



Coccidiostat residues in withdrawal feed for broilers – implications of cross-contamination in feed production lines

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ABSTRACT

This study investigated the presence of residues of eleven coccidiostats in unmedicated feed intended for broilers in the withdrawal phase, with the aim of assessing potential cross-contamination during feed production. Between 2023 and 2024, a total of 341 feed samples were analysed, using validated LC-MS/MS methodology, for amprolium, diclazuril, halofuginone, lasalocid, salinomycin, maduramycin, monensin, narasin, nicarbazin, robenidine, semduramycin. Detected concentrations ranged from below the limit of quantification to 85.0 mg/kg. Non-compliant samples were identified for diclazuril (2/243), lasalocid (1/328), salinomycin (12/329), maduramycin (11/324), monensin (6/324), narasin (6/315), nicarbazin (2/327), and robenidine (7/333). The highest detected concentrations per analyte were: diclazuril 0.133 mg/kg, lasalocid 1.33 mg/kg, salinomycin 85.0 mg/kg, maduramycin 0.834 mg/kg, monensin 25.30 mg/kg, narasin 58.15 mg/kg, nicarbazin 33.70 mg/kg, and robenidine 35.2 mg/kg. Amprolium, halofuginone, semduramycin were not detected in any feed sample. Overall, 42 out of 341 feed samples (12.3%) exceeded the maximum permitted levels, indicating insufficient cleaning of production lines and inadequate separation between medicated and non-medicated feed. The occurrence of such residues in feed intended for broilers in the withdrawal phase poses a risk of non-compliant residues in edible tissues, highlighting the need for stricter hygiene protocols, improved quality control in feed mills, and continuous monitoring to safeguard food safety and public health.

1. Introduction

Coccidiosis is a parasitic disease caused by protozoa of the genus *Eimeria*, affecting poultry worldwide, particularly broilers in intensive production systems. The disease remains a major threat to animal health and welfare, often causing significant economic losses due to reduced productivity,

increased mortality, and treatment costs (Bampidis *et al.*, 2022; EFSA, 2021). Preventive use of coccidiostats in feed is still considered indispensable for maintaining flock health and production efficiency (EFSA, 2008; EFSA, 2021).

In the European Union and Serbia, coccidiostats are authorized as feed additives for poultry

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and include ionophores (lasalocid, monensin, salinomycin, maduramycin, narasin, semduramycin) and synthetic compounds (amprolium, diclazuril, nicarbazin, decoquinate, robenidine, halofuginone). These substances are administered during early and mid-production phases, but are withdrawn several days before slaughter to avoid residues in edible tissues. During the withdrawal phase, animals should be fed non-medicated feed; however, cross-contamination can occur if medicated and non-medicated feeds are produced sequentially without adequate cleaning of production lines. This so-called unavoidable carry-over may result in detectable coccidiostat residues in non-target feed and subsequently in animal products (Mortier et al., 2005; Stolker et al., 2007; Galarini et al., 2021).

To manage this risk, the European Union has established maximum permissible levels of unavoidable carry-over in non-target feed, set at 1% of the authorized concentration in target feed (*Directive 2021/808/EC*). Serbia has harmonized its national legislation with these requirements (*Official Gazette RS*, Nos. 4/2010, 113/2012, 27/2014, 54/2017). Table 1 presents the current maximum levels of unavoidable carry-over for the 12 coccidiostats included in this study.

The presence of coccidiostats in withdrawal feed above these maximum levels increases the likelihood of residues in edible tissues, potentially leading to non-compliance with established maximum

Table 1. Maximum levels of unavoidable carry-over (1%) of coccidiostats in non-target feed (mg/kg) (Source: Commission Regulation (EU) 2020/499)

Maximum level (mg/kg)	
Amprolium	n/a (no EU carry-over limit set, national rules may apply)
Diclazuril	0.03
Halofuginone	0.03
Lasalocid	1.25
Salinomycin	0.70
Maduramycin	0.05
Monensin	1.25
Narasin	2.10
Nicarbazin (DNC)	3.75
Robenidine	0.70
Semduramycin	0.25

residue limits (MRLs) in meat and other animal products. This risk is particularly relevant for ionophores, such as lasalocid, monensin, narasin and nicarbazin, which have relatively high carry-over limits and can persist in production lines if flushing procedures are inadequate. Continuous monitoring and strict feed mill hygiene protocols are, therefore, essential to ensure compliance with food safety regulations and protect public health.

2. Materials and methods

Between 2023 and 2024, 341 withdrawal feed samples for broilers were collected from various feed mills, including rinsing batches used when switching from medicated to unmedicated feed. Eleven coccidiostats were analysed: amprolium (AMP), diclazuril (DICL), halofuginone (HAL), lasalocid (LAS), salinomycin (SAL), maduramycin (MAD), monensin (MON), narasin (NAR), nicarbazin (DNC), robenidine (ROBN), semduramycin (SEM). Sample preparation involved homogenization, extraction of 5.0 g feed with 25 mL acetonitrile, shaking for 60 min, and filtration through a 0.22 µm filter. LC-MS/MS analysis was performed on a Shimadzu triple quadrupole system with a Kinetex C18 column (100 × 2.1 mm, 2.6 µm) using gradient elution (water/acetonitrile with 0.1% formic acid) at 0.55 mL/min. Electrospray ionization was applied in positive or negative mode, depending on the analyte. Quantification was done using matrix-matched calibration at four levels. The method was validated according to Commission Implementing Regulation (EU) 2021/808 (*European Commission*, 2021), achieving recoveries between 80–110% and LOQs of 0.005–0.02 mg/kg. Internal quality control included fortified blank feed samples in each batch.

3. Results

The analysis of 341 feed samples labelled as withdrawal feed for broilers revealed the presence of residues from 8 of the 11 investigated coccidiostats. Amprolium, halofuginone and semduramycin were not detected in any feed sample. Non-compliant results (above the maximum permissible levels for unavoidable carry-over) were recorded for diclazuril, lasalocid, salinomycin, maduramycin, monensin, narasin, nicarbazin, and robenidine. Overall, 42 out of 341 feed samples (12.3%) exceeded the legal limits. Salinomycin showed the highest proportion of non-compliant feed samples (3.6%), followed by

Table 2. Concentration of coccidiostats in withdrawal feed for broilers (2023–2024)

Coccidiostat	n (samples)	Min–Max (mg/kg)	Positive* n (%)	Non-compliant** n (%)
Amprolium (AMP)	84	<LOQ – n.d.	0 (0.0)	0 (0.0)
Diclazuril (DICL)	243	<LOQ – 0.133	6 (2.47)	2 (0.82)
Halofuginone (HAL)	85	<LOQ – n.d.	0 (0.0)	0 (0.0)
Lasalocid (LAS)	328	<LOQ – 1.33	27 (8.23)	1 (0.30)
Salinomycin (SAL)	329	<LOQ – 85.0	197 (59.88)	12 (3.65)
Maduramycin (MAD)	324	<LOQ – 0.834	56 (17.28)	11 (3.40)
Monensin (MON)	324	<LOQ – 25.30	124 (38.27)	6 (1.85)
Narasin (NAR)	315	<LOQ – 58.15	95 (30.16)	6 (1.90)
Nicarbazin (DNC)	327	<LOQ – 33.70	98 (29.97)	2 (0.61)
Robenidine (ROBN)	333	<LOQ – 35.20	65 (19.52)	7 (2.10)
Semduramycin (SEM)	83	<LOQ – n.d.	0 (0.0)	0 (0.0)

*Positive: detected residues above LOQ but below maximum permissible carry-over level.

**Non-compliant: detected residues above maximum permissible carry-over level as defined in Regulation (EU) No. 574/2011 (European Commission, 2011).

maduramycin (3.4%) and robenidine (2.1%). The highest detected concentration was 85.0 mg/kg for salinomycin, corresponding to more than 100 times the allowable carry-over limit. Such elevated concentrations strongly indicate inadequate cleaning protocols between the production of medicated and unmedicated feed batches.

The majority of feed samples with detectable residues were below the maximum permissible levels, yet a significant portion (approximately 80%) contained more than one coccidiostat, typically in low concentrations. This finding aligns with previous studies (Mortier *et al.*, 2005; Galarini *et al.*, 2021) showing that cross-contamination in feed mills often involves multiple analytes, reflecting sequential production of different medicated feeds without adequate line flushing. The detection of high concentrations (e.g., 85.0 mg/kg salinomycin, 58.15 mg/kg narasin, 35.2 mg/kg robenidine) in withdrawal feed raise concern over possible residues in edible tissues, particularly when withdrawal periods are not respected. These results highlight the importance of strict hygiene protocols, physical separation of production lines, and targeted monitoring of high-risk compounds, such as salinomycin, narasin, and robenidine.

4. Discussion

The detection of residues from multiple coccidiostats in withdrawal feed for broilers, includ-

ing concentrations exceeding the maximum permissible levels for unavoidable carry-over, indicates that while most producers follow regulatory requirements, some hygiene gaps persist within feed manufacturing processes. The overall non-compliance rate of 12.3% observed in this study is relatively low considering the total number of samples, but still highlights that cleaning procedures between medicated and non-medicated batches are not always fully effective. Similar challenges have been reported in some EU countries, where non-compliance rates typically range between 6–9% (Mortier *et al.*, 2005; Roila *et al.*, 2019; Annunziata *et al.*, 2020), indicating that the current findings are consistent with trends observed across Europe.

Salinomycin was the most frequently detected analyte, present in 59.9% of feed samples, with the highest individual concentration (85.0 mg/kg) and non-compliance rate (3.65%). Maduramycin and robenidine also showed notable non-compliance rates (3.40% and 2.10%, respectively). The frequent detection of multiple coccidiostats in the same feed sample, usually at low levels, likely reflects sequential production of different medicated feeds without complete line clearance. While these residues do not necessarily indicate intentional misuse, they underline the need for consistent application of hazard analysis and critical control point (HACCP) principles to prevent cross-contamination at all stages.

From a food safety perspective, the presence of coccidiostats above regulatory limits in withdrawal feed is concerning, because this feed should be completely free of these compounds to ensure compliance with MRLs in meat. Although the majority of feed samples were compliant, the occurrence of exceedances—particularly at high concentrations—confirms that risks remain if preventive measures fail. Maintaining strong HACCP-based monitoring, regular verification of cleaning protocols, and staff training across the feed production chain will help sustain compliance levels and further reduce contamination risks. The feed producers generally adhered to established legal and safety standards, with non-compliance rates similar to those reported in EU countries. However, continuous improvement in good manufacturing practice (GMP), good hygiene practice (GHP), and HACCP implementa-

tion is essential to ensure that carry-over remains within permissible limits, thereby safeguarding food safety and consumer trust.

5. Conclusion

This study showed that 87.7% of withdrawal feed samples for broilers complied with legal carry-over limits for coccidiostats, while 12.3% exceeded these thresholds. Although most feed was compliant, high concentrations in some non-compliant feed samples confirm that preventive measures are not always fully effective. Strengthening hygiene protocols, quality control, and HACCP implementation across the feed production and distribution chain is essential to ensure residue-free withdrawal feed, protect food safety, and maintain consumer trust.

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