



Article Efficacy and Growth Performance between Two Different Ionophore Coccidiostats (Narasin and Salinomycin) in Broiler Chickens after Challenge with *Eimeria* spp.

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Simple Summary: There has been an exponential increase in demand for poultry meat and eggs worldwide. Therefore, it is essential to keep coccidiosis under control in broilers to fulfill the increase in demand for protein. The objective of this study was to assess the efficacy of two ionophore coccidiostats against coccidiosis and their impact on broiler gut health and performance. Both the ionophores were effective in treatment against coccidiosis, and out of the two, narasin demonstrated superiority in terms of improved performance parameters compared with salinomycin. This finding is highly important as it significantly focuses on sustainable poultry and, in turn, can help prevent economic losses and maintain broiler health.

Abstract: The objective of this study was primarily to assess the different performance impacts of two ionophore coccidiostats (narasin and salinomycin) used to manage coccidiosis. While both products may be efficacious in controlling disease challenges, previous literature has suggested that some ionophores are less well tolerated by the broiler chickens. In this study, we were particularly interested to know how the use of different coccidiostat programs translates into broiler health and performance, as measured by zootechnical parameters such as the feed conversion ratio, average daily gain, and final body weight. A total of 352 male Ross 308 one-day-old broilers were randomly divided into two treatment groups (T1 and T2). Treatment 1 included a basal diet (BD) + nicarbazin/narasin (Maxiban[®], Elanco) at 100 ppm 0–24 days, narasin at 70 ppm 25–42 days, and (2) Treatment 2 included basal diet + nicarbazin/narasin at 100 ppm 0-24 days, salinomycin (Sacox[®], Huvepharma) at 70 ppm 25-42 days. Efficacy and performance parameters, slaughter analysis, dry matter (DM) in litter, and intestinal integrity (I^2) were measured for the broilers from both treatment groups. The findings demonstrated more favorable results for broilers reared in the group diet fed with narasin (in the finisher phase), including higher daily body weight gain, higher final body weight, lower feed conversion ratio value (improved feed efficiency), and higher European Production Efficiency Factor value, compared with the salinomycin-supplemented group.

Keywords: broiler chickens; ionophore; anticoccidials; coccidiostats; efficacy; broiler performance; narasin; salinomycin

1. Introduction

Avian coccidiosis caused by obligate intracellular parasites of the genus *Eimeria* is perhaps the most prevalent disease in the poultry industry, resulting in great financial pressures and economic losses as high as USD 3–4 billion per year due to impaired feed



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Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). conversion rate, morbidity, intestinal lesions, poor weight gain, and, in some cases, mortality [1–6]. *Eimeria* spp. are highly adaptable and can survive in the same environmental conditions as the host poultry species [7].

Seven species of *Eimeria*, namely, *E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox*, and *E. tenella*, are associated with infections in chickens through colonization of the gastrointestinal tract [8]. *Eimeria acervulina*, *Eimeria necatrix*, *Eimeria maxima*, and *Eimeria tenella* are reported to be the most pathogenic species [9]. Coccidiosis leads to decreased average daily weight gain, greater feed conversion, lower growth/final body weight, and increased mortality in poultry [10]. The control of coccidiosis has been an important parameter in the growth and success of the worldwide poultry meat industry [11]. Between the years 1961 and 2020, there was an exponential increase in the demand for poultry meat (9–133 million tons) and eggs (15–93 million tons) [12]. Therefore, it is critical to keep coccidiosis under control in commercial poultry to fulfill the increasing demand for protein [13].

Also, the widespread historical use of synthetic compounds in combating coccidiosis has resulted in anticoccidial resistance. In recent decades, ionophore coccidiostats added as feed additives have offered an additional solution for stable control of coccidiosis [14].

Ionophores (IONs) are polyether ionophorous antiparasitics derived by fermentation (*Streptomyces* and other actinomycetes) or synthetic/chemicals derived by chemical (CHE) synthesis. The ionophore coccidiostats approved for use globally are monensin, lasalocid, narasin, salinomycin, and semduramicin. Synthetic chemicals include halofuginone, decoquinate, robenidine, zoalene, and nicarbazin [14–16]. Maxiban[®] (classified as a CHE for the purpose of analysis) is a feed additive from Elanco (Elanco Animal Health, 2500 Innovation Way, Greenfield, IN, USA), which is a combination product of narasin (ionophore) and nicarbazin (synthetic compound) in a 1:1 ratio. Combining low levels of narasin and nicarbazin creates a synergistic effect that results in a dual mode of action, protecting birds against coccidiosis and subsequent enteritis [17,18].

Ionophore coccidiostats were first licensed over 50 years ago in 1971 and have been commercially used since then. Monensin was the first chemically characterized and commercialized ionophore [19,20]. While they remain highly effective, there have been several attempts to further improve their efficacy through combinations with other drugs [19].

The growth of the poultry industry today is mostly due to the extensive use of ionophore coccidiostats [20]. Narasin is a polyether coccidiostat (produced by fermentation) obtained from *Streptomyces aureofaciens* and a derivative of salinomycin with an additional methyl group [21]. Salinomycin, an ionophore coccidiostat, is another commonly used poultry feed supplement against *Eimeria* spp. [22].

The objective of this comparative study was to assess the efficacy and performance of two different potentiated monovalent ionophores, i.e., narasin and salinomycin, in a shuttle program with Maxiban[®] in chickens. The birds received a coccidia challenge by using a $40 \times$ dose of a commercial anti-coccidial vaccine. Performance and health were evaluated to assess how the two coccidiostats differed in their management of this challenge.

2. Materials and Methods

2.1. Ethical and Study Approvals

All procedures in this study were evaluated and approved by the Local Animal Care and Ethics Committee in Olsztyn (UWM), Poland (Resolution No. 12/2022 from 16 March 2022), and were performed in accordance with the principles of the EU (recommendation 2007/526/CE) and the Polish Law on Animal Protection.

2.2. Experimental Design, Housing, and Management of Birds

A total of 352 male Ross 308 one-day-old broilers from a commercial hatchery were randomly divided into 2 treatment groups. Every treatment consisted of 16 pens (16 replicates) with 11 birds each—resulting in 176 birds per treatment. The two treatments were as follows: (1) Treatment 1—basal diet (BD) + nicarbazin/narasin (Maxiban[®], Elanco) at 100 ppm for 0–24 days, narasin (Monteban[®], Elanco) at 70 ppm for 25–42 days, and (2) Treatment 2—BD + nicarbazin/narasin (Maxiban[®], Elanco) at 100 ppm for 0–24 days, salinomycin (Sacox[®], Huvepharma) at 70 ppm 25–42 days. Both treatment groups were challenged with a 40-dose Evant vaccine (Hipra, Amer, Spain) provided by poultry veterinarians (Avipoint, Olsztyn, Poland), containing *Eimeria acervulina*, *Eimeria maxima*, *Eimeria praecox*, *Eimeria mitis*, and *Eimeria tenella* strains, into the crop on day 14 and 21 of the trial.

As stated in the study objectives, the primary goal of this study was to compare the direct impact of two of the most widely used coccidiostats in commercial poultry. Both narasin and salinomycin have been used to control coccidiosis in broiler production for many years, and many studies have demonstrated their efficacies in controlling this disease [23,24]. While one might assume that if both products are efficacious, then they are comparable, the literature suggests that some ionophore molecules have an effect on broiler feed intake and thus could impact broiler performance parameters [25,26]. This study primarily aimed to quantify any differences between narasin and salinomycin. This study was designed to maximize the value of any comparison; thus, we limited the treatment groups to enable a direct comparison and increase the replicates of each group to give greater power to the data generated.

The birds were kept in floor pens, 32 pens in total and 0.75 m^2 each, with netting walls to avoid migration and pelleted straw as bedding material. Prior to the arrival of the birds, the pens were thoroughly cleaned and disinfected with a non-toxic solution. This was performed 7 days before the birds' arrival on an "all in–all out" basis. The final body weight (BW) per pen was not higher than foreseen in EU regulations—39 kg/m².

The trial was conducted in a poultry house with windows. The house was provided with artificial programmable lights and climate, automated electric heating, and forced ventilation. The heating and light program was in accordance with the recommendations of the Ross Broiler Management Manual [27]. The house was lit by programmable artificial light. This study followed a standard lighting program (per day) including 23 h of light and 1 h of dark (0–7 days of age) and 18 h of light and 6 h of dark (from day 7 to 3 days prior to slaughter). The total length of the study period was 42 days.

2.3. Basal Diet and Feed Additives

A two-phase feeding scheme was used (starter phase: 0–24 days and grower phase: 25–42 days), and feed and water were provided to the birds ad libitum. Wheat/corn/soya/ rapeseed-based mash diets were provided to the broilers from feeders (one per pen; 15 cm \times 30 cm). Basal feed mixtures (3 tons of starter and 6 tons of grower diet) were prepared by Agrocentrum Sp. z o.o. (Pisz, Poland) and delivered to the University of Warmia and Mazury in Olsztyn, Poland, where the experimental diets were prepared, and the feeding experiment was conducted. The composition and nutritional value of the basal diets are shown in Table 1.

Basal diets were analyzed for crude protein, crude fiber, crude fat, dry matter, ash content (UWM Olsztyn, Poland), and coccidiostats content (Eurofins laboratory, Barcelona, Spain). The nutritional value of all diets corresponded to the nutrient requirements of Ross 308 broiler chickens [27].

| Feed Composition | Starter (0–24 Days) | Grower (25–42 Days) | |
|--------------------------|---------------------|---------------------|--|
| Wheat | 45.00 | 55.00 | |
| Soybean meal | 25.50 | 21.50 | |
| Corn | 20.04 | 10.80 | |
| Rapeseed meal | 3.00 | 5.00 | |
| Soya oil | 2.74 | 4.63 | |
| NaCl | 0.33 | 0.34 | |
| Limestone | 1.26 | 1.17 | |
| Mono calcium phosphate | 0.97 | 0.65 | |
| Methionine | 0.32 | 0.23 | |
| L-Lysine | 0.38 | 0.21 | |
| L-Threonine | 0.08 | 0.09 | |
| Ronozyme [®] P | 0.01 | 0.01 | |
| Ronozyme [®] WX | 0.02 | 0.02 | |
| Choline chloride | 0.10 | 0.10 | |
| Vit-Min-premix | 0.25 | 0.25 | |
| ME, kcal/kg | 2950 | 3050 | |
| Crude protein | 21.00 | 20.00 | |
| Lysine | 1.30 | 1.10 | |
| Methionine | 0.62 | 0.52 | |
| Methionine + Cystine | 1.00 | 0.90 | |
| Threonine | 0.83 | 0.80 | |
| Calcium | 0.85 | 0.75 | |
| Available phosphorus | 0.33 | 0.27 | |
| Sodium | 0.15 | 0.15 | |

Table 1. Calculated composition and nutritional value of basal diets (BDs)—starter and grower, % (as-fed basis).

ME, metabolizable energy.

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2.4. Applied Experimental Challenges

The experimental treatment groups (T1 and T2) were challenged with a coccidia vaccine and an addition of coccidiostats to the diet (Table 2). The sources of coccidiostats used in the diets were the following commercial preparations: Maxiban in both groups Monteban[®] by Elanco as a source of narasin (T1), and Sacox[®] by Huvepharma as a source of salinomycin (T2). Both coccidiostats were used at a dose of 70 ppm. Maxiban was used at a dosage of 100 ppm.

Table 2. Experimental treatments.

| Treatment Group | Product and Coccidiostats | 0–24 Days | 25–42 Days |
|-----------------|---|------------------------------|------------------------------|
| | Maxiban [®] /Monteban [®] | Maxiban [®] 100 ppm | Monteban [®] 70 ppm |
| | Maxiban [®] /Sacox [®] | Maxiban [®] 100 ppm | Sacox [®] 70 ppm |

The birds in the two treatment groups were challenged into crops with an Evant vaccine (40 times the recommended dose) on days 14 and 21 of the trial, according to Kozlowski et al. [28]. The vaccine was provided by poultry veterinarians (Avipoint, Olsztyn, Poland) and contained *Eimeria acervulina* (003), *Eimeria maxima* (013), *Eimeria mitis* (006), *Eimeria praecox* (007), and *Eimeria tenella* (004) strains of *Eimeria*.

The coccidia vaccine was administered directly into the crop with the use of a cannula. The degree of intestinal mucosa damage was evaluated by a poultry disease specialist based on any gross pathological changes on day 21 and day 28 of age, according to a previously described study using the health tracking system (HTSi) protocol established by Elanco Animal Health [29].

Health Tracking System (HTSi)

The health tracking system (HTSi) is an independently verified broiler benchmarking platform that provides a record of bird health based on necropsies, enabling producers to track bird performance and health prior to reaching processing. HTSi was developed by Elanco and has been in operation since 1995. The system was established to improve the understanding of flock health, support timely data-based decisions, and provide robust benchmarking with the overall goal of better performance, profitability, and animal welfare.

The I² index is a weighted score, unique to HTSi, that analyzes the overall intestinal health of flocks. The index can function as a driver of broiler health and performance as a link has been established between better intestinal integrity and improved average daily gain (ADG), the feed conversion ratio (FCR), and the European production efficiency factor (EPEF) [30]. The score established in the I² index is based on the evaluation of 23 key lesions, including coccidia, reviewed during necropsies. Data from the lesions tracked are used to calculate an I² score, with a perfect intestinal integrity index score of 100 meaning no potential loss from gut health is detected.

2.5. Efficacy and Performance Parameters

The body weight of broilers (pen basis) was measured on days 1, 21, 35, and 42, whereas feed consumption and the feed conversion ratio were analyzed for the two treatment groups over experimental periods, i.e., 0–21, 22–35, 0–35, 36–42, and 0–42 days.

The average weight gain per bird, in each period (AWG), as calculated as:

$$AWG = F - S \tag{1}$$

where F = average weight of the live birds in the pen on the day of weighing;

S = average weight of live birds in the pen at the first weighing.

The average feed consumed per bird, per day (ADFI) for the period, was calculated according to the following formula:

$$ADFI = \frac{A}{(B * C) + (D)}$$
(2)

A = Total feed consumed per pen for that period;

B = Number of surviving birds;

C = Day of study or the number of days for that period;

D = The sum of the days on which birds that died (+culled) were alive (in this study). The feed conversion ratio for the period (FCR) was calculated as follows:

$$FCR = \frac{Feed \text{ consumed for the period in each replicate}}{Total weight gain for the period}$$
(3)
(including the WG from dead and euthanized birds)

The European Production Efficiency Factor (EPEF) was calculated as:

$$EPEF = \frac{\text{Liveweight } (kg) \times \text{Liveability}(\%)}{\text{Age at depletion } (days) \times \text{Feed conversion ratio } (kg/kg)} \times 100$$
(4)

The results of slaughter analysis (day 42, one bird/pen with BW close to average BW of each treatment group) included carcass weight (CW), dressing percentage, breast meat yield, and bowel content (heart, gizzard, and liver).

Lesion scores were evaluated using method of Johnson and Reid [31] by randomly selecting one bird from each pen (16 birds per treatment) on days 21 and 28; 64 birds in total were necropsied. HTSi data were carried out for the birds on days 21 and 28. HTSi was developed by ElancoTM and has been in operation since 1995. The system was established to improve the understanding of flock health, support timely data-based decisions, and

provide robust benchmarking with the overall goal of better performance, profitability, and animal welfare.

Dry matter (DM) in the litter was measured at the end of this study by taking five samples of about 0.1 kg from 5 different points (4 corners and the center of the pen, excluding the areas directly under the heater and the drinker), which were mixed. Moisture was determined after being kept in a forced air oven at 75 $^{\circ}$ C for 48 h.

2.6. Disposition of Birds

All pens were assessed once daily with pen-side observations for general health. Employees handling birds in pens used face masks and gloves. Birds found dead were removed from the pen. Visibly sick birds were humanely euthanized and necropsied. The euthanasia method was according to the EU directive. Euthanasia was performed by trained personnel deemed proficient in the method. Birds that died or were removed from the experiment were weighed, and body weight and feeding period were considered in the calculation of growth performance indices.

2.7. Statistical Analysis

Comparisons of mean differences between the two groups for all analyzed means were performed by *t*-tests using SAS/STAT[®] software (Version 9.4 of the SAS System for Windows, Copyright[©] 2002–2012 by SAS Institute Inc., Cary, NC, USA). Significance was declared at *p* (probability) < 0.05, and $0.05 \le p < 0.10$ was considered a near-significant trend.

3. Results

3.1. Feed Analysis

Both the starter and the grower basal meals, as well as experimental diets, were analyzed (Tables 3 and 4). The analyzed values were found to be in line with the calculated values.

Table 3. Analysis of starter and grower basal diets, % (as fed basis).

| Diet | DM | СР | EE | CA | CF |
|------------------|------|-------|------|------|------|
| Starter (0–21 d) | 89.9 | 21.10 | 5.58 | 4.64 | 3.26 |
| Grower (22–42 d) | 90.0 | 20.16 | 6.69 | 4.53 | 3.05 |

DM-dry matter; CP-crude protein; EE-ether extract; CA-crude ash; CF-crude fiber.

Table 4. Analysis of coccidiostat content in the experimental diets.

| Treatment | Coccidiostat Content (mg/kg) |
|--------------|---|
| T1 (0–21 d) | Maxiban [®] (narasin-51 and nicarbazin-43) |
| T1 (22–42 d) | Monteban [®] (narasin-68) |
| T2 (0–21 d) | Maxiban [®] (narasin-49 and nicarbazin-50) |
| T2 (22–42 d) | Sacox [®] (salinomycin-74) |

The litter in all the pens was of good quality. The dry matter (DM) in the litter was high, from 84.43% in T1 to 84.24% in T2 (day 42), and the mean difference was not statistically significant between the two treatment groups (p = 0.737).

3.2. Gut Health and Intestinal Integrity (I²)

On day 21, the chickens in the T1 group had a total intestinal integrity of 94.7, while the chickens in the T2 group had a total of 95.2. On day 28, the I² was 95.4 for T1 and 95.0 for T2 birds. The overall HTSi results are presented in Table 5. The intestinal integrity in both groups was not significantly different. The other intestinal integrity components were measured, and the only significant difference was observed in the level of mucus content.

| Intestinal Integrity Lesions | Treatment (Me | | |
|------------------------------|-------------------|-------------------|-------------------|
| - | T1 | T2 | - <i>p</i> -Value |
| I ² | 95.07 | 95.09 | 0.979 |
| Cecal Foamy Material | 0.19 ^y | 0.38 ^x | 0.098 |
| Cellular Sloughing | 0.09 | 0.13 | 0.694 |
| Feed Passage | 0.09 | 0.13 | 0.694 |
| Hyperemia | 0.00 | 0.03 | 0.321 |
| Mucus Content | 0.41 ^b | 0.66 ^a | 0.046 |
| Thin Intestinal Walls | 0.25 ^y | 0.47 ^x | 0.070 |
| Watery Content | 0.25 | 0.22 | 0.772 |

Table 5. Mean scores of intestinal integrity lesions in both treatment groups.

T1—Maxiban[®]/Monteban[®]; T2—Maxiban[®]/Sacox[®]. Values in the same row with no common superscript (a,b) are significantly different (p < 0.05), and $0.05 \le p < 0.10$ (x,y) is considered a near-significant trend.

3.3. Growth Performance of Birds

The growth performance results are presented in Table 6.

| Table 6. Growth performance of broilers fed the experimentation | ıl diets. |
|--|-----------|
|--|-----------|

| с. :с. <i>и</i> : | Treatment | | | | |
|-------------------|--------------------|--------------------|--------|-----------------|--|
| Specification – | T1 T2 | | SEM | <i>p</i> -Value | |
| BW, g | | | | | |
| Day 1 | 0.039 | 0.039 | 0.001 | 0.212 | |
| Day 21 | 0.813 | 0.818 | 0.012 | 0.685 | |
| Day 35 | 2.174 ^a | 2.083 ^b | 0.252 | 0.017 | |
| Day 42 | 2.937 ^x | 2.858 ^y | 0.217 | 0.077 | |
| DWG, g | | | | | |
| Days 0-21 | 36.9 | 37.1 | 0.601 | 0.679 | |
| Days 0-35 | 61.0 ^a | 58.4 ^b | 7.220 | 0.017 | |
| Days 0-42 | 69.0 ^x | 67.1 ^y | 5.171 | 0.077 | |
| Days 22-35 | 97.2 ^a | 90.3 ^b | 19.189 | 0.011 | |
| Days 36-42 | 111.0 | 111.0 | 0.026 | 0.996 | |
| DFI, g | | | | | |
| Days 0-21 | 46.7 | 47.4 | 2.022 | 0.126 | |
| Days 0-35 | 94.5 | 92.7 | 4.967 | 0.127 | |
| Days 0-42 | 122.4 ^x | 119.4 ^y | 8.556 | 0.078 | |
| Days 22-35 | 148.5 ^a | 143.0 ^b | 15.715 | 0.022 | |
| Days 36-42 | 257.3 | 256.2 | 3.094 | 0.727 | |
| FCR, kg/kg | | | | | |
| Days 0–21 | 1.267 | 1.280 | 0.037 | 0.397 | |
| Days 0–35 | 1.454 | 1.461 | 0.019 | 0.588 | |
| Days $0-42$ | 1.681 | 1.683 | 0.006 | 0.845 | |
| Days 22-35 | 1.581 | 1.595 | 0.039 | 0.605 | |
| Days 36-42 | 2.350 | 2.324 | 0.071 | 0.434 | |
| EPEF | 410.1 | 402.0 | 22.073 | 0.271 | |
| Liveability, % | 99.4 | 99.4 | 0.000 | 1.000 | |

T1—Maxiban[®]/Monteban[®]; T2—Maxiban[®]/Sacox[®]; BW—body weight; DFI—daily feed intake; DWG—daily weight gain; FCR—feed conversion ratio; EPEF—European Production Efficiency Factor; SEM—standard error mean. Values in the same row with no common superscript (a,b) are significantly different (p < 0.05), and $0.05 \le p < 0.10$ (x,y) is considered a near-significant trend.

At the start, the average body weight of day-old chickens was 39.3 g. During the experimental period on days 22–35, the birds from the T1 group gained significantly (p = 0.011) more weight than the T2 birds. Throughout the experimental phase (0–35 days), the birds in the T1 group were significantly heavier (p = 0.017) than the T2 birds. During the entire experiment (0–42 days), the T1 group birds were nearly significantly higher in

BW and DWG (p = 0.077) in comparison with the T2 group birds. During the starter period (0–21 days), the results of feed intake (DFI) of both coccidiostat-supplemented groups (T1 and T2) were similar in values. During the experimental period on days 22–35, the birds in the T1 group consumed significantly more feed (p = 0.022) than the birds in the T2 group. During the experimental period on days 0–35, the birds in the T1 group consumed a higher amount of feed than the birds in the T2 group. For the entire experiment (0–42 days), the DFI calculated for the T1 birds was nearly significantly (p = 0.078) higher than for the T2 birds.

FCR was almost similar. Negligible differences were observed between the two treatments during the entire study, but the difference was not statistically confirmed.

The EPEF value calculated for the T1 birds was higher compared with the T2 birds; however, the difference was not statistically confirmed.

The liveability in the flock was 99.4% for both treatment groups throughout the experiment.

3.4. Slaughter Analysis

Representative birds from each treatment group were selected for slaughter analysis (Table 7). The results indicated that birds and carcasses from group T1 were nearsignificantly and significantly heavier than T2 birds (p = 0.061 and 0.046, respectively). The dressing percentage (DP) was nearly significantly (0.062) higher in the T1 group birds than in the T2 group birds. There were no significant differences in breast muscle or edible bowel (heart, gizzard, and liver) content between the two groups.

| Specification — | Treatment * | | SEM | <i>p</i> -Value | |
|------------------|--------------------|--------------------|-------|-----------------|--|
| | T1 | T1 T2 | | | |
| BWbs, kg | 2.976 × | 2.881 y | 0.270 | 0.061 | |
| CW, kg | 2.163 ^a | 2.083 ^b | 0.225 | 0.046 | |
| DP, % | 72.7 ^x | 71.8 ^y | 2.567 | 0.062 | |
| Breast muscle, % | 20.1 | 19.7 | 1.038 | 0.293 | |
| Heart, % | 0.44 | 0.42 | 0.057 | 0.149 | |
| Gizzard, % | 0.79 | 0.82 | 0.092 | 0.184 | |
| Liver, % | 1.95 | 2.01 | 0.160 | 0.402 | |

Table 7. Slaughter analysis on day 42 of the trial.

* Sixteen replicates/birds per treatment; T1—Maxiban[®]/Monteban[®]; T2—Maxiban[®]/Sacox[®]; BWbs = body weight before slaughter = 100%, CW = carcass weight, DP = dressing percentage. Values in the same row with no common superscript (a,b) are significantly different (p < 0.05), and $0.05 \le p < 0.10$ (x,y) is considered a near-significant trend.

3.5. Lesion Scores

The lesion scores taken from the intestine of one randomly selected bird per pen are summarized in Table 8. No lesion scores were seen in birds from either group with *Eimeria tenella*. Fewer scattered lesions were seen in the birds from both groups with *Eimeria acervulina*. With *Eimeria maxima*, there were >five cases in the T1 group on day 21 and day 28.

| | Day of HTSi Trea | | Lesion Score ¹ | | | |
|-----------------------|------------------|------------------------|---------------------------|----|---|---|
| <i>Eimeria</i> Strain | | Treatment ² | 0 | 1 | 2 | 3 |
| | Day 21 | T1 | 14 | 2 | 0 | 0 |
| F 1' | Day 21 | T2 | 15 | 1 | 0 | 0 |
| E. acervulina | Day 28 | T1 | 16 | 0 | 0 | 0 |
| | Day 20 | T2 | 14 | 2 | 0 | 0 |
| | Day 21 | T1 | 5 | 11 | 0 | 0 |
| - · | | T2 | 10 | 6 | 0 | 0 |
| E. maxima | Day 28 | T1 | 8 | 7 | 0 | 1 |
| | , | T2 | 13 | 2 | 0 | 1 |
| E. tenella | Day 21 | T1 | 16 | 0 | 0 | 0 |
| | - | T2 | 16 | 0 | 0 | 0 |
| | Day 28 | T1 | 16 | 0 | 0 | 0 |
| | 2 | T2 | 16 | 0 | 0 | 0 |

Table 8. Lesion scores of challenged chickens on days 21 and 28.

Sixteen replicates/birds per treatment, for lesion scores by randomly selecting one bird per pen, which were examined on day 21 and day 28. T1—Maxiban[®]/Monteban[®]; T2—Maxiban[®]/Sacox[®], HTSi—health tracking system. ¹ Lesion score, 0 = gross lesions absent, 1 = few scattered lesions, 2 = greater number of discrete lesions involving more of the affected zone of the intestine and marked bleeding with *Eimeria*, 3 = higher number of lesions. ² The broilers were challenged into the crop with an Evant vaccine (40 times the recommended dose) on days 14 and 21 of the trial. The coccidia vaccine contained *Eimeria acervulina* (003), *Eimeria maxima* (013), *Eimeria mitis* (006), *Eimeria praecox* (007), and *Eimeria tenella* (004) strains of *Eimeria*.

4. Discussion

In this paper, we report the efficacy and performance of two different monovalent ionophores, i.e., narasin and salinomycin, in a shuttle program with Maxiban[®] in chickens challenged by using a $40\times$ the recommended dose of a commercial anti-coccidial vaccine. In addition, differences in performance and health were evaluated between the two coccidiostats in terms of impact on broiler gut health and performance.

Eimeria protozoa, which are responsible for causing parasitic infections such as coccidiosis, have major impacts on the morphology of the intestine, affecting digestion, microabsorption of nutrients, and absorptive surface area [32–35]. The intestinal integrity in both groups was comparable, as the majority of factors were included in the composition of this index. Anti-protozoal protection, supported by the immunity of the birds in both groups, was probably maintained at similar levels. The vaccine challenge, in contrast to regular use, did not cause the clinical outbreak of coccidiosis, which indicates that the protective activity of the anticoccidials in both groups was maintained. The only statistically significant difference was observed in the level of excessive mucus in the intestines. In some cases, excessive mucus can be a good environment for anaerobic bacteria development, which may have a huge impact on the final performance of fast-growing broilers [36].

Growth performance is the most valuable index for monitoring poultry production [37]. The narasin-supplemented group (T1) showed improved performance parameters in terms of average body weight, daily feed intake, and EPEF. The improvement in growth parameters with narasin could have been due to its ability to restore the intestinal microbial balance, which then invigorated the secretion of endogenous digestive enzymes, improved the gut passage rate, and boosted intestinal morphology and nutrient absorption. Narasin may have also indirectly mitigated the inflammatory processes of coccidiosis by disrupting the replication of *Eimeria* [38,39]. It was also reported previously that narasin supplementation led to an improved feed conversion ratio in broilers [40]. A recent study by Abdelhady et al. reported that ionophores have the power to disrupt the ion gradients across the parasitic cell membrane [39].

However, the lower daily feed intake found in the salinomycin-supplemented broilers is in line with findings from previous studies that found decreased body weight, poor feed consumption, and a lower feed conversion ratio with therapeutic doses of salinomycin and even with higher doses of salinomycin in broilers [25,41,42]. Our study did not identify any significant differences in the lesion score between the two ionophores, but a previous study indicated that narasin supplementation led to a reduction in gizzard lesions in broilers [25,42,43]. In addition, previous studies have indicated that the use of narasin increasingly reduced intestinal damage in broilers, which is also the largest immune organ in broilers.

Additionally, these substances are highly efficacious against *Eimeria* and are, therefore, expected to reduce intestinal loads of parasites and secondary pathogenic bacteria, leading to a decrease in the corresponding host inflammatory responses. Under the conditions of the present study, both the examined ionophore coccidiostats were effective in treating coccidiosis and reinstating the measured parameters to optimum levels [39].

5. Conclusions

In our study, both coccidiostats were effective in treatment of coccidiosis and mitigation of the damage caused by the parasite, enabling desirable broiler performance. However, among the two coccidiostats, the results suggest that narasin (70 ppm) should be considered as a superior coccidiostat in comparison with salinomycin (70 ppm) in the finisher period (25–42 days) because of the enhanced performance parameters specifically in terms of average body weight, daily feed intake, intestinal integrity, and EPEF.

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Institutional Review Board Statement: This study was conducted in accordance with the principles of the EU (recommendation 2007/526/CE) and the Polish Law on Animal Protection. All procedures in this study were evaluated and approved by the Local Animal Care and Ethics Committee in Olsztyn (UWM), Poland (Resolution No. 12/2022 from 16 March 2022).

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare conflicts of interest. The authors are currently employed at the University of Warmia and Mazury in Olsztyn, Poland, and Elanco Animal Health (Warsaw, U.K., and India), but these employers had no influence on the decision to publish the data.

References

- 1. Williams, R.B. A compartmentalised model for the estimation of the cost of coccidiosis to the world's chicken production industry. *Int. J. Parasitol.* **1999**, *29*, 1209–1229. [CrossRef] [PubMed]
- Blake, D.P.; Tomley, F.M. Securing poultry production from the ever-present *Eimeria* challenge. *Trends Parasitol.* 2014, 30, 12–19. [CrossRef] [PubMed]
- Kadykalo, S.; Roberts, T.; Thompson, M.; Wilson, J.; Lang, M.; Espeisse, O. The value of anticoccidials for sustainable global poultry production. *Int. J. Antimicrob. Agents* 2018, 51, 304–310. [CrossRef]
- Lawal, J.R.; Jajere, S.M.; Geidam, Y.A.; Bello, A.M.; Wakil, Y.; Mustapha, M. Antibiotic Residues in Edible Poultry Tissues and Products in Nigeria. *Int. J. Anim. Vet. Adv.* 2015, 7, 55–61. [CrossRef]
- 5. Chapman, H.D. Origins of coccidiosis research in the fowl--the first fifty years. Avian Dis. 2003, 47, 1–20. [CrossRef] [PubMed]
- Pinard-Van Der Laan, M.H.; Monvoisin, J.L.; Pery, P.; Hamet, N.; Thomas, M. Comparison of outbred lines of chickens for resistance to experimental infection with coccidiosis (*Eimeria tenella*). *Poult. Sci.* 1998, 77, 185–191. [CrossRef] [PubMed]
- Belli, S.I.; Smith, N.C.; Ferguson, D.J. The coccidian oocyst: A tough nut to crack! *Trends Parasitol.* 2006, 22, 416–423. [CrossRef] [PubMed]

- De Gussem, M. Coccidiosis in poultry: Review on diagnosis, control, prevention and interaction with overall gut health. In Proceedings of the 16th European Symposium on Poultry Nutrition, Strasbourg, France, 26–30 August 2007.
- Chengat Prakashbabu, B.; Thenmozhi, V.; Limon, G.; Kundu, K.; Kumar, S.; Garg, R.; Clark, E.L.; Srinivasa Rao, A.S.R.; Raj, D.G.; Raman, M.; et al. *Eimeria* species occurrence varies between geographic regions and poultry production systems and may influence parasite genetic diversity. *Vet. Parasitol.* 2017, 233, 62–72. [CrossRef]
- 10. Peek, H.W.; Landman, W.J. Coccidiosis in poultry: Anticoccidial products, vaccines and other prevention strategies. *Vet. Q.* **2011**, *31*, 143–161. [CrossRef] [PubMed]
- 11. Parker, C.D.; Lister, S.A.; Gittins, J. Impact assessment of the reduction or removal of ionophores used for controlling coccidiosis in the UK broiler industry. *Vet. Rec.* 2021, *189*, e513. [CrossRef] [PubMed]
- FAO. Gateway to Poultry Production and Products. Available online: https://www.fao.org/poultry-production-products/ production/en/ (accessed on 26 May 2023).
- 13. Kart, A.; Bilgili, A. Ionophore Antibiotics: Toxicity, Mode of Action and Neurotoxic Aspect of Carboxylic Ionophores. J. Anim. Vet. Adv. 2008, 7, 748–751.
- Noack, S.; Chapman, H.D.; Selzer, P.M. Anticoccidial drugs of the livestock industry. *Parasitol. Res.* 2019, 118, 2009–2026. [CrossRef] [PubMed]
- 15. Chapman, H.D. Use of anticoccidial drugs in broiler chickens in the USA: Analysis for the years 1995 to 1999. *Poult. Sci.* 2001, *80*, 572–580. [CrossRef] [PubMed]
- 16. Chapman, H.D. Rotation programmes for coccidiosis control. Int. Poult. Product. 2007, 15, 7–9.
- 17. Bentué, M.; Jeffers, T.K. The Role of Narasin in Poultry Health: A Review. In Proceedings of the 14th European Poultry Conference, Stavanger, Norway, 23–27 June 2014.
- 18. Oehme, F.W.; Pickrell, J.A. An analysis of the chronic oral toxicity of polyether ionophore antibiotics in animals. *Vet. Hum. Toxicol.* **1999**, *41*, 251–257.
- Chapman, H.D. Studies on the sensitivity of field isolates of *Eimeria* maxima to combinations of anticoccidial drugs. *Avian Pathol.* 1980, 9, 67–76. [CrossRef]
- 20. Jeffers, T.K. Anticoccidials: Past to present to future. Feedstuffs 2011, 83, 16–18.
- Jeffers, T.K.; Tonkinson, L.V.; Callender, M.E. Anticoccidial Efficacy of Narasin in Battery Cage Trials. *Poult. Sci.* 1988, 67, 1043–1049. [CrossRef]
- Bolder, N.M.; Wagenaar, J.A.; Putirulan, F.F.; Veldman, K.T.; Sommer, M. The effect of flavophospholipol (Flavomycin) and salinomycin sodium (Sacox) on the excretion of Clostridium perfringens, Salmonella enteritidis, and Campylobacter jejuni in broilers after experimental infection. *Poult. Sci.* 1999, 78, 1681–1689. [CrossRef]
- 23. Yvorě, P.; Raynaud, J.P.; Conan, L.; Naciri, M. Evaluation of the Efficacy of Salinomycin in the Control of Coccidiosis in Chicks. *Poult. Sci.* **1980**, *59*, 2412–2416. [CrossRef]
- Ruff, M.D.; Reid, W.M.; Rahn, A.P.; McDougald, L.R. Anticoccidial Activity of Narasin in Broiler Chickens Reared in Floor Pens. Poult. Sci. 1980, 59, 2008–2013. [CrossRef] [PubMed]
- Keshavarz, K.; McDougald, L.R. Anticoccidial drugs: Growth and performance depressing effects in young chickens. *Poult. Sci.* 1982, 61, 699–705. [CrossRef] [PubMed]
- Ebrahimnezhad, Y.; Pourreza, J. Effects of Ionophorous Anticoccidial Drugs, Salinomycin and Lasalocid, on the Performance of Broiler Chicks and the Relationship of These Drugs to Supplementary Methionine. *Inter. J. Poult. Sci.* 2005, *4*, 911–916. [CrossRef]
- 27. Aviagen. Ross 308 Broiler: Management Manual; Aviagen Ltd.: Midlothian, UK, 2018.
- 28. Kozłowski, K.; Vervenne-Zetteler, P.; Konieczka, P.; Szymanski, Ł.; van Vilsteren, A. *Yucca schidigera* Improves Performance and Lowers Oocyst Counts in *Eimeria* Challenged Broilers. *Animals* **2022**, *12*, 1668. [CrossRef]
- Konieczka, P.; Sandvang, D.; Kinsner, M.; Szkopek, D.; Szyryńska, N.; Jankowski, J. Bacillus-based probiotics affect gut barrier integrity in different ways in chickens subjected to optimal or challenge conditions. *Vet. Microbiol.* 2022, 265, 109323. [CrossRef] [PubMed]
- 30. Kasab-Bachi, H.; Arruda, A.G.; Roberts, T.E.; Wilson, J.B. The use of large databases to inform the development of an intestinal scoring system for the poultry industry. *Prev. Vet. Med.* **2017**, *146*, 130–135. [CrossRef]
- Johnson, J.; Reid, W.M. Anticoccidial drugs: Lesion scoring techniques in battery and floor-pen experiments with chickens. *Exp. Parasitol.* 1970, 28, 30–36. [CrossRef]
- Gottardo, E.T.; Prokoski, K.; Horn, D.; Viott, A.D.; Santos, T.C.; Fernandes, J.I.M. Regeneration of the intestinal mucosa in *Eimeria* and *E. coli* challenged broilers supplemented with amino acids. *Poult. Sci.* 2016, 95, 1056–1065. [CrossRef]
- Su, S.; Miska, K.B.; Fetterer, R.H.; Jenkins, M.C.; Wong, E.A. Expression of digestive enzymes and nutrient transporters in *Eimeria*-challenged broilers. *Experim. Parasitol.* 2015, 150, 13–21. [CrossRef]
- 34. Morris, B.C.; Danforth, H.D.; Caldwell, D.J.; Pierson, F.W.; McElroy, A.P. Intestinal Mucosal Mast Cell Immune Response and Pathogenesis of Two *Eimeria acervulina* Isolates in Broiler Chickens. *Poult. Sci.* 2004, *83*, 1667–1674. [CrossRef]
- 35. Adams, C.; Vahl, H.A.; Veldman, A. Interaction between nutrition and *Eimeria acervulina* infection in broiler chickens: Development of an experimental infection model. *Br. J. Nutr.* **1996**, *75*, 867–873. [CrossRef] [PubMed]
- 36. Van der Sluis, W. Clostridial enteritis is an often underestimated problem. World's Poult. Sci. J. 2000, 16, 42–43.
- Chen, Y.; Aorigele, C.; Yan, F.; Li, Y.; Cheng, P.; Qi, Z. Effect of production system on welfare traits, growth performance and meat quality of ducks. S. Afr. J. Anim. Sci. 2015, 45, 173–179. [CrossRef]

- Bozkurt, M.; Ege, G.; Aysul, N.; Akşit, H.; Tüzün, A.E.; Küçükyılmaz, K.; Borum, A.E.; Uygun, M.; Akşit, D.; Aypak, S.; et al. Effect of anticoccidial monensin with oregano essential oil on broilers experimentally challenged with mixed *Eimeria* spp. *Poult. Sci.* 2016, *95*, 1858–1868. [CrossRef] [PubMed]
- Abdelhady, A.Y.; El-Safty, S.A.; Hashim, M.; Ibrahim, M.A.; Mohammed, F.F.; Elbaz, A.M.; Abdel-Moneim, A.-M.E. Comparative evaluation of single or combined anticoccidials on performance, antioxidant status, immune response, and intestinal architecture of broiler chickens challenged with mixed *Eimeria* species. *Poult. Sci.* 2021, 100, 101162. [CrossRef] [PubMed]
- 40. Yan, L.; Lv, Z.Z.; An, S.; Xing, K.; Wang, Z.G.; Lv, M.B.; Choct, M.; Guo, Y.M.; Zhou, G.L. Effects of rearing system and narasin on growth performance, gastrointestinal development, and gut microbiota of broilers. *Poult. Sci.* **2021**, *100*, 100840. [CrossRef]
- 41. Paulillo, A.C.; Chieri, A.B.; Ariki, J.; Ferreira, R.D.; Panobianco, M.A. Anticoccidialagents (monensian, salinomycin andd halofuginone) in broiler rations. *Ars. Vet.* **1986**, *2*, 5–12.
- 42. Rizvi, F.; Din Anjum, A. Effect of salinomycin on broiler health. Vet. Arh. 1999, 69, 39–47.
- Kaldhusdal, M.; Hetland, H.; Gjevre, A.G. Non-soluble fibres and narasin reduce spontaneous gizzard erosion and ulceration in broiler chickens. *Avian Pathol.* 2012, 41, 227–234. [CrossRef]

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