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Abstract

Coccidiosis remains a significant threat to the welfare of game farm-reared pheasants in the United States. Although lasalocid has been demonstrated to be effective against pheasant specific coccidia, information regarding its safety in this species is lacking. The purpose of this study was to gather data on the safety of lasalocid when fed to Chinese ring-necked pheasants at one, two, and three times the recommended high dose of lasalocid used for prevention of coccidiosis in other poultry at three times the normal treatment period. Pheasant chicks (approximately 1 day-old; $n = 160$) were randomly blocked by sex into four treatment groups and given their respective diets continuously for 6 wk. No significant differences were observed in overall feed consumption, weight gain, feed conversion rates, clinical pathology measurements, or tissue gross and histopathologic evaluations between controls and treatment groups associated with lasalocid administration. Based on the results of this study it appears that lasalocid fed at the recommended rate of 125 ppm is safe in Chinese ring-necked pheasants.

Keywords

coccidiosis, lasalocid, pheasants, safety

Disciplines

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Safety Evaluation of Lasalocid Use in Chinese Ring-Necked Pheasants (*Phasianus colchicus*)

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SUMMARY. Coccidiosis remains a significant threat to the welfare of game farm-reared pheasants in the United States. Although lasalocid has been demonstrated to be effective against pheasant specific coccidia, information regarding its safety in this species is lacking. The purpose of this study was to gather data on the safety of lasalocid when fed to Chinese ring-necked pheasants at one, two, and three times the recommended high dose of lasalocid used for prevention of coccidiosis in other poultry at three times the normal treatment period. Pheasant chicks (approximately 1 day-old; $n = 160$) were randomly blocked by sex into four treatment groups and given their respective diets continuously for 6 wk. No significant differences were observed in overall feed consumption, weight gain, feed conversion rates, clinical pathology measurements, or tissue gross and histopathologic evaluations between controls and treatment groups associated with lasalocid administration. Based on the results of this study it appears that lasalocid fed at the recommended rate of 125 ppm is safe in Chinese ring-necked pheasants.

RESUMEN. Evaluación de la seguridad del uso de la lasalocida en faisanes comunes (*Phasianus colchicus*).

La coccidiosis sigue siendo una amenaza importante para el bienestar de las aves de caza criadas en granja en los Estados Unidos. Aunque la lasalocida ha demostrado ser eficaz contra las coccidias específicas para los faisanes, no se cuenta con la información referente a la seguridad en esta especie. El propósito de este estudio fue recopilar datos sobre la seguridad de la lasalocida suministrada a los faisanes en una, dos y tres veces la dosis máxima recomendada de lasalocida utilizada para la prevención de la coccidiosis en otras aves de corral en un periodo tres veces mayor al periodo de tratamiento normal. Ciento sesenta polluelos de faisán de un día de edad fueron asignados aleatoriamente en cuatro grupos de tratamiento bloqueados por sexo y administrando sus respectivas dietas continuamente durante seis semanas. No se observaron diferencias significativas en el consumo del alimento general, el aumento de peso, las tasas de conversión del alimento, las mediciones de patología clínica, o en las evaluaciones macroscópicas e histopatológicas de tejidos entre los controles y los grupos tratados, asociados con la administración de la lasalocida. Con base en los resultados de este estudio se deduce que la lasalocida proporcionada en el alimento a la tasa recomendada de 125 ppm es segura en faisanes comunes.

Key words: coccidiosis, lasalocid, pheasants, safety

Abbreviations: FDA/CVM = U.S. Food and Drug Administration/Center for Veterinary Medicine; ppm = parts per million

Farm-reared Chinese ring-necked pheasants (*Phasianus colchicus*) are routinely infected with coccidia such as *Eimeria colchici*, *Eimeria duodenalis*, and *Eimeria phasianii* (5,8,10). Fatal infections are highest between 2 and 6 weeks of age and may result in high morbidity and mortality and consequently severe economic losses. Options available for treatment of coccidiosis in pheasants are limited, with amprolium (Corid®, Merial Limited, Duluth, GA) being the only approved medication for prevention of disease (9,12). Although safe and effective at 175 ppm, amprolium has a limited species activity spectrum and resistance has been reported (11).

Lasalocid (Avatec®, Pfizer Animal Health, Madison, NJ) is a carboxylic polyether ionophore that has been shown to be safe and efficacious against species-specific coccidia that cause disease in broiler chickens, growing turkeys, and chukar partridges. At 120 ppm, lasalocid has been shown to decrease oocyst shedding and allow improved weight gain in infected pheasants when compared to untreated pheasants (3,8). Before lasalocid can be approved for clinical application, undesirable side effects that may occur as a result of its use must be documented and reviewed by the U.S. Food and Drug Administration/Center for Veterinary

Medicine (FDA/CVM). The objective of this study was to assess the safety of lasalocid in Chinese ring-necked pheasants, to support the addition of pheasants to the Avatec label. It was hypothesized that continuous feeding of lasalocid at higher than recommended levels for 6 wk would be toxic to Chinese ring-necked pheasants.

MATERIALS AND METHODS

Pheasants. One hundred sixty Chinese ring-neck pheasant chicks were purchased from Oakwood Game Farm (Princeton, MN). There were 80 males and 80 females. Pheasant chicks were 1 day old at study initiation and 6 wk old at study termination.

Pheasant housing and maintenance. Pheasants were housed at the poultry research facility at the Iowa State University Poultry Science Farm (Ames, IA). No acclimation period was used, and pheasant chicks were placed in the experiment on the day of arrival. Pheasants were raised in floor pens that measured 1.22 × 1.22 × 1.22 m separated by wire partitions. Heat lamps suspended approximately 0.35 m were used to provide artificial heat and light for the pheasant chicks up to 2 wk of age. Once heat lamps were removed, room lighting was the only source of light for the pheasants. Pheasants were provided with approximately 16 hr of light and 8 hr of darkness per day. Wood shavings were used as bedding and were replenished as needed to maintain a sanitary and comfortable environment.

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Table 1. Test group diet analyses reported by Alpharma Inc.

Diet	Target dose g/t (ppm)	Pre-trial lasalocid analysis g/t (ppm)	Post-trial lasalocid analysis g/t (ppm)
Control	0 (0)	<0.5 (0)	<0.5 (0)
1×	113 (125)	133.6 (147)	110.3 (122)
2×	204 (250)	231.8 (256)	244.7 (270)
3×	340 (375)	424.7 (468)	272 (300)

Treatment and control group layout. Pheasant chicks were blocked by sex and randomly assigned to four test groups of 40 pheasants each (20 males and 20 females). Eight pens (replicates) were used per test group and each pen housed five pheasants. There were a total of 32 pens of pheasants.

Test article and diet formulation. The basal diet used was a commercial starter preparation, (Game Bird Startena™ Purina Mills, LLC, Gray Summit, MO), formulated to meet the nutritional requirements of pheasants from 0 to 6 wk of age. Lasalocid (Avatec) was obtained from Alpharma Inc. (Bridgewater, NJ) as a premix with 20% (199.54 g/kg) lasalocid sodium. Lasalocid was formulated for mixing with the basal diet using the following formula:

$$\text{Dose(mg/kg)} \times \text{Weight of basal diet(kg)} / \\ \text{Concentration of lasalocid(g lasalocid/g premix)} =$$

$$\text{Test Article Dose(g) of premix per 45.4 kg bag of premix}$$

Approximately 0.5 kg of the medicated feed was collected from each prepared batch and submitted to Alpharma Inc. for assay at study initiation and at study termination.

Experimental design. There were four test groups in this investigation. Table 1 shows the formulation of the four test group diets. No lasalocid was added to the diet fed to the pheasants in test group one, the controls. Pheasants in test groups two, three, and four were fed the basal diet with approximately 125, 250, and 375 ppm lasalocid added, respectively. These doses were equivalent to 1×, 2×, and 3× the approved dose of lasalocid in broiler or fryer chickens, growing turkeys, and chukar partridges. Fresh feed was provided to the pheasants once daily for 6 wk. Water was provided *ad libitum* via automatic drip tubes. Blinding was maintained by ensuring that persons who were involved at each level of clinical or pathologic evaluations had no knowledge of dosing. All phases of this study were conducted under Good Laboratory Practices (21 CFR Part 58) guidelines for nonclinical laboratory studies, and were approved by the Institutional Animal Care and Use Committee of Iowa State University. Critical phases were monitored by quality assurance personnel appointed by the Minor Use Animal Drug Program, National Research Support Project-7 (NRSP-7).

Clinical observations. Study inclusion physical examinations were conducted by a veterinarian on each pheasant on day 0, and only normal and healthy pheasant chicks were included in the study. Pheasants were observed twice daily throughout the study to determine their general appearance as well as monitor consistency of fecal output, death, and/or normal conditions. A veterinarian confirmed any unusual observations. Moribund and/or dead pheasants were removed and necropsied. The chicks were weighed as a group at the time of placement into pens and individually at euthanasia at 6 wk of age.

Feed consumption and feed efficiency. Fresh feed was weighed daily prior to being fed to pheasants. Feed remaining in feed containers or that may have inadvertently spilled during the day or night was noted in the morning, weighed and discarded. Overall feed consumption and feed conversion rates were determined and reported as average of the pen at the end of the study. The following formula was used to calculate adjusted feed conversion rate:

$$\text{Total feed disappearance} / \\ [(\text{total terminal pen bird weight} \\ + \text{all dead and removed bird weights}) \\ - \text{total bird weights in the pen at day 0}]$$

Total feed disappearance (feed consumption) was defined as the sum of all feed additions to a pen minus the sum of all feed weighed back from that pen and the estimated or actual wastage.

Clinical pathology. At 6 wk, pheasants were randomly sacrificed by direct intramuscular injection of pentobarbital solution (Beuthanasia®, Intervet Schering-Plough, Summit, NJ) into the left breast muscle. As soon as the birds were unconscious and prior to cardiac arrest, approximately 2 ml of blood was collected via the vena cava or heart chamber. The blood was divided between EDTA and heparinized vacutainer tubes and submitted to the Iowa State University, Veterinary Clinical Pathology Laboratory for hematology and serum biochemistry analyses. The following hematology variables were determined: red blood cell count (RBC); packed cell volume (PCV); mean corpuscular volume (MCV); white blood cell count (WBC); and heterophil, eosinophil, lymphocyte, monocyte, and thrombocyte counts. The following serum biochemistry values were determined: glucose, total protein (TP), albumin, creatine kinase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), calcium, phosphorous, sodium, potassium, chloride, magnesium, amylase, uric acid, lactate dehydrogenase (LDH), alkaline phosphatase (ALP), total bilirubin, cholesterol, and globulin.

Organ weights, necropsy, and histopathology. Organ weights were obtained on heart, liver, spleen, and thymus of the sacrificed birds. The following organs and tissues were examined grossly and histologically for any lesions or abnormalities: skin, eyes, liver, kidney, heart, lungs, trachea, adrenal gland, pancreas, esophagus, crop, spleen, proventriculus, ventriculus, intestines (upper, middle, and cecum), bursa of Fabricius, ovaries and oviducts, testes, bone, bone marrow, thyroid gland, thymus, parathyroid gland, brain, spinal cord, and pituitary body. If lesions were noted on gross necropsy, a full description was made and tissues collected for histologic examination. If no lesions were present, a representative sample of tissues was collected for histologic examination. The selected tissues were preserved in 10% neutral buffered formalin and prepared as paraffin-embedded sections on glass microscopic slides.

Table 2. Test group means (SD) for live weights, organ weights, overall weights, feed consumption, and feed conversion rates.

Variable	Control, mean (SD)	1× Group, mean (SD)	2× Group, mean (SD)	3× Group, mean (SD)
Beginning body weight (g)	90.38 (2.77)	90.13 (1.73)	93.50 (4.72)	88.63 (5.15)
Ending body weight (g)	358.50 (43.95)	368.41 (46.80)	372.85 (47.44)	362.48 (51.30)
Feed consumption (g)	3992.63 (254.05)	4024.25 (385.63)	4054.00 (456.73)	4114.38 (333.66)
Feed conversion rate	2.35 (0.08)	2.36 (0.09)	2.35 (0.07)	2.39 (0.07)
Liver weight (g)	7.88 (1.07)	8.28 (1.04)	8.65 (1.18)	8.61 (1.30)
Heart weight (g)	1.69 (0.29)	1.81 (0.28)	1.78 (0.27)	1.80 (0.27)
Spleen weight (g)	0.25 (0.08)	0.33 (0.14)	0.32 (0.13)	0.34 (0.12)
Thymus weight (g)	1.22 (0.40)	1.34 (0.39)	1.36 (0.30)	1.37 (0.44)

Table 3. Test group means (SD) from clinical pathology sample testing performed in controls and pheasants treated with lasalocid sodium.

Variable (units)	Control mean (SD)	1× Mean (SD)	2× Mean (SD)	3× Mean (SD)
RBC ($\times 10^6/\mu\text{l}$)	3.05 (0.52)	2.87 (0.41)	2.77 (0.41)	2.72 (0.31)
PCV (%)	35.86 (2.44)	34.30 (2.17)	34.34 (2.89)	33.87 (2.45)
MCV (fl)	120.27 (17.01)	121.85 (17.43)	126.23 (17.62)	125.60 (11.85)
WBC ($\times 10^3/\mu\text{l}$)	9.07 (4.96)	8.24 (4.45)	9.4 (4.33)	11.17 (9.38)
Heterophils ($\times 10^3/\mu\text{l}$)	2.81 (3.27)	1.99 (2.43)	2.01 (2.23)	3.86 (6.80)
Eosinophils ($\times 10^3/\mu\text{l}$)	0.05 (0.09)	0.07 (0.10)	0.07 (0.12)	0.08 (0.13)
Basophils ($\times 10^3/\mu\text{l}$)	0.49 (0.42)	0.60 (0.32)	0.58 (0.35)	0.63 (0.39)
Lymphocytes ($\times 10^3/\mu\text{l}$)	5.60 (3.22)	5.45 (3.29)	6.39 (2.72)	6.04 (3.29)
Monocytes ($\times 10^3/\mu\text{l}$)	0.20 (0.21)	0.17 (0.16)	0.34 (0.48)	0.56 (1.24)
Glucose (mg/dl)	344.60 (26.32)	344.05 (36.76)	349.26 (30.48)	340.75 (25.77)
TP (mg/dl)	2.82 (0.24)	2.84 (0.23)	2.86 (0.26)	2.92 (0.46)
Albumin (mg/dl)	1.44 (0.15)	1.42 (0.10)	1.43 (0.13)	1.42 (0.13)
Creatine kinase (IU/L)	3376.40 (941.28)	3395.92 (1379.59)	3199.92 (926.82)	2896.08 (801.91)
AST (IU/L)	402.43 (93.11)	371.47 (57.27)	362.38 (42.79)	374.05 (48.93)
ALT (IU/L)	7.33 (6.12)	6.87 (3.24)	5.49 (1.90)	6.55 (4.21)
GGT (IU/L)	4.20 (1.70)	4.08 (1.94)	3.74 (1.73)	3.93 (1.56)
Calcium (mg/dl)	10.01 (0.57)	9.88 (0.94)	10.37 (0.58)	10.29 (0.87)
Phosphorus (mg/dl)	9.76 (1.91)	9.19 (1.98)	9.20 (1.28)	8.40 (0.92)
Sodium (mEq/L)	153.33 (3.00)	152.44 (2.16)	153.13 (2.96)	152.93 (2.53)
Potassium (mEq/L)	4.26 (1.54)	4.08 (1.50)	3.55 (0.92)	3.43 (0.74)
Chloride (mEq/L)	112.08 (2.15)	112.13 (2.32)	111.56 (3.00)	111.93 (2.68)
Magnesium (mg/dl)	2.53 (0.27)	2.42 (0.30)	2.52 (0.28)	2.40 (0.23)
Amylase (mg/dl)	2582.83 (856.96)	2607.03 (680.19)	2421.33 (657.68)	2438.48 (627.80)
Uric acid (mg/dl)	20.41 (8.47)	15.12 (6.07)	18.43 (6.81)	16.06 (7.87)
LDH (IU/L)	776.25 (328.70)	683.92 (174.04)	660.42 (136.33)	649.60 (196.99)
ALP (IU/L)	1286.68 (269.31)	1205.21 (242.75)	1211.74 (221.95)	1044.75 (259.68)
Total bilirubin (mg/dl)	0.21 (0.10)	0.25 (0.15)	0.26 (0.17)	0.26 (0.19)
Cholesterol (mg/dl)	104.23 (14.94)	111.55 (13.58)	108.18 (15.88)	112.08 (16.71)
Globulin (mg/dl)	1.36 (0.20)	1.42 (0.18)	1.78 (2.05)	1.49 (0.44)

Tissue sections stained with hematoxylin and eosin were examined by light microscopy. Necropsy and histopathologic examinations were performed by a board-certified veterinary anatomic pathologist.

Data analyses. Statistical analyses were performed using SAS Enterprise Guide statistical software (version 4.3; SAS Institute, Inc., Cary, NC, USA). A mixed effect two-way analysis of variance was performed on the variables live weights, organ weights, feed consumption data, serum chemistry, and hematology data. Least square means were used to compare the treated test groups to the control test groups. All statistical tests were conducted at a level of 0.05 for this publication, but a level of 0.10 was required to meet FDA/CVM protocol specifications.

RESULTS

Diet analyses. Analyses performed on each dietary treatment group to confirm the target test article composition are shown in Table 1. Lasalocid was incorporated into three out of four test group diets. Beginning and ending trial composition for diets 2, 3, and 4 were within 20% of the targeted dose.

Pheasant observations, morbidity, and mortality. One hundred fifty-eight out of 160 pheasants received their intended doses for the entire 6 wk. Two pheasants were observed to be moribund on days 17 (2× group) and 27 (3× group), respectively, and these were humanely euthanized. Tissue evaluations of these pheasants revealed that the first bird had a generalized bacterial infection and the second bird had a dislocation of the spinal cord between cervical vertebrae C3 and C4. These deaths were not attributed to lasalocid administration. All other pheasants remained clinically healthy based on a normal general appearance and normal fecal score for the duration of the study. None of the signs of lasalocid toxicity described in other poultry were observed. The 1.25% mortality rate observed was within historical values of the facility and statistical analyses were not necessary for the variables morbidity and mortality.

Live weights, feed consumption and feed conversion rates.

Test group means are summarized in Table 2. All pheasant groups gained weight over the course of the study and consumed approximately equal amounts of feed. Mean ending weights were slightly higher for each treatment group compared to controls, but these were not significant ($P \geq 0.05$).

Clinical pathology. Hematology and serum biochemistry parameter test group means are summarized in Table 3. There were no significant differences in any of the clinical pathology variables between treatment and control groups ($P \geq 0.05$).

Tissue evaluations. There were no differences in respective mean terminal organ weights between treatment and control groups ($P \geq 0.05$). No treatment-related gross lesions were observed on any of the pheasants. Occasionally, mild focal heterophilic and lymphocytic infiltrates were seen microscopically on various tissues including myocardia, kidneys, lungs, and spleens. These were evenly distributed amongst the test groups and not associated with lasalocid administration. One pheasant (3× group) had moderate subacute myocardial hemorrhage, necrosis, and mineralization of the myocardium.

DISCUSSION

In general, lasalocid is safe in poultry if given at recommended inclusion rate in feed. Lasalocid is used as a preventive for coccidiosis, not a therapeutic treatment of infections. Toxic effects described for older chickens and turkeys are commonly encountered as a result of feed mixing errors (1,2,6,13). These are due to a cellular dysfunction because lasalocid carries ions across host cell surface membranes disrupting normal physiologic gradients (4). Depending on the severity and duration of exposure, the effects of lasalocid exposure may be reversible once removed from feed (7). Feeding

lasalocid for 6 wk at dietary levels up to 375 ppm was well tolerated, and signs reported in other poultry were not seen. The microscopic changes suggestive of lasalocid toxicity seen in the single pheasant were likely incidental since no other pheasants in this group showed similar lesions. The pheasant weighed 316 g (group mean was 362.48 g) and had appeared clinically healthy, its clinical pathology parameters were within established reference ranges, and no other tissue gross or microscopic lasalocid-related lesions were seen.

As part of the approval process, lasalocid must be shown in controlled trials to be safe at the claimed effective dose and progressively higher doses, for an extended period of time beyond the duration it would ordinarily be used. Information regarding any adverse reactions, including death, associated with these overdoses or increased duration of administration must be documented and reported to FDA/CVM. Based on physical, clinical pathologic, and tissue evaluations (gross and microscopic), we were unable to demonstrate adverse effects of feeding lasalocid continuously to Chinese ring-necked pheasants at a rate of at least three times the effective dose of 125 ppm.

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