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The residue levels of narasin in eggs of laying hens fed with unmedicated and medicated feed

Mervi Rokka[1], Susanna Eerola[1], Ulla Perttilä[1], Laila Rossow[2], Eija Venäläinen[3], Eija Valkonen[3], Jarmo Valaja[3] and Kimmo Peltonen[1]

[1]National Veterinary and Food Research Institute, EELA, Department of Chemistry, Helsinki, Finland
[2]National Veterinary and Food Research Institute, EELA, Department of Pathology, Helsinki, Finland
[3]MTT Agrifood Research Finland, Animal Nutrition, Jokioinen, Finland

Laying hens were fed contaminated feed containing narasin 2.5 mg/kg for 21 days followed by a 7 day withdrawal period, hens in the control group were fed unmedicated feed. Eggs were collected during trial days 0, 3, 7, 14, 21 and after the withdrawal period of 7 days. The concentration of narasin in yolks and egg whites was analyzed by a liquid chromatography-mass spectrometry method. Narasin was found to accumulate in yolks, where the narasin concentration increased during the treatment. The concentration of narasin varied from 5.9 to 13.8 μg/kg (mean 10.6 μg/kg) in yolks after 21 day feeding periods. The concentrations of narasin ranged from <0.9 to 1.4 μg/kg after the withdrawal period. Narasin residues were not found in egg whites of the laying hens fed contaminated feed nor in either yolks or egg whites of the laying hens fed unmedicated feed. The effect of cooking was also tested on the amount of narasin residues in eggs. Cooking for 10 min did not significantly influence the narasin residues in eggs. Traces of lasalocid were also found in the yolks. The traces of lasalocid are attributable to an accidental contamination of the feed during its manufacture.

Keywords: Coccidiostats / Eggs / Narasin

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1 Introduction

The ionophoric coccidiostats lasalocid, monensin, salinomycin, and narasin (Fig. 1) are widely used in the poultry industry to prevent and control coccidiosis caused by the genus *Eimeria*. In Finland they are added into feeds as their sodium salts at concentration levels of 10–100 mg/kg. Anticoccidial drugs are also given to other farm animals to improve feed efficiency, *i.e.*, to increase the body mass. Broiler chickens receive coccidiostats in the feed during their entire lifespan. In order to avoid residues in edible tissues it is recommended that there is a withdrawal period of 5 days for lasalocid, salinomycin, and narasin and 3 days for monensin. Lasalocid, monensin, and salinomycin are used for laying hens up to the age of 16 weeks. None of the coccidiostats including narasin are licensed for use in egg-laying birds and therefore eggs should be free from coccidiostats contamination. Nonetheless, residues of coccidiostats have been detected in eggs. These have been attributed either to contamination of unmedicated feed at the feed manufacturer [1–3] or accidental feeding of salinomycin [4] or lasalocid [5] to the laying hens.

Coccidiostats are not used in human medicine because of their toxicity [6] principally due to their cardiovascular effects [7], *e.g.*, lasalocid has been found to cause contraction of human heart under laboratory conditions [8]. The toxic effects of coccidiostats have been noted in many animal studies. An accidental feeding with lasalocid has caused leg weakness, ataxia, and microscopic muscular damage in broiler breeder chickens [5]. Also an accidental feeding with salinomycin has caused weight loss, cannibalism, and necrotic hepatitis with infarcts for in breeders [4]. Salinomycin toxicity seems to be mostly neurological in mice, rats, and rabbits. In pigs, bulls, and horses salinomycin damaged both liver and myocardium [9]. Even low concentrations of monensin and narasin can increase coronary blood flow in dogs [10, 11]. Monensin has also been found to interfere with feather growth in young chicks and to evoke hyperexcitability [12], whereas narasin has caused anorexia, hypoactivity, leg weakness, ataxia, depression, and diarrhea in animals of various species [11]. Even though coccidiostats are rather toxic, the residues in meat and other animal products have been found to be so low, that they have not induced any observable clinical symptoms in humans [13].

Correspondence: Dr. Mervi Rokka, National Veterinary and Food Research Institute (EELA), Department of Chemistry, P.O.BOX 45, FI-00581 Helsinki, Finland
E-mail: mervi.rokka@eela.fi
Fax: +358-9-393-1920

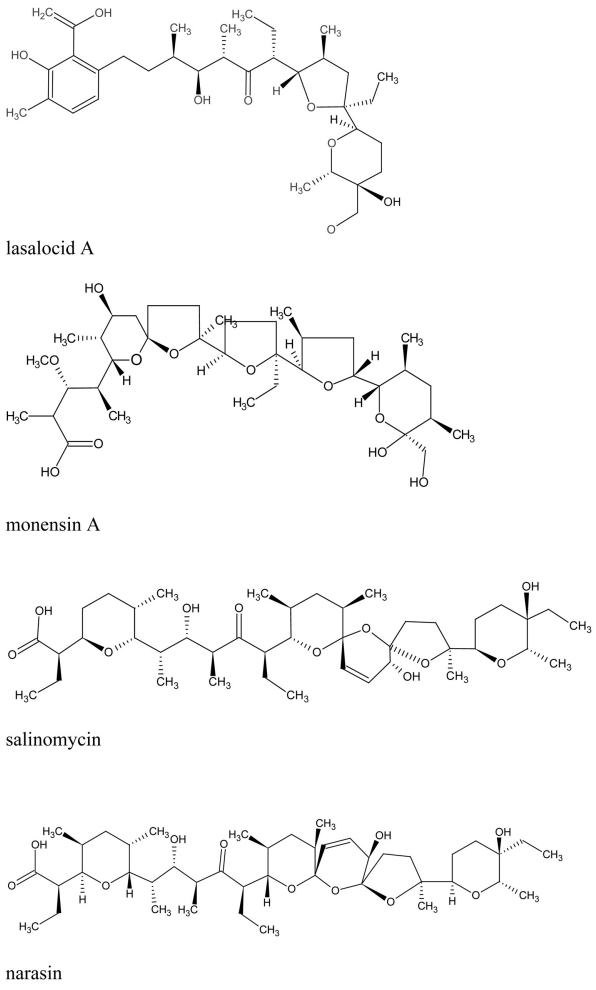

Figure 1. Chemical structures of lasalocid A, monensin A, salinomycin, and narasin.

Coccidiostats have a variety of effects on the production and the quality of eggs, effects which appear to be concentration-dependent. Egg production did not decrease, even if broiler-breeders or laying hens were fed with feed containing maximum levels of monensin (100 mg/kg), narasin (70 mg/kg), or salinomycin (60 mg/kg) [14 – 16]. However, if higher concentrations of salinomycin (90 – 150 mg/kg) or lasalocid (115 – 150 mg/kg) are administered, then a significant decrease in the egg production is observed [4, 5] although narasin had only a minor influence on egg weight of broiler-breeders at a level of 70 mg/kg [14]. In that same study, narasin and salinomycin were noted to reduce hatchability. The shells of eggs of broiler-breeders fed with monensin and salinomycin were slightly depigmented, but no association could be established between depigmentation and the drug concentration. The same experiments were carried out with White Leghorn laying hens using monensin, salinomycin, and narasin, but they did not demonstrate any significant effect on egg weight, hatchability and shell thickness [14, 15].

Narasin and lasalocid residues were detected in a few egg samples in Finland in 2000 and 2002. Narasin was detected in three egg samples (>10 μg/kg) [17] and lasalocid was detected in two egg samples (34 and 51 μg/kg, respectively) [18]. The contamination had occurred in the feed production process. The object of this study was to assess the accumulation of narasin into eggs, when laying hens were fed with medicated feed for 21 consecutive days followed by a 7-day withdrawal period. A control group of laying hens received unmedicated feed. The yolks and egg whites of the eggs were separated and analyzed individually using a liquid chromatography-mass spectrometry method (Rokka and Peltonen, submitted for publication in *Food Additives and Contaminants*). The influence of cooking on narasin residues was also investigated.

2 Materials and methods

2.1 Feeding trial

The trial was conducted using 16 LSL laying hens (69 weeks of age), which were housed with three hens per cage. The cages were separated from the others in the henhouse. The unmedicated feed for laying hens was prepared first. The contaminated feed was prepared by adding narasin to the unmedicated feed, *i.e.*, the concentration of narasin (2.5 mg/kg) was equal to the situation where 5% of medicated feed (containing 0.005% narasin) would be mixed with unmedicated feed. The ingredients and the chemical composition of the feed are listed in Table 1. The laying hens were first fed with unmedicated feed which was changed to the contaminated feed for three weeks. Feeding was continued with unmedicated feed for a one week withdrawal period. A control group of the laying hens were fed with unmedicated feed. The eggs were collected on 0 d, 3 d, 7 d, 14 d, 21 d, and 28 d and marked accordingly with the group and date. Eggs laid on the other days were destroyed. Six eggs from every group were selected randomly. Yolks and egg whites were separated and they were analyzed separately. Three eggs from one group were also selected randomly and cooked for 10 min. After cooking, the yolks and the egg whites were separated and analyzed separately.

2.2 Determination of coccidiostats

The concentrations of narasin and lasalocid in yolks and egg whites were measured as described by Rokka and Peltonen (submitted). Briefly, a sample was extracted with acetonitrile and cleaned up with solid-phase extraction using a silica column. The purified sample was analyzed by liquid chromatography-mass spectrometry. The detection capability (CCß) for narasin was 0.9 μg/kg and for lasalocid 2.0 μg/kg. Tukey's test and analysis of variance (ANOVA) were

Table 1. Composition of the diet for layers

Ingredient	Percentage
Barley	41.77
Wheat	10.00
Oat	15.00
Soybean	20.20
Rapeseed oil	2.10
Monocalcium phosphate	1.55
Limestone	8.50
Salt	0.38
Trace-mineral mix	0.20
Vitamin premix	0.20
Methionine	0.10
Narasin (only in contaminated feed)	0.0025
Lasalocid	< 0.001

Chemical composition	g/kg
Protein	175.31
Fat	42.49
Fiber	41.45
Nitrogen free extracts	500.72
Ash	29.15
Lysine	8.54
Methionine	3.78
Cystine	3.32
Calcium	39.36
Phosphate	3.82
Sodium	1.54
ME[a)] (MJ/kg)	10.30

a) ME, metabolized energy

used to evaluate the statistical significance of the differences.

3 Results and discussion

In our study narasin was not detected in any yolks of the eggs from the control group. At the beginning of this trial narasin was not detected in yolks from the eggs from the hens given the medicated feed. However, the concentration of narasin increased in the yolks, reaching a plateau after 7 days. The mean concentration of narasin was 6.3 μg/kg on day 7 and 7.1 μg/kg on day 14, respectively. Thereafter, the mean concentration of narasin increased up to 10.6 μg/kg (at day 21). The mean concentration levels of narasin in yolks of laying hens fed with medicated feed are shown in Fig. 2. The concentration of narasin in yolks was at the same level in this trial as found in another study [3]. The source of the residues in that study was explained by probable contamination of unmedicated feed.

Similar feeding trials with low levels of other coccidiostats, such as lasalocid, salinomycin, and monensin, have been reported [1, 2]. In these studies, the concentrations of lasalocid in the feed varied from 0.1 to 5.0 mg/kg, salinomycin from 0.9 to 13.9 mg/kg, and monensin from 1.1 to 12.9 mg/kg. The coccidiostats were not determined at the beginning of the trial, but they were present after 1 day of feeding. Also, in these studies it was noted that the concentration of lasalocid, salinomycin, and monensin in eggs reached a plateau after 7 days of exposure. The concentration of coccidiostats in eggs was explained by the amount of coccidiostats present in the feed [1, 2]. The concentration of lasalocid in eggs ranged from 7 to 200 μg/kg, salinomycin from 1 to 60 μg/kg and monensin varied from 0.1 to 4.0 μg/kg. As can be seen in Fig. 2, the concentration of narasin increased in our study linearly from day 14 to 21. This finding is different compared with other coccidiostats as discussed above.

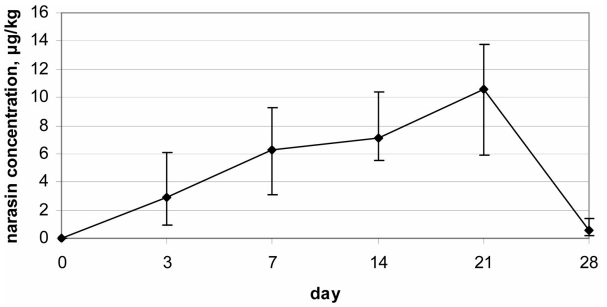

Figure 2. Mean, the lowest and the highest concentrations of narasin in egg yolks of laying hens fed with medicated feed (concentration of narasin 2.5 mg/kg) for 21 days followed by a 7-day withdrawal period. Narasin was not found in egg whites analyzed.

In our study the hens were fed unmedicated feed during the withdrawal period (7 days). After this withdrawal period we measured narasin concentrations, which ranged from less than 0.9 μg/kg up to 1.4 μg/kg in yolks. These results are different compared with other coccidiostats. It was shown that salinomycin is detectable in eggs up to 3 days after withdrawal [16]. Lasalocid, which transfers into eggs more readily than, *e.g.*, salinomycin, could still be found in eggs 10 days after withdrawal [1]. In this earlier reported feeding study, the laying hens had received lasalocid at a level of 5 mg/kg in their feed.

In our study the low concentration of narasin (2.5 mg/kg) in feed did not lead to any detectable residues in the egg whites. Kan and Petz [19] reported a very high concentration of narasin (250 μg/kg) in egg whites. However, in that experiment the laying hens had received narasin at a level of 70 mg/kg in feed. Other coccidiostats have also been detected in egg whites, but once again the reported levels of coccidiostats in feed have been much higher than used in our trial [5, 14, 17, 18]. Akhtar *et al.* [16] detected concentrations levels of salinomycin ranging from < 10 μg/kg to 200 μg/kg in egg whites in a study in which the hens were

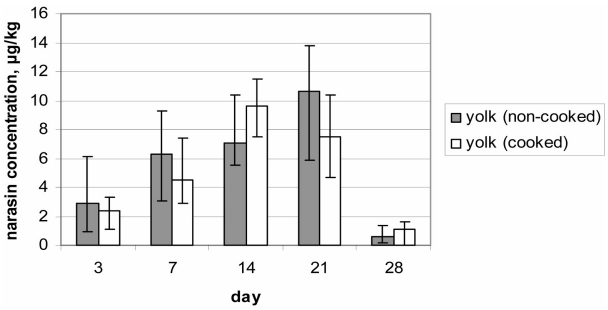

Figure 3. Concentration of narasin in noncooked egg yolks and in yolks heated for 10 min. Narasin was not found in egg whites analyzed.

fed with feed containing salinomycin at the level from 30 to 150 mg/kg. Sinigoj-Gacnik [20] reported that the salinomycin content was < 10 µg/kg in egg whites, if the salinomycin in feed was 60 mg/kg. In the study of Kan and Petz [19] egg whites contained a 100 µg/kg concentration of monensin, when the laying hens had been exposed to feed containing monensin 110 mg/kg.

We tested the influence of heating on the narasin residue level by cooking the eggs for 10 min. There were some differences between the mean concentrations of narasin in non-cooked and cooked yolks, but the difference was not statistically significant ($p < 0.05$). This is in agreement with the results of Sinigoj-Gacnik [20] who heated salinomycin contaminated eggs for 5 min, but did not observe any alterations in the levels of salinomycin. The effect of heating for 10 min on the concentration of narasin in our study is shown Fig. 3.

The LC-MS/MS method used in this study was able to detect four coccidiostats, lasalocid, monensin, salinomycin, and narasin. In addition to narasin we found traces of lasalocid in all of the yolk samples that we analyzed. The amounts of lasalocid were always less than 2.0 µg/kg. Lasalocid was not detected in egg whites. Lasalocid was not used in this trial, but it has been applied earlier in the same feed manufacture. The concentration of lasalocid in feed using this study was below 1.0 mg/kg. Lasalocid is known to be a very dusty compound, which easily can contaminate feed during the manufacturing processes. Kennedy *et al.* [1] found traces of lasalocid in approximately 66% of the eggs they examined in the Northern Ireland. Lasalocid residues were attributed to an accidental cross-contamination of the feed consumed by the laying hens. The same study also found a carry-over of lasalocid from medicated feed to unmedicated batches of feed. Lasalocid was found at levels as high as 0.5 – 1.0 mg/kg in the ninth batch of unmedicated feed, a level high enough to lead to residues in eggs [1].

4 Concluding remarks

We conducted a feeding trial, in which laying hens were fed with medicated feed with narasin followed by a withdrawal period. The concentration of narasin 2.5 mg/kg in the feed resulted in narasin residues higher than 10 µg/kg in the egg yolks. In this trial the content of residues was at the same level as those found in some Finnish egg samples analyzed in 2000. In the yolks analyzed also the traces of lasalocid were detected, though this compound had not been intentionally added to the feeds. Lasalocid had been used earlier in the feed manufacture and thus its presence pointed to accidental contamination. Thus, the feed industry needs to pay attention to the risk of cross-contamination, when medicated and unmedicated feed are being manufactured on the same production lines. The Finnish feed industry has already initiated changes in its production protocols to reduce the risk of the contamination and thus eliminate coccidiostat residues from eggs for human consumption.

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