Impacts of antibiotic reduction strategies on zootechnical performances, health control, and *Eimeria* spp. excretion compared with conventional antibiotic programs in commercial broiler chicken flocks

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ABSTRACT Increasing efforts have been made in recent years to reduce antimicrobial use in animal production. The objective of this prospective study was to evaluate, in commercial broiler chicken farms, 2 antibiotic reduction strategies that eliminated the use of antibiotics important for human medicine, in comparison with the conventional use of antibiotics. On 7 broiler chicken farms, a house was allocated to the antibiotic reduction treatments for 6 consecutive flocks, whereas a similar house on the same premises was assigned to the conventional use of antibiotics (**CONV**) for 6 consecutive flocks. The antibiotic reduction strategies consisted of continuous in-feed use of ionophores (**TX1**) and continuous in-feed use of ionophores with butyric acid (**TX2**). In the 84 flocks, zootechnical performance was recorded, lesion scoring at 21 and 28 D of age was performed, and fecal samples were recovered during grow out for *Eimeria* spp. oocysts counts. There was no statistical difference between TX1, TX2, and CONV for weights at slaughter, feed conversion ratios, average daily gains, age at slaughter, total mortalities, and condemnations. The probability of identifying oocysts in the fecal samples significantly increased with the age of the flock, but there was no significant treatment effect between 7 and 16 D of age. At 19 D of age, the probability of a sample containing oocysts was higher in TX1 than in CONV, but TX2 was not statistically different from TX1 and CONV. Predicted oocysts per gram in CONV flocks were significantly lower between 22 and 34 D of age than in TX1 and TX2 flocks, whereas there were no significant differences between TX1 and TX2 for all ages. Lesion scoring of the gastrointestinal system showed no differences for coccidiosis scores between TX1, TX2, and CONV. No lesions of necrotic enteritis were observed. In conclusion, it was possible to adequately control intestinal diseases and maintain zootechnical performances by relying exclusively on ionophores, when compared with broiler chicken flocks using standard shuttle programs with antibiotic growth promoters.

Key words: antibiotic reduction, broiler, intestinal health, zootechnical performance, *Eimeria* spp

INTRODUCTION

Awareness about the growing incidence of antimicrobial resistance (**AMR**) of important bacterial pathogens and its impacts on human and animal health has increased in recent years. The World Health Organization describes this phenomenon as one of the biggest threats to global health, which could cause 10 million human deaths each year by 2050 if no action is taken (Tangcharoensathien et al., 2017). To help meliorate this issue, the international agency is calling for a ''One Health'' approach, which includes various recommendations such as urgently phasing out the use of critically important antimicrobials for growth promotion and prevention of diseases in agriculture. Indeed, the extensive use of antibiotics has been shown to be associated with an increased abundance of resistance genes to many antibiotics. For instance, metagenomic analyses of broiler chickens and slaughter pigs' fecal samples in European countries showed a positive association between the abundance of resistance genes and antimicrobial

use (**AMU**) (Munk et al., 2018). This emphasizes the importance of reducing AMU in animal production to decrease the selective pressure on antibiotic-resistant bacteria and the prevalence of AMR gene.

In Europe, concerns about AMR have led to the ban of antibiotics used for growth promotion in farm animals (Castanon, 2007; Cogliani et al., 2011). However, the transition has been associated with increased *Clostridium perfringens*-associated enteritis and cholangiohepatitis infections (Van Immerseel et al., 2004). Substantial losses were observed in broiler chicken flocks affected by these conditions, leading to poor zootechnical performance and increased condemnations at slaughter (Lovland and Kaldhusdal, 1999, 2001). In the United States(**US**), consumer demand has recently driven the broiler chicken industry to reduce AMU (Karavolias et al., 2018). Three categories of broiler production can be identified based on AMU: 1) Flocks using antibiotics that were considered medically important for human medicine are reported as conventional flocks in the present article; 2) flocks using exclusively antibiotics that were nonmedically important for human medicine are reported as antibiotic-reduced flocks in the present article, and 3) no antibiotics ever (**NAE**), also named "raised without the use of antibiotics (**RWA**)" or "antibiotic-free" flocks. Between 2013 and 2017 in the US, a substantial decrease has been recorded for the use of most of the antibiotic classes administered to broilers for diseases treatment or prevention (Singer and Porter, 2019). Most importantly, these authors also reported a shift from antimicrobial drugs medically important to humans toward antibiotic classes considered not important for human medicine. In Canada, where there is no antibiotic ban, the conventional use of antibiotics is comparable with the use of medically important antibiotics in the US. The Canadian federal authorities labeled a type of production called RWA (CFIA, 2016) similar to the NAE programs in the US. In a study comparing drug-free to conventional broiler chicken flocks, significant production losses were associated with the RWA flocks (Gaucher et al., 2015) and many farms experienced recurring outbreaks of necrotic enteritis (**NE**) caused by pathogenic and clonal *C. perfringens* strains carrying the *netB* gene (Gaucher et al., 2017; Parent et al., 2017). Thus, there is a global trend for AMU reduction in broiler chicken production, but it has often been associated with increased intestinal health disorders and production losses. Controlling intestinal diseases such as NE has become critical to successfully raise healthy broiler chicken flocks with antibiotic-reduction programs.

The epidemiology of NE has been extensively studied and risk factors, for instance coccidiosis, play a significant role in the pathogenesis of the disease (Lee et al., 2011; Shojadoost et al., 2012; Moore, 2016; Prescott et al., 2016; Van Waeyenberghe et al., 2016). Indeed, coccidiosis has been shown to exacerbate NE in challenge models (Al-Sheikhly and Al-Saieg, 1980; Rodgers et al., 2015) and to impair zootechnical performance of broiler chickens (Rochell et al., 2016). Various products, including chemical anticoccidials, ionophores, and vaccines, are currently used in commercial farms to control this protozoal disease. Each product has a different effect on the excretion and cycling of *Eimeria* spp., the causative agent of coccidiosis (Williams and Gobbi, 2002; Chapman et al., 2016; Jenkins et al., 2017; Parent et al., 2018). Various alternatives to antibiotics such as herbal products or organic acids have also been reviewed as compounds with prophylactic/therapeutic potential for coccidiosis control (Ali et al., 2014; Muthamilselvan et al., 2016).

The use of nonmedically important antibiotics may provide an adequate control of diseases, most likely equivalent to the use of medically important antibiotics in broiler chickens (Karavolias et al., 2018). The hypothesis of this study is that antibiotic reduction strategies using nonmedically important antibiotics in commercial broiler chicken flock prevention programs can provide similar zootechnical performance and offer similar health control to the prevention programs using medically important antibiotics for human medicine. The objective of the study was to evaluate, in commercial broiler chicken farms, 2 antibiotic reduction strategies eliminating the use of antibiotics considered critically important for human medicine, in comparison with the conventional use of antibiotics. More specifically, production performance, flock health, and *Eimeria* spp. excretion were compared between antibiotic reduction and conventional strategies.

MATERIALS AND METHODS

Study Design

Care and Use of Animals The committee on animal care in research (Comité d'éthique pour l'utilisation des animaux) of the Faculté de médecine vétérinaire of the Université de Montréal approved the study and protocols involving animal use with the project number 16-Rech-1850.

Farm Eligibility Criteria and Description The prospective study was conducted in commercial broiler chicken farms owned by chicken farmers in the province of Quebec, Canada. Producers from the Poultry Farmers Association of Quebec ("Éleveurs de volailles du Québec"), who owned at least 2 houses on the same farm, were contacted to participate in this year-long study. Seven broiler chicken farms were included on a voluntary basis. Each farm was visited before the beginning of the study to inspect the facilities. The premise was required to have 2 broiler chicken houses, with similar stocking densities, surface areas, feeding systems, water equipment, and ventilation systems. A summary of the 7 broiler chicken farms characteristics is presented in Table 1. The houses capacity ranged from 9,800 to 22,000 broiler chickens per flock. Three hatcheries provided chicks to the farms, 4 feed mills prepared the feed during the study, and 3 processing plants slaughtered the chickens at market weight. A premise was required to keep the same hatchery, feed mill, and

slaughterhouse for the duration of the study. All flocks raised were male. The average downtime between flocks on a farm ranged from 11.4 to 20.0 D for the duration of the study.

Rearing and Housing Conditions On each farm, a broiler house was randomly allocated to the antibiotic reduction treatments for 6 consecutive flocks, whereas the other house was allocated to the conventional treatment for 6 consecutive flocks. Flocks were raised simultaneously in the 2 houses at each farm, that is the flocks in both houses of a same farm were always placed on the same day with chicks originating from the same hatchery and breeder flocks. As more than 1 breeder flock contributed to the chicks placed in each house, the same proportion of chicks from each breeder flock were placed in both houses. A specific breeder flock age was not required for the study, but the placements in each paired broiler houses needed to be identical to control for chick quality. Chicks were vaccinated against Marek's disease and received lincomycin-spectinomycin *in ovo*. The brooding method described in Chick Champs was used (Chicken Farmers of Canada, 2015). Management on each farm followed rearing standards in the broiler chicken flock industry, which can be found in the Aviagen and Cobb broiler management guides (Aviagen, 2018; Cobb-Vantress, 2018). On a farm, daily care of chickens and management of both houses were performed by the same employees. Shipping to processing plants was individually determined for each flock to meet target average BW. For this reason, the slaughtering day was allowed to vary between 2 paired flocks. More precisely, the processing of a flock would be pre-empted or delayed to reach a target weight depending on its weight a few days before slaughter, a common practice within the Quebec industry to standardize carcass weights at processing. After each flock, as per the Chicken Farmers of Canada On-Farm Food Safety Assurance Program manual (Chicken Farmers of Canada, 2014), litter was removed from broiler houses, and a dry cleaning (dust removal) was performed. Fresh pine wood shavings were used as bedding material by the 7 participating farms for each lot. The drinking water of all flocks was acidified with an inorganic acid (phosphoric acid [H_3PO_4] 17%) at an inclusion rate targeting an end-of-line pH between 5 and 6. Water lines were flushed daily for the first 7 D, then weekly until shipping to the processing plant. Producers washed and disinfected lines as per the standard operating procedures on each farm.

Antibiotic Reduction Treatments Two antibiotic reduction strategies were randomly allocated to the 6 consecutive flocks of the first broiler house of each farm, for a total of 3 repetitions per farm for each strategy. The first strategy consisted of the continuous use of monovalent ionophores from placement to shipping to the processing plant, in accordance with the Veterinary Drugs Directorate of the Health Canada's Health Products and Food Branch (Government of Canada, 2018). The second strategy used the same monovalent ionophores from placement to shipping, and an inorganic

acid (butyric acid 65%) was also added as a feed additive at the concentration of 0.7 kg of premix per ton of feed. No antibiotics other than ionophores were used in the prevention programs of this broiler house for each farm. A summary of medication programs used in each flock is presented in Supplementary Table 1.

Conventional Treatment The conventional treatment consisted of the normal use of shuttle anticoccidial programs with antibiotics considered critically important for human medicine in the feed for the 6 consecutive flocks of the second broiler house in each farm. The programs used in the study were prepared by the referring veterinarian and were not modified by the research team. Briefly, veterinarians used chemical anticoccidial until 3 wk of age, followed by a monovalent ionophore until shipping to slaughter. From placement to shipping, antibiotics were included in the feed as a common practice to prevent NE during rearing. Product rotations were performed every 2 flocks as a common practice within the industry to prevent the development of pathogen resistance against anticoccidials and antibiotics. The antimicrobials and rotations used in the conventional programs were considered as the best practices to maximize production performances and health control in broiler chicken flocks. Details of medication programs are included in Supplementary Table 1.

Feed and Nutritional Guidelines Nutritional guidelines were provided to each participating feed mill (Supplementary Table 2). Feed formulation and ingredient inclusion rates were identical between 2 flocks raised simultaneously on a premise, but it was allowed to vary within the nutritional guidelines for flocks on different premises.

Zootechnical Performances

For the 84 flocks, BW at slaughter (kg) and total condemnations (%) were retrieved from the slaughterhouse data. Age at slaughter (days) and total mortality (%) were recovered from farm data. Feed conversion ratio (**FCR**) was calculated with the formula: FCR = Total feed consumed (kg)/Total chickens' weight at slaughter (kg). The average daily gain (**ADG**) was calculated with the formula: ADG (g/D) = Mean BW at slaughter (g)/Age at slaughter (D).

Flock Health

Eimeria *spp. Oocysts Fecal Counts* Fresh feces from all flocks were sampled every 3 D, starting at 7 D of age until the end of the grow out period. One pooled sample of fecal content was taken per time point in each flock, consisting of 20 to 25 fresh fecal droppings evenly distributed in the house. A total of 84 samples per sampling day, 21 from the ionophores group, 21 from the ionophores with butyric acid group, and 42 from conventional flocks, were planned for collection. Fecal droppings were recovered in a Whirl-Pak bag and stored immediately at 4°C until processing for oocyst counts. In accordance with the procedures of the Faculty

of Veterinary Medicine parasitology diagnostic laboratory, a slightly modified McMaster technique was used to count total oocysts per gram of fecal content (**OPG**) in each sample. After homogenization of the pooled sample, 10 g of fecal content were weighed and mixed with 100 mL of water. The mixture was stored at 4°C for 24 h and then filtered with a sieve. The filtrate (15 mL) was transferred in a Falcon tube to be centrifuged at 1,500 rpm for 10 min. The pellet was resuspended in 5 mL of 35.5% NaCl solution, vortexed, and the solution transferred to a 50 mL beaker. Two additional rinses with 5 mL of 35.5% salt solution were performed to recover all oocysts in the tube. A Pasteur pipette was used to fill a McMaster chamber (Partnar Animal Health, Ilderton, Ontario, Canada) with the homogenized solution. Readings were performed 1 min after filling the chamber. All oocysts in the limits of each chamber were recorded. To express the results in OPG, the following formula was used: OPG = (Oocyst counts in chamber 1 + Oocyst counts in chamber 2)/2 × 66.6.

Lesion Scoring Postmortem sessions, based on the lesion scores in the Elanco Animal Health's Health Tracking System described in the Broiler Disease Reference Guide (Elanco Animal Health, 2010; Kasab-Bachi et al., 2017), were conducted at 21 and 28 D of age to evaluate the health condition of all flocks. Twelve live chickens per flock at each time point were randomly selected across the house to represent the flock. Chickens were humanely euthanized by a standard cervical dislocation (American Veterinary Medical Association, 2013) and weighed. The same observer then performed the Elanco Animal Health's Health Tracking System scoring method to identify and score each lesion or condition described in the aforementioned guide for the 2016 individual chickens selected across the 84 flocks.

Fecal and Litter Humidity In each flock, fecal and litter samples were recovered at 21 D of age, 28 D of age, and before shipping to slaughter. Approximately 20 g of fresh fecal droppings and litter were sampled across the broiler chicken house and put in separate hermetic Whirl-Pak bags. Then, samples were refrigerated at 4°C until processing at the laboratory. From each sample, 5 g of feces or litter was put in a moisture analyzer (Denver IR-120 Moisture Balance; Laboratory Instrument Specialists, CA) for the determination of moisture content by infrared radiation and measuring the weight loss on drying.

Statistical Analysis

Zootechnical Performance, Lesion Scoring, Fecal Humidity, and Litter Humidity The flock was considered the experimental unit for all analyses. All statistical analyses were performed with R statistical software (R Core Team, 2017) using the lme4 package (Bates et al., 2015) and the function lmer to fit linear mixed-effect models with the restricted maximum likelihood approach for coefficients estimation. Six different models were built for the zootechnical performances outcomes: BW at slaughter (kg), FCR, ADG (g/D), age at slaughter (days), total mortality (%), and percentage of condemnations at slaughter (%). Data from the percentage of condemnations at slaughter were log transformed to improve model fit. Results were back transformed to their original scale for presentation of results. For the lesion scoring, the mean lesion scores were calculated from the 12 chickens evaluated at 21 or 28 D of age in each flock by adding each individual score and by dividing the total by 12. For the litter and fecal humidity analyses, models were built for each sampling time point at 21 D of age, 28 D of age, and before slaughter. For all models, treatment was included as a fixed effect, and the farm was used as a random intercept. Coefficients with a P-value ≤ 0.05 were considered significant. Models validity were assessed by the visual inspection of quantile–quantile plots for normality and by scatter plots of the standardized residuals as a function of the adjusted outcome values for homoscedasticity.

Oocysts Excretion Modeling Two different statistical models were built to model the dynamics of *Eimeria* spp. oocysts excretion during grow out for the 3 treatments. A mixed multivariable logistic regression model for the first 5 sampling ages (7, 10, 13, 16, and 19 D of age) and a mixed multivariable linear regression model for the next 5 time points (22, 25, 28, 31, and 34 D of age) were built as the data distribution differed between early and late flocks' ages. Samples at 37 and 40 D of age were not considered in the analyses owing to most flocks being slaughtered before these ages. A small number of samples (33 of 840) were missing between 7 and 34 D of age because of sampling omission, and these were not considered in the analyses because missing samples were evenly distributed across the 7 farms and 3 treatments for all ages. In the early grow out, many samples contained no *Eimeria* spp. oocysts, whereas most of the later ages' samples did contain oocysts. Owing to many zero values in the early ages, linear regression models did not fit the data. Hence, the OPG were dichotomized for the presence (≥ 1 oocyst) or absence (0 oocyst) of *Eimeria* spp. in each sample. Using the glmer function from the lme4 package in the R statistical software (Bates et al., 2015), a mixed logistic regression model was fitted with the inclusion of the treatment and age as fixed effects and the farm as random intercept. The outcome was the presence or absence of *Eimeria* spp. oocysts in the samples. The logistic regression model validity was assessed by determining the goodness of fit with the Hosmer-Lemeshow test and by evaluating the accuracy of the model to correctly predict the outcome. In later ages, a mixed linear regression model with the restricted maximum likelihood approach for coefficients estimation was built to model the excretion of *Eimeria* spp. oocysts. The OPG values were log transformed to improve model fit. Treatments and flocks' age were considered as fixed effects, and the farm was included as a random intercept. Owing to the curvilinear relationship between the log10 OPG values and flocks' age, a quadratic variable of age (age^2) was added to improve the final model fit. Coefficients with a P-value ≤ 0.05 were considered significant. The mixed linear regression model validity was assessed by the visual inspection of

Table 1. Summary of the participating farms.

Farm ID	Houses capacity (# chickens)	Hatchery ID	Feed mill ID	Processor ID	Average downtime between flocks (D)
1	12,000	1	1	1	11.4
2	22,000	2	2	2	17.4
3	15,000	2	3	3	14.4
4	13,500	2	2	2	18.2
5	19,000	3	3	2	20.0
6	13,500	2	2	2	18.0
7	9,800	3	4	2	19.4

quantile–quantile plots for normality and by scatter plots of the residuals as a function of the adjusted outcome values for homoscedasticity. Predicted probabilities of identifying *Eimeria* spp. oocysts and predicted log10 OPG in the droppings were computed from the logistic and regression models, respectively, and then plotted against flocks' age to display differences between treatments for the excretion of oocysts in each treatment during grow out.

Prestudy Power Analysis Prestudy statistical power analyses on sample size were performed while devising the study to determine the number of flocks required to adequately evaluate group differences. For example, the ability to detect significant differences between groups for the FCR was based on SE of 0.05 and means of 1.65. A sample size per group of 16 was determined to be sufficient to detect a difference between groups with a significance threshold of 0.05.

RESULTS

Influence of the Treatments on Zootechnical Performance

There were no significant differences between treatments ($P > 0.05$) for the BW at slaughter, FCR, ADG, age at slaughter, total mortality, and total condemnations (Table 2).

Influence of the Treatments on Flocks' Health

Eimeria *Excretion* Predicted probabilities of a fecal sample containing *Eimeria* spp. oocysts from 7 to 19 D of age for each treatment are presented in Figure 1, and the model results are presented in Supplementary Table 3. The mixed multivariable logistic regression model showed an accuracy of 79.4% to correctly identify

the presence or absence of oocysts in a fecal sample. The probability of identifying oocysts in the fecal samples significantly increased with the age of the flock, but there was no significant effect of the treatment between 7 and 16 D of age based on the 95% confidence intervals. At 19 D of age, the probability of having a sample containing oocysts was higher in the ionophores group than in the conventional flocks, but the flocks receiving ionophores and butyric acids were not statistically different from the 2 other groups. Predicted log10 OPG values from 22 to 34 D of age for each treatment are displayed in Figure 2, and results of the mixed multivariable linear regression model are shown in Supplementary Table 3. The predicted OPG in the conventional flocks were significantly lower for all ages compared with the 2 other groups, whereas there was no significant difference between the 2 antibiotic-reduced groups for all ages. The predicted OPG increased from 22 to 28 D of age for all treatments, then decreased at 31 and 34 D of age.

Lesion Scoring per Treatment at 21 D of Age Average BW ranged from 952.3 to 964.9 g with no significant differences between groups ($P > 0.05$) (Table 3). For the gastrointestinal system, mean scores for the Intestinal Integrity Index, *Eimeria acervulina*, *Eimeria maxima*, *Eimeria tenella*, microscopic *E. maxima*, NE, and gizzard erosions were not statistically different between groups ($P > 0.05$). For the evaluation of the integumentary and skeletal systems, scores of burned feet (pododermatitis), femoral head necrosis, and tibial dyschondroplasia were recorded. For all mean scores, there was no difference between treatments ($P > 0.05$). Burned feet lesions were present in nearly all flocks, but low scores on the 0 to 3 scale were mostly recorded. The presence of femoral head necrosis and tibial dyschondroplasia was infrequent; hence, the mean scores were close to 0 for all groups. The bursal diameter was evaluated to evaluate the immune system. The mean diameter ranged from 1.82 to 2.05 cm in the 3 groups, and no

Table 2. Impacts of treatments on zootechnical performances.

Zootechnical parameter	Ionophores (n = 21)		Ionophores and butyric acids (n = 21)		Conventional (n = 42)		P-value
	Mean	SE	Mean	SE	Mean	SE	
Slaughter weight (kg)	2.44	0.07	2.41	0.04	2.43	0.03	0.40
Feed conversion ratio	1.62	0.04	1.64	0.02	1.64	0.02	0.23
Average daily gain (g/D)	66.7	1.1	65.7	1.1	66.1	0.9	0.34
Age at slaughter (days)	36.7	1.0	36.7	0.3	36.6	0.3	0.66
Mortality (%)	2.92	0.58	3.03	0.38	3.18	0.33	0.43
Total condemnations (%)	1.74	1.17	1.62	1.17	1.70	1.15	0.64
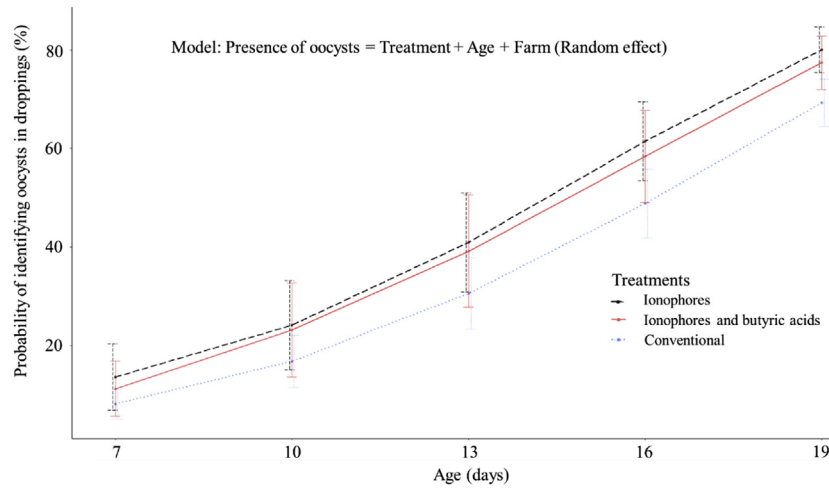
Figure 1. Predicted probabilities of a fecal sample containing *Eimeria* spp. oocysts from 7- to 19-day-old flocks for each treatment based on the mixed multivariable logistic regression model (n = 412). Error bars are corresponding to the 95% confidence intervals computed for each predicted probability by the model.

statistical difference was noted ($P > 0.05$). Finally, the respiratory system was evaluated by scoring the presence of airsacculitis and tracheal lesions. No significant difference was observed between the 2 antibiotic-reduction groups and the conventional group ($P > 0.05$).

**Lesion Scoring per Treatment at 28 D of Age** Average BW in all groups ranged from 1596.4 to 1617.3 g, with no statistical differences between groups ($P > 0.05$) (Table 3). Scores in the gastrointestinal and respiratory systems were not statistically different between all groups ($P > 0.05$). In the integumentary and skeletal systems, the mean score of pododermatitis was significantly higher in the ionophores with butyric acid group than in the ionophores-only and conventional groups ($P = 0.05$). Femoral head necrosis and tibial dyschondroplasia lesions were sporadic, resulting in mean scores close to 0 for all groups. There was no statistical difference between groups for these 2 parameters ($P > 0.05$). The average bursal diameter of 2.02 cm in the ionophores-only group was significantly higher than

the mean diameter of 1.79 cm in the ionophores-plus-butyric-acid group ($P = 0.05$). The conventional group was not statistically different ($P > 0.05$) from the other groups with a bursal mean diameter of 1.86 cm.

Litter and Fecal Humidity There was no significant difference between the 3 treatments for the humidity in the litter and fecal samples at 21 D of age, 28 D of age, and before slaughter ($P > 0.05$ for all comparisons) (Supplementary Table 4).

DISCUSSION

This study aimed to evaluate the impacts of reducing AMU in a commercial context of broiler chicken production by removing medically important in-feed antibiotics from disease prevention programs. The 7 selected farms followed management and vaccination procedures commonly used within the industry for the 6 paired consecutive flocks, and feed formulation did not differ from current industry standards. Hence, the results
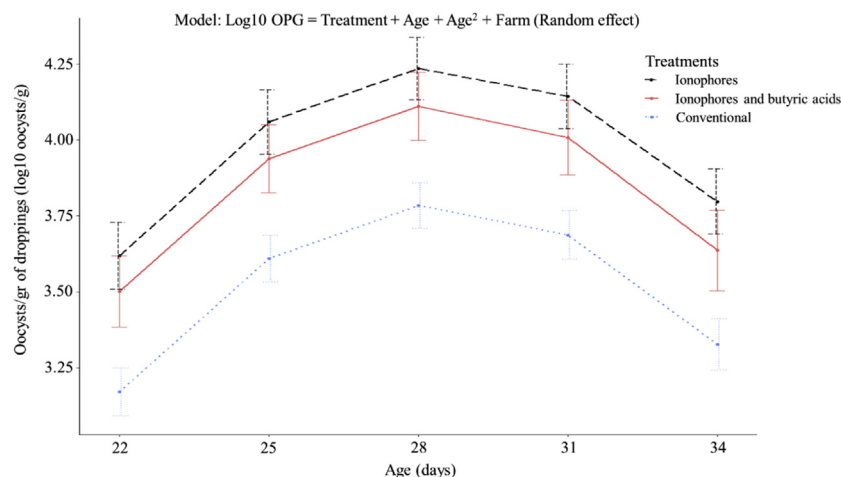
Figure 2. Predicted log10 OPG values from 22- to 34-day-old flocks for each treatment based on the mixed multivariable linear regression model (n = 395). The error bars are corresponding to the 95% confidence intervals computed for each predicted OPG value by the model. Abbreviation: OPG, oocysts per gram of fecal content.

Table 3. Results of the lesion scoring by treatment at 21 and 28 D of age.

Lesion score	Ionophores		Ionophores and butyric acids		Conventional		
	Mean	SE	Mean	SE	Mean	SE	*P*-value
BW (g)							
21 D	952.3	19.9	964.9	22.4	953.8	19.4	0.57
28 D	1605.4	38.2	1596.4	35.2	1617.3	30.5	0.70
Gastrointestinal system							
Intestinal Integrity Index							
21 D	93.9	0.8	93.9	0.8	93.9	0.7	0.95
28 D	92.5	0.9	91.8	1.1	92.7	1.0	0.52
Eimeria acervulina							
21 D	0.30	0.09	0.21	0.12	0.25	0.11	0.48
28 D	0.44	0.10	0.36	0.13	0.44	0.12	0.52
Eimeria maxima							
21 D	0.03	0.01	0.02	0.01	0.01	0.01	0.06
28 D	0.02	0.02	0.05	0.03	0.03	0.02	0.12
Eimeria tenella							
21 D	0.04	0.03	0.04	0.03	0.01	0.03	0.2
28 D	0.01	0.03	0.07	0.05	0.00	0.04	0.13
Microscopic *E. maxima*							
21 D	0.21	0.09	0.18	0.11	0.15	0.09	0.52
28 D	0.5	0.13	0.49	0.16	0.35	0.14	0.27
Necrotic enteritis							
21 D	0.0	0.0	0.0	0.0	0.0	0.0	1.00
28 D	0.0	0.0	0.0	0.0	0.0	0.0	1.00
Gizzard erosions							
21 D	0.08	0.06	0.06	0.05	0.08	0.04	0.59
28 D	0.07	0.06	0.15	0.05	0.14	0.05	0.14
Intertegumentory and skeletal systems							
Burned feet (pododermatitis)							
21 D	0.64	0.18	0.80	0.12	0.61	0.11	0.21
28 D	0.84[a]	0.20	1.11[b]	0.14	0.83[a]	0.12	0.05
Femoral head necrosis							
21 D	0.02	0.01	0.01	0.01	0.01	0.01	0.11
28 D	0.003	0.010	0.002	0.010	0.013	0.09	0.15
Tibial dyschondroplasia							
21 D	0.11	0.04	0.09	0.03	0.08	0.03	0.25
28 D	0.11	0.06	0.08	0.03	0.08	0.03	0.36
Immune system							
Bursal diameter (cm)							
21 D	2.05	0.13	1.82	0.19	1.88	0.16	0.22
28 D	2.02[a]	0.08	1.79[b]	0.10	1.86[a,b]	0.08	0.05
Respiratory system							
Airsacculitis							
21 D	0.13	0.11	0.34	0.13	0.23	0.11	0.09
28 D	0.17	0.11	0.26	0.11	0.34	0.10	0.08
Tracheal mucosa reddening							
21 D	0.63	0.08	0.75	0.11	0.70	0.09	0.29
28 D	0.75	0.11	0.72	0.11	0.69	0.09	0.52

[a,b]Means ± SE within a row without a common letter are significantly different ($P < 0.05$).

obtained from this trial can be extrapolated across the current broiler chicken production system. Our data support the concept of replacing medically important antibiotics by nonmedically important antibiotics in the feed without impacting performance in commercial broiler chicken flocks. However, the removal of lincomycin and spectinomycin from *in ovo* injection would need to be separately evaluated; it was logistically impossible to remove it from the present study. Indeed, this antimicrobial product was used as a standard procedure by the hatcheries to prevent early mortalities caused by *Escherichia coli* septicemia and omphalitis. Our results contrast with those of a previous study in a similar context, where RWA broiler chicken flocks showed significantly decreased zootechnical performances compared with conventionally raised flocks (Gaucher et al., 2015). The

major difference between the 2 studies was the use of a live coccidial vaccine for the prevention of coccidiosis in the RWA flocks from Gaucher et al. (2015), whereas the present study used ionophores, considered nonmedically important antibiotics (World Health Organization, 2017). No antibiotic growth promoters were used in the antibiotic-free or antibiotic-reduced flocks in both studies. However, preventive programs used in the present study are not consistent with Canadian RWA or US NAE standards because ionophores are antibiotics. Compared with the study by Gaucher et al. (2015), it can be hypothesized that ionophores have a critical role in the maintenance of zootechnical performance. More specifically, the results of this study suggest the shuttle programs using antibiotic growth promoters for NE prevention provide similar zootechnical performances to

programs relying exclusively on ionophores. This could also represent an economic advantage to the industry by decreasing antibiotic use.

The advantages of using antibiotics as growth promoters have been extensively studied and reviewed (Jones and Ricke, 2003; Dibner and Richards, 2005). Although the exact mechanism of action still needs to be elucidated, the administration of subtherapeutic doses of antibiotics in chickens' diet is believed to alter the intestinal microbiota to improve feed efficiency (Broom, 2017). Because various antibiotics will have different effects on the bacterial membership in the ceca (Costa et al., 2017), the exact microbiota composition that leads to increased performances with the use of antibiotic growth promoters is still unclear. More investigations would be needed to clarify the influence of the chickens' intestinal microbiota on growth performances in chickens as dissimilar intestinal microbiota compositions can result in similar growth performances (Stanley et al., 2016).

Numerous prebiotic products, for instance essential oils and organic acids, have been reviewed as alternatives to antibiotic growth promoters in broiler chicken production owing to their positive effect on growth performances (Ducatelle et al., 2015; Zeng et al., 2015; Khan and Iqbal, 2016). For example, butyric acids were shown to improve zootechnical performances such as BW gain and FCR without a disease challenge (Kaczmarek et al., 2016; Bortoluzzi et al., 2017). Although not completely understood, the growth-promoting mechanism of these products is thought to be related to the specific changes induced in the intestinal microbiota composition and function (Ducatelle et al., 2015). For instance, the cecal diversity was shown to be significantly impacted by the use of sodium butyrates, which could be associated with a decreased relative abundance of Lactobacillaceae (Zou et al., 2019). In a study by Bortoluzzi et al. (2017), the altered microbiota composition by butyrates showed improved carbohydrate and lipid pathways as analyzed by predicted functional composition (PICRUSt analysis from 16S rRNA sequencing). However, butyrates seems to be less effective when facing a disease, as shown in studies evaluating the efficacy of butyrates to improve growth performances with a NE challenge (Liu et al., 2017, 2019). In the present study, clinical enteric diseases were absent at the flock level based on the postmortem intestinal evaluations at 21 and 28 D of age in the 3 groups, where no NE lesions and low coccidiosis scores were recorded. Based on the aforementioned studies, it would have been expected to observe improved growth performances in the antibiotic-reduced flocks receiving butyric acids compared with the antibiotic-reduced flocks without these organic acids. However, no beneficial effect was observed; zootechnical performances were similar between the 2 groups. From our results, it could be assumed that butyric acids would not be required in broiler chicken flocks receiving exclusively ionophores in their prevention program.

The antibiotic-reduction strategies were associated with a higher excretion of Eimeria spp. oocysts after 22 D of age than the conventional strategy. Indeed, the statistical model showed constantly higher OPG counts through the different time points than the conventional flocks. The peak of excretion, which is thought to be related to the immunization of the flocks against coccidiosis (Chapman et al., 2016), occurred at 28 D of age in all treatments. This observation was also reported from previous studies, where the quantity of oocysts in the feces or litter is maximal around 4 wk of age when using anticoccidials (Chapman et al., 2016; Parent et al., 2018). However, it has also been reported that OPG in the litter of flocks treated with anticoccidial drugs were not seen until 34 D of age (Williams and Gobbi, 2002). This observation might have been explained by the environment because the studies from Chapman et al. (2016) and Parent et al. (2018) took place in commercial farms, whereas the study from Williams and Gobbi (2002) was conducted in a research facility. Indeed, a commercial environment is known to harbor viable Eimeria spp. oocysts at placement (Jenkins et al., 2019), which may contribute to an earlier infection compared with a clean environment such as a research facility. However, the higher excretion of oocysts in the fecal content is most likely not affecting the flocks, as zootechnical performances and postmortem examinations were not affected by the antibiotic-reduction treatments.

The reduction and elimination of antibiotic use in poultry production has often been associated with health disorders. For example, a large US poultry company experienced important technicoeconomical losses associated with the transition to a drug-free program (Smith, 2011). These problems associated with NE were also reported in another study, where 27.45% of the RWA flocks experienced outbreaks of clinical NE and 49.02% of these flocks were affected by subclinical NE, which was significantly different from conventional flocks using antibiotics as none of these flocks experienced issues with clinical or subclinical NE (Gaucher et al., 2015). In contrast, none of these conditions were observed during the present study. This observation can be supported by the absence of generalized clinical signs of enteritis in the 3 groups but also by normal mortality rates, standard growth rates, and low intestinal lesion scores. In a US study comparing NAE (no antibiotic), antibiotic-reduced (use of nonmedically important antibiotic), and conventional (use of medically important antibiotics) broiler chicken flocks in a commercial context, NAE flocks showed higher odds of eyes burns (i.e., corneal erosion or ulceration), footpad lesions and airsacculitis than the 2 other groups using antibiotics (Karavolias et al., 2018). When flocks receiving nonmedically important antibiotics were compared with flocks receiving medically important antibiotics, only footpad lesions showed slightly higher odds, whereas eyes burns had similar odds and airsacculitis lesions had lower odds. Similarly to the results from the study by Karavolias et al. (2018), there were no significant differences in the incidence of eye burns between flocks using nonmedically and medically important antibiotics. The slightly higher odds of having footpad lesions was

partially seen in our results; the antibiotic-reduced group receiving butyrates showed higher pododermatitis mean scores at 28 D of age. This condition could be an indicator of an impaired gastrointestinal system because any disease that induce watery droppings and diarrhea can cause wet litter problems (Dunlop et al., 2016). The presence of wet litter can be an important factor contributing to pododermatitis (Tullo et al., 2017), thus the importance of monitoring foot pad lesions during grow out to assess the presence of intestinal disorders. However, the litter and fecal humidities in our study were similar between the 3 groups for the 3 time points recorded. Hence, this hypothesis would not be consistent with pododermatitis caused by intestinal diseases and wet litter in the group receiving butyrates. The slightly lower odds of identifying airsacculitis lesions in the flocks receiving nonmedically important antibiotics than the flocks receiving medically important antibiotics from Karavolias et al. (2018) was not observed in our study. Bacterial airsacculitis lesions being mostly related to an infection by the gram-negative bacterium *E. coli* (El-Sukhon et al., 2002), the prevention programs for coccidiosis and NE are most likely not influencing this condition because most antibiotics in these programs are active against gram-positive bacteria (Agunos et al., 2017). Bursal diameter is a variable rarely evaluated in studies evaluating the impact of antibiotic use on performance and health even if its size can be related to immunosuppression caused by the chicken infectious anemia virus (Haridy et al., 2012), infectious bursal disease virus (Withers et al., 2005), Marek's disease virus (Chang et al., 2011), reovirus (Wang et al., 2007), or mycotoxin contamination in the feed (Peng et al., 2014), all potentially decreasing bursal size and impacting health and performance. At 21 D of age, bursal diameter was similar between the 3 groups, but a statistical difference has been identified at 28 D of age between the group receiving ionophores only and the group receiving ionophores and butyrates. Although significant, the 0.23 cm difference is most likely marginal because of the absence of differences between the 2 groups on zootechnical performance or the severity of lesions evaluated in other systems.

This study presented a disease prevention program relying on the continuous use of ionophores as a potential replacement of current conventional shuttle programs using antibiotics medically important for human medicine. Discontinuing the use of medically important antibiotics is a crucial step to decrease the selective pressure on bacteria harboring resistance genes to antibiotics and decrease the likelihood of animal production transferring antibiotic-resistant bacteria to the human population. The results obtained during this study in a commercial context do not support the extensive use of medically important antibiotics in disease prevention programs of broiler chicken flocks. Indeed, zootechnical performance and control of intestinal diseases were shown to be similar between conventional shuttle programs using medically important antibiotics and antibiotic-reduction strategies using ionophores continuously. The addition of in-feed butyric acids as a replacement of antibiotic growth promoters did not result in zootechnical performance improvement or better health control compared with flocks receiving exclusively ionophores in their prevention program. In a "One-Health" perspective, this study provides to the broiler chicken industry a successful strategy to decrease the impacts of agriculture on AMR by using nonmedically important antibiotics in broiler chicken production.

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SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.psj.2020.05.037.

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