxin A                          | 1.9   | 3.0      | 1.6   |
| Fumonisin B1+B2                       | 45.9  | 56.6     | 42.0  |
| Zearalenone                           | 335   | 428      | 583   |
| Alternariol                           | -     |  -       |  -    |
| Beauvericin                           | 6.6   |  -       |  -    |

Below detection level in all diets: Aflatoxin B1, B2, G1, and G2, T2 & HT2 Toxin, Diacetoxyscirpenol, Cytochalasine E, Sterigmatocystin, Alternariol ME, Citrinin, Roquefortine C, Enniatin A, A1, B and B1, Moniliformin.
3. Results and discussion

All birds had an initial body weight of 42 g at the start of the trial, and no symptoms of disease were observed during the experiment. No differences among the treatments were observed when comparing body weight (BW). During the starter period (0–13d), broilers fed the diet with the Moderate DON had the poorest feed conversion ratio (FCR), and no other significant differences in performance parameters were observed. However, during the grower period (13–28d), feed intake (FI) was significantly increased when broilers were fed a diet contaminated with the moderate or high mycotoxins levels without affecting body weight gain (BWG), resulting in a significant increase of FCR when the birds were fed the highly contaminated diet. During the finisher period (28–37d) or when considering the complete feeding period (0–37d), no significant differences were observed among the treatments (Table 3). No differences between the pens were observed on the litter score, which had good quality (dry and friable). Besides DON and its derivatives, the diets were also contaminated with fumonisins B1+B2 and zearalenone (ZEN; Metzler, 2011), while ochratoxin A was detected in the finisher diet, but at extremely low levels (<2 μg/kg), NIV was present in the starter low DON diet at a negligible level (70.8 μg/kg), and aflatoxins were not detected. Fumonisins were found at a concentration of ~30 μg/kg, which is far lower than the maximum recommended levels of 20,000 μg/kg in broilers diets; Commission Recommendation 2006/576/EC), while ZEN levels ranged from 290 to 602 μg/kg, but poultry is not sensitive to this mycotoxin, which explains the absence of maximum recommendation levels of ZEN for poultry. The presence of a low amount of NIV in one of the diets is a result of the heterogeneous distribution of this toxin, which was not the primary one interfering with broilers performance. In broiler chickens, NIV has low oral bioavailability (~4%) and is rapidly eliminated via feces (Kongkapan et al., 2016).

As demonstrated by Broekaert et al. (2015a), the oral bioavailability of 3-Ac-DON and 15-Ac-DON in broiler chickens is 18.2 and 42.2%, respectively. Also, 100% from 3-Ac-DON and 75% from 15-Ac-DON can be deacetylated to DON. Furthermore, acetylated forms of DON have a low polarity facilitating their passive diffusion into the blood circulation (Broekaert et al., 2015b). In the present study, the levels of 3 + 15-Ac-DON were below 100 μg/kg, but their dietary levels increased together with DON levels. Therefore, the contribution of these DON acetylated forms on impaired animal performance should not be neglected. Although in the present study the levels of DON-3-G ranged from 356 to 787 μg/kg, this mycotoxin is not toxic to the intestine because of its inability to bind the main target of the DON toxicity, i.e. the ribosome peptidyl transferase center (Pierrot et al., 2015). Differently from pigs, DON-3-G is not hydrolyzed to DON in broilers chickens, and its oral bioavailability is very low (<4%) (Broekaert et al., 2017).

While in the starter period some differences were observed regarding FCR, feed consumption in these first two weeks of life is very small, but probably the deleterious effects of the mycotoxins are already taking place. The negative effects of the mycotoxins on performance were mainly found in the grower period (13-28d), where an increased FI was observed in broilers fed the moderate and high DON diets, with an impaired FCR in broilers fed the high DON diet. Usually, it is expected that DON will decrease FI, as reported in broilers fed high DON levels in the diets (>5,000 μg/kg) (Lucke et al., 2017; Kolaowale et al., 2020). It was noteworthy that in a study from Dietrich et al. (2011) a numerical increase of FI was observed with the increase of DON in the diet (from 1,000 to 2,500 μg/kg), but it decreased when birds were fed 5,000 μg/kg DON. Likewise, Ghereeb et al. (2014) also observed a numerically increased FI in broilers in the first three weeks being fed diets contaminated with 10,000 μg/kg DON. In the present study, FI was significantly increased with the increase of mycotoxins levels in the diets. It seems that the birds were attempting to compensate for some deficiency in nutrient absorption caused by the mycotoxins.

During the finisher period, no differences were observed among the treatments, which might be a result of bird adaptation to mycotoxin exposure. Similarly, Wang and Hogan (2019) found that production

| Table 3. Effect of mycotoxins levels on broiler performance and on mortality rate. |
|---|---|---|---|---|---|
| D0-13 | DON and derivates’ levels | Low | Moderate | High | P-value | LSD |
| BW D13 | 474 | 473 | 487 | 0.19 | 16.8 |
| BWG (g) | 431 | 313 | 145 | 0.19 | 16.7 |
| F1 (g) | 469 | 478 | 481 | 0.50 | 15.7 |
| FCR (g/g) | 1.09 a | 1.19 b | 1.081 a | 0.006 | 0.0160 |
| Mortality (%) | 0.00 | 0.48 | 0.48 | 0.63 | 1.18 |
| D13-28 | 1176 | 1806 | 1810 | 0.10 | 34.1 |
| BW D28 | 1302 | 1333 | 1323 | 0.12 | 29.3 |
| BWG (g) | 1765 a | 1825 b | 1842 b | 0.001 | 37.4 |
| F1 (g) | 1.355 a | 1.369 a | 1.392 b | 0.006 | 0.0216 |
| FCR (g/g) | 0.48 | 0.95 | 0.48 | 0.74 | 1.466 |
| Mortality (%) | 0.48 | 0.95 | 0.48 | 0.74 | 1.466 |
| D28-37 | 2760 | 2779 | 2807 | 0.41 | 57.4 |
| BW D37 | 984 | 973 | 997 | 0.05 | 38.4 |
| BWG (g) | 1594 | 1554 | 1577 | 0.15 | 0.05 |
| F1 (g) | 1.624 | 1.600 | 1.588 | 0.06 | 0.095 |
| FCR (g/g) | 1.43 | 2.38 | 1.43 | 0.59 | 2.212 |
| Mortality (%) | 1.91 | 3.81 | 2.39 | 0.49 | 3.412 |

a,b Values without a common letter within a row differ significantly (P < 0.05).
losses are more severe if broilers are fed contaminated diets with 7,900 µg/kg DON between 22 and 34-days-old. In this later study, DON was the major mycotoxin in the diet and its derivates were not detected. These tested levels are much higher than those tested in our study, showing that natural contamination with DON and its derivates may be sufficient to impair broilers’ performance at very realistic and commercially acceptable levels.

In conclusion, DON at levels just above 3,000 µg/kg combined with its derivates 3-15 Ac-DON and DON-3-G impair broiler FCR in the first 28 days of age. The negative effects of DON are less intense when this mycotoxin is present in the finisher feeding period, suggesting that moderately contaminated corn should be avoided in the starter and grower phases and could be added in the last feeding phase. Together with the challenge caused by the presence of DON and its derivates in broiler diets, it is necessary to consider that diet composition, e.g. type of feedstuffs and risks to increase intestinal viscosity, as well as by management system or diseases, may enhance the negative effects caused by these mycotoxins.

Declarations

Author contribution statement
Regiane R. Santos: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Francesc Molist: Analyzed and interpreted the data; Wrote the paper.

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