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Original Article

Meta-Analysis of Butyric Acid: a Performance-Enhancing Additive to Replace Antibiotics for Broiler Chickens

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ABSTRACT

Butyric acid has been studied and utilized intensively in broiler chicken production in order to substitute growth-promoting antibiotics. However, the comprehensive literature on this topic makes it difficult to understand overall results, since there is a noticeable number of studies with conflicting conclusions. Although several research studies have shown that butyric acid may increment broiler chicken's performance, several other studies show the opposite. This work aimed to organize information to understand, through a meta-analysis, whether butyric acid can be used as a performance-enhancing additive for broiler chickens, and whether it can replace growth-promoting antibiotics. It was clear from the results that the effect of butyric acid depends on the microbiological challenge that broilers receive. When broilers are raised without health challenge, butyric acid and even antibiotics do not influence growth performance. However, when broilers are challenged, butyric acid provided an increase in weight gain and a significant improvement in feed conversion, matching the results of antibiotics. We conclude that butyric acid improves broiler performance and can be used to replace growth-promoting antibiotics.

INTRODUCTION

For decades, antibiotics have been widely used in poultry feed as growth-promoting substances. However, the excessive and indiscriminate use of antimicrobials can lead to the emergence of antibiotic-resistant microorganisms in humans (Marshall & Levy, 2011). For similar reasons, the European Union opted to ban the use of antibiotics as growth promoters in broiler chickens feed in 2006. On the other hand, the absence of growth promoters can cause several challenges concerning enteritis due to the imbalance in intestinal microbiota (Huyghebaert *et al.*, 2011). However, this problem can be overcome with natural additives replacing antibiotics.

Organic acids are among the most studied natural additives, with butyric acid standing out for its antimicrobial potential and the number of studies carried out (Polycarpo *et al.*, 2017). Nonetheless, there are numerous results with inconsistent information on the effect of butyric acid on broiler performance. Some studies show that butyric acid can improve broiler performance (Song *et al.*, 2017; Jazi *et al.*, 2018; Saki *et al.*, 2018; Raza *et al.*, 2019), while others have not reported the same significant results (Sayrafi *et al.*, 2011; Barbieri *et al.*, 2015; Wu *et al.*, 2018; Araujo *et al.*, 2019; Makled *et al.*, 2019; Isroli *et al.*, 2020). We believe that the main explanation for these differences is the heterogeneity of conditions in which each experiment was carried out. For these situations, meta-analysis is a powerful tool, as it allows to quantitatively identify some factors that influence the results of the primary research studies (St-Pierre, 2001, 2007).

According to Sauvant *et al.* (2005, 2008), meta-analysis integrates different variables to establish systematic responses adjusted to the diversity of publications. Therefore, the use of meta-analysis refers to the transformation of research results into applicable knowledge, as it considers heterogeneity among studies in a systematic way, while a single experiment reflects only the experimental conditions under which it was carried out (Lovatto *et al.*, 2007).

Thus, we aimed to evaluate through a meta-analytical study the effect of butyric acid as a performance-enhancing additive as an alternative to antibiotics for broilers chickens, as well as to identify and quantify the main factors that interfere in the results.

MATERIAL AND METHODS

Search and Data Filtering

To perform the meta-analysis, the search in digital media for studies included scientific articles published in specialized journals in the Scopus database. As a result of the advanced genetic improvement of poultry and its constant evolution, we limited the search between 2015 and December 2019. Three main keywords (broiler, butyric acid and performance) and their synonyms were inserted in the search strategy: ((TITLE-ABS-KEY (broiler*) OR TITLE-ABS-KEY (chick*)) AND DOCTYPE (ar) AND PUBYEAR > 2014) AND ((TITLE-ABS-KEY ("butyric acid") OR TITLE-ABS-KEY ("butanoic acid") OR TITLE-ABS-KEY (butyrate)) AND DOCTYPE (ar) AND PUBYEAR > 2014) AND ((ALL (performance) OR ALL ("body weight") OR ALL (bw) OR ALL ("average daily gain") OR ALL (adg) OR ALL ("weight gain") OR ALL ("average daily feed intake") OR ALL (adfi) OR ALL ("feed intake") OR ALL ("feed consumption") OR ALL ("feed conversion") OR ALL ("feed to gain") OR ALL ("feed efficiency") OR ALL ("gain to feed") OR ALL (mortality) OR ALL (viability)) AND DOCTYPE (ar) AND PUBYEAR > 2014). The use of synonyms expands the scope of the search for articles that will comprise the dataset. Three selection criteria were created: 1) *in vivo* experiments with broilers, 2) broilers supplemented with butyric acid, and 3) presenting zootechnical performance data (weight gain, feed intake, feed conversion, or viability). Once identified, the selected studies were subjected to a filtering process to control and ensure the quality of each publication.

After searching Scopus, 188 articles were identified, 142 of which were disregarded because they did not meet the pre-established selection criteria, resulting in 46 remaining articles. During the eligibility process, 12 articles were excluded according to the criteria: nine articles were eliminated due to the administration of antibiotics or chemotherapy in the diets, whether via inclusion as an ingredient in the feed or premix, since that may interfere with the effect of butyric acid; cases where antibiotics were used as a treatment (positive control) were still considered; one article was excluded because butyric acid was associated in the same treatment with a phytogenic additive, making it impossible to identify what was the contribution of butyric acid within the treatment (two sources of variation); and two articles were excluded for presenting performance data in graphs, preventing data collection (absence of numbers). After subtracting the 12 articles, 34 articles were inserted into the dataset, adding up to a total of 36 experiments available to perform the meta-analysis. Thirty-six (36) experiments were used to evaluate the effect of butyric acid, *i.e.*, control treatment *versus* treatment supplemented with butyric acid (trial 1). From the 36 experiments, a subgroup of 10 experiments was selected, which also contained a positive control treatment containing antibiotics to evaluate the effect of butyric acid as an alternative to growth promoters (trial 2). Experiments with antibiotics were evaluated separately, dividing the analysis into two trials. The objective was preventing a sharp imbalance between the number of observations and, consequently, in the degrees of freedom between treatments, in order to avoid inconsistencies during the comparison tests of adjusted means. In figure 1, the process was summarized according to the suggestions of the PRISMA Group (Moher *et al.*, 2009).

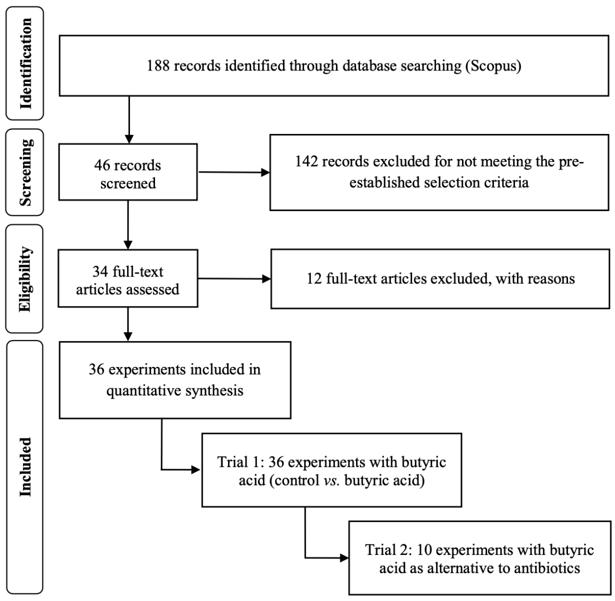

Figure 1 – Four-phase flow diagram of the systematic review process of the meta-analysis (Moher *et al.*, 2009).

Data Systematization and Coding

All the information available in the selected articles that could be applied to the goals of the meta-analysis was critically explored, extracted, and recorded in an online electronic spreadsheet. The treatments were arranged in the rows, and the exploratory variables in the columns. Each experiment inserted in the dataset was coded with a sequential number to identify the effect of each experiment (inter-experimental effect).

The groups with responses measured repeatedly over time were coded to observe intra-experiment effects. Some codifications were used to group common characteristics, that is, to join homogeneous groups that were included in the statistical models as a source of variation. The main codes used to classify the groups of additives were: 1- control (without additive), 2-butyric acid, and 3-antibiotics. The microbiological challenge, which was another important factor explored in the analysis, received the following code: 1- absence of challenge (chickens not inoculated with pathogens, -) and 2- presence of challenge (chickens inoculated with pathogens, +).

Dataset Description

The data were organized in a spreadsheet of 378 rows and 72 columns consisting of 34 articles (36 experiments) published between early 2015 and December 2019 (mode = 2016). The experiments included in the dataset totaled 13,218 broilers, with an average of 367 per experiment (mode = 120) and 95 per treatment (mode = 60). The average duration of each evaluation period was 23 days, with a maximum and minimum of 42 and 7 days, respectively. The initial average age of the chickens was 10 days (range between 1 and 36 days) and the final average age was 32 days (range between 11 and 49 days). The facilities used for the development of the experiments were as follows: 17% conducted on the floor, 22% in cages, and 61% did not describe this information. Only 17% of the experiments involved chickens inoculated with some type of microbiological challenge. The relative frequency of the (genetic) strains used was: 50% Ross, 14% Cobb, 11% Arbor Acres, 9% Hubbard, 3% other strains, and 8% of the experiments did not describe this information. With regard to the sex of chickens, 8% were mixed (females and males subjected to the same treatments), 69% were male, 3% were females, and 20% did not specify this information. While 58% of the experiments contained diets with a low fiber content, 31% used ingredients rich in non-starch polysaccharides (PNA's), and 11% did not describe this information. The averages of the nutritional values of the diets were: 3,077.1 kcal / kg of metabolizable energy, 20.2% of crude protein, 1.15% of digestible lysine, 0.86% of digestible methionine + cystine, 3.50% of fiber, 0.91% calcium, 0.71% total phosphorus, 0.43% available phosphorus, 10.32mg of copper / kg diet, and 73.85 mg of zinc / kg diet. The forms of butyric acid found in the experiments that made up the dataset were: free (pure butyric acid), calcium butyrate, sodium butyrate, tributyrin, DL-2-hydroxy- (4-methylthio) butanoic acid (DL-HMTBA) and β-hydroxy-β-methylbutyrate (DL-HMB). Butyric acid was used with some form of protection in 36% of the experiments. The antibiotics present in the dataset as a positive control were: aureomycin, avilamycin, bacitracin, enrofloxacin, maduramycin, salinomycin, and sulfamethoxazole.

Data Analysis

The data analysis was carried out using the software SAS University Edition, version 9.4 (SAS, 2019), considering 5% or less of error as significant probability value.

Graphical Analysis, Correlations, and Residual Variations

Graphical analysis was used to assess the structure of the general distribution of the data and, thus, to identify the heterogeneity of the information making up the dataset. Based on the visual assessment of different types of graphs, hypotheses were established that contributed to the choice of statistical model. As suggested by Sauvant *et al.* (2005, 2008), inter and intra-experimental relationships were assessed. Correlation hypotheses (PROC CORR) were tested to investigate relationships between variables that influenced performance results and, thus, identify and consider these factors in the adjustments of the models. The normality of the residues was analyzed using the UNIVARIATE procedure. Studentized deviations greater than 3 and less than -3 were considered outliers (Lovatto *et al.*, 2007).

Variance-Covariance Analyses

In all analyzes of variance-covariance, the effect of the experiment was inserted into the model as a random-effect class variable (St-pierre, 2001), due to differences in experimental conditions between researches (inter-experimental effect). In this context, MIXED procedure was used to perform mixed models as proposed by St-Pierre (2007). The effect of the additives (groups: control, butyric acid or antibiotics) and the microbiological challenge (presence or absence), as well as the interaction between them, were considered as fixed effect factors. The data were obtained at different ages of broiler rearing and the average age (average between the initial and final ages of each evaluation) was therefore inserted in the model as a fixed effect covariate (except for VB). The quadratic effect of average age was used when significant. Additionally, in order to adjust the data of average daily weight gain (ADG) and average daily feed intake (ADFI) from trial 1, the country effect (where the experiment was carried out) was also used as a fixed-effect class variable. The Tukey-Kramer test was used to compare least square means. The response variables analyzed were: ADG, ADFI, feed conversion ratio (FCR), and viability (VB).

The variation of ADG and ADFI of butyric acid or antibiotics in relation to the control group was calculated and expressed as a percentage: ΔADG and ΔADFI, respectively [*i.e.*, ΔADG = (((ADG of the additive × 100) / ADG of the control) - 100)]. The relationship between ΔADG and ΔADFI was studied and adjusted in a linear relationship, as shown below:

$$\Delta ADGij = \beta_0 + \beta_1 \times \Delta ADFIij + Si + bi \times \Delta ADFIij + eij$$

Interpretation of the fixed effects above: the general intercept (β_0) shows the variation in weight gain caused by supplementation of the additive (butyric acid or antibiotic) when the variation in consumption is equal to zero, and can be interpreted as a maintenance indicator. The slope [slope (β_1)] indicates the extent to whichΔADG change is associated with ΔADFI in broilers fed with butyric acid or antibiotics. The significance of the fixed effect parameters of the regression was assessed by the t test. The parameters making up the random effects part are: S_i = effect of the ith experiment, assuming $\sim_{iid}N(0, \sigma^2_s)$, b_i = effect of the experiment on the regression coefficient, assumed $\sim_{iid}N(0, \sigma^2_b)$ and e_{ij} = residual errors, assumed $\sim_{iid}N(0, \sigma^2_\varepsilon)$. S_i, b_i, and e_{ij} were considered as independent random variables.

RESULTS AND DISCUSSION

Several factors were considered in the elaboration of meta-models. Researchers report that the effectiveness of organic acids is influenced by dietary components (Dibner & Buttin, 2002; Kim *et al.*, 2015); however, we did not find a significant effect of ingredients in the models, not even of antimicrobial ingredients (copper and zinc) or those with buffering capacity (phosphate and bicarbonate). Other factors such as genetics, sex, facilities (floor or cage), and material used as litter did not influence the results. The predominant factor with great influence on the results of butyric acid was the microbiological challenge, which is discussed below in the presentation of the performance results.

Trial 1: Control versus Butyric Acid

It was found that the interaction between butyric acid and the microbiological challenge was significant on ADG (Figure 2a., *p*=0.046) and FCR (figure 2c., *p*=0.014). In the absence of the microbiological challenge, the performance variables were not influenced by the supplementation of butyric acid (*p*>0.05). On the other hand, under microbiological challenge, broilers fed with butyric acid showed an increase of 13.4% in ADG (*p*=0.018) and an improvement of 18.3% in FCR (*p*=0.006). The evaluated factors did not influence ADFI (figure 2b.) and VB (figure 2d.) (*p*>0.05).

The interaction of butyric acid with the microbiological challenge showed that the efficiency of butyric acid as a growth promoter is associated with the presence of pathogenic bacteria. This is an important premise that is very evident in our work. However, most researchers who design experiments to evaluate antimicrobial additives ignore the issue of the health challenge, leading to mistaken and contradictory results that occur due to this methodological flaw. Some recent research studies have not observed the effect of butyric acid on broiler performance (*e.g.*: Wu *et al.*, 2018; Makled *et al.*, 2019; Gonzáles-Ortiz *et al.*, 2019; Isroli *et al.*, 2020), but the question is: did these results occur due to the absence of butyric acid effect as a performance-enhancing additive or due to the lack of microbial challenge?

Our results demonstrate the potential of butyric acid in promoting the performance of broiler chickens. Butyric acid has an antimicrobial effect related to the reduction of pH by its ability to release hydrogen ions from its carboxylates (Cherrington *et al.*, 1991). The non-dissociated form of the acid crosses the bacterial cell membrane by reducing the intracellular pH, inhibiting its growth. In this way, the acid causes the loss of bacterial cellular energy and interrupts specific metabolic functions such as replication and protein synthesis. Some studies have demonstrated the efficiency of butyric acid as an antimicrobial additive against pathogens that cause major losses in poultry production, such as *Eimeria* spp. (Ali *et al.*, 2014; Song *et al.*, 2017), *Salmonella* (Van Immerseel *etal.*, 2004, 2005; Fernandez-Rubio *et al.*, 2009; Menconi *et al.*, 2013; Cerisuelo *et al.*, 2014), and *Clostridium* (Timbermont *et al.*, 2010; Mohamed *et al.*, 2014).

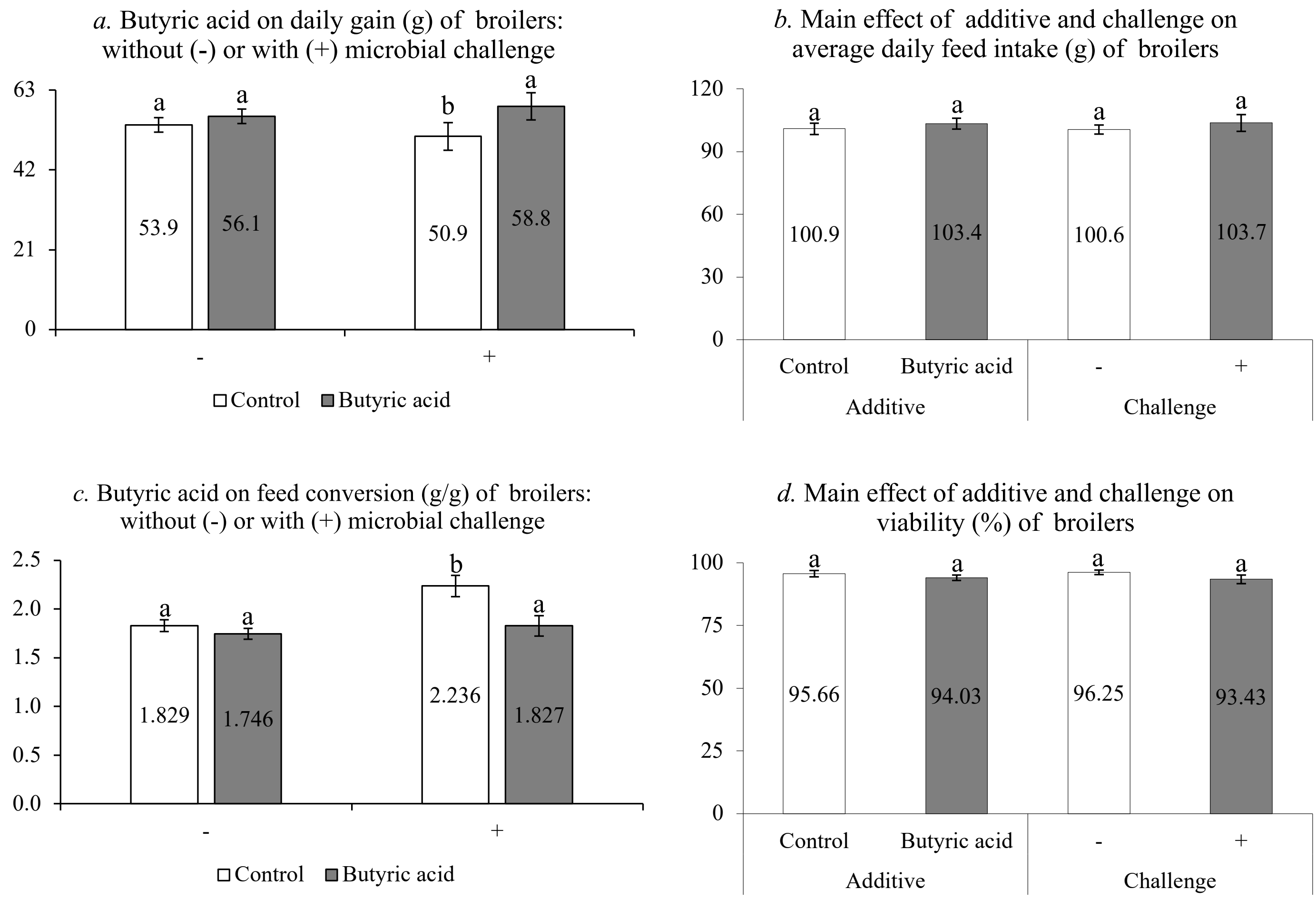

Figure 2 – Performance of broilers supplemented with butyric acid as a performance-enhancing additive.

The antimicrobial action of butyric acid ends up being positive for the intestinal mucosa, favoring the health of the epithelium. The dissociation of butyric acid in the intestine produces mucin glycoproteins (mainly MUC2) in the intestinal epithelium, creating a defense barrier in the colon mucosa that prevents the colonization by pathogenic bacteria (Barcelo *et al.*, 2000; Schauber *et al.*, 2003; Van Immerseel *et al.*, 2004). In this way, butyric acid favors the development of the intestinal mucosa, provides a greater absorptive area and, consequently, facilitates the absorption of nutrients with the prevention and regeneration of epithelial lesions. A recent review discusses in details the benefits of butyric acid in the development of the intestinal mucosa, improving intestinal integrity (Elnesr *et al.*, 2020). In addition, butyric acid can be used as an energy source by intestinal cells (Dalmasso *et al.*, 2008). After being metabolized in the liver, butyric acid is the main source of energy for the cells of the superficial layer of the intestine (enterocytes), necessary for the development of lymphatic tissue associated with the intestine (Friedman & Bar-shira, 2005). All of these effects combined can explain the best results obtained with butyric acid on ADG and on FC with microbiologically challenged broilers.

Trial 2: Butyric Acid as an Alternative to Antibiotics

Performance data

The analysis of butyric acid as an alternative to antibiotics showed again the interaction between the additive effect and microbiological challenge impacting ADG (figure 3a, p=0.042) and on CA (figure 3c, p=0.008). Another time, in the absence of the microbiological challenge, there was no effect of butyric acid on ADG and CA (p>0.05). However, broilers inoculated with microbiological challenge and supplemented with butyric acid or antibiotics showed an increase in comparison to the control group of 33.2% (p=0.039) and 36.5% (p=0.017) in the ADG, respectively.

It is important to highlight that there was no difference between butyric acid and antibiotics on ADG and FCR (p>0.05), confirming with strong evidence that this organic acid can be used as a replacement for growth-promoting antibiotics for broilers rearing with health challenge. There was no effect on ADFI (figure 3b, p>0.05). Due to the smaller number of experiments that comprised trial 2, no VB results were presented, since the data were "intractable" in the analysis.

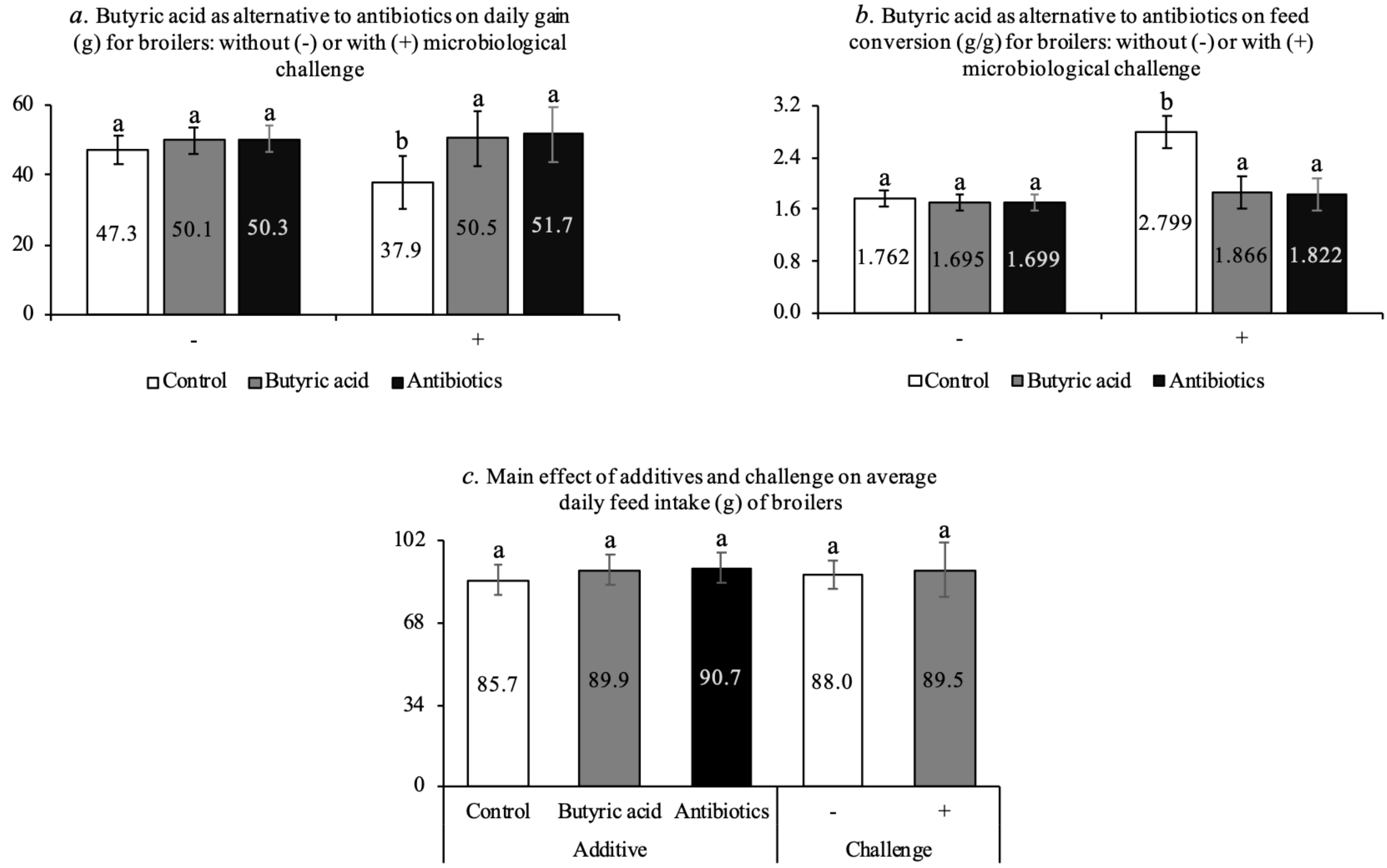

Figure 3 – Performance of broilers supplemented with butyric acid as an alternative to growth-promoting antibiotics.

The withdrawal of antibiotics as growth promoters is constantly associated with enteric diseases, which cause worsening of weight gain, feed conversion, mortality, among others problems. However, in the analysis carried out by Smith (2011), drug-free production programs with natural additives may overcome the challenge of removing antibiotics. Our results show that butyric acid can provide growth performance similar to that of antibiotics. The use of antimicrobial additives promotes a healthy balance of the intestinal microbiota by reducing the growth of undesirable microorganisms, such as *Eimeria* spp. (Abbas *et al.*, 2011; Galli *et al.*, 2020) and *Clostridium perfringens* (Granstad *et al.*, 2020), the infections of which are related to induction of necrotic enteritis with intestinal lesions, causing loss of weight gain (Lu *et al.*, 2020). Evidence from our work and the literature shows that natural additives such as butyric acid are promising as replacement of growth-promoting antibiotics; and that in the not too distant future, antibiotics will be used in broiler production only for therapeutic purposes, contributing to a more sustainable poultry industry in the long term.

Relationship between ΔADFI and ΔADG

The relationship between ΔADG and ΔADFI of butyric acid and antibiotics is presented in Figure 4. In the butyric acid equation, if ΔADFI is equal to zero, ΔADG will be the value of the intercept. In other words, with the same amount of nutrients consumed (ΔADFI = 0), broilers supplemented with butyric acid had more ADG [+ 3,02% (intercept value), p=0.002]. This is an interesting insight, as it allows for valuable physiological interpretation. We can interpret that body maintenance requirements are lower for broilers that receive butyric acid - less destination of nutrients for metabolism and, consequently, more nutrients for growth performance. The lower maintenance requirements may be due to the beneficial effects of butyric acid on gastrointestinal tract health of the, which have already been mentioned in the discussion of trial 1. Other reports mention that organic acids can provide higher metabolic rates (Abdel-Fattah *et al.*, 2008) and can stimulate energetic metabolism (Kim *et al.*, 2015). The intercept of the antibiotic equation was not significant (p>0.05), showing that antibiotics do not cause variation in ADG when ADFI is equal to the control group. We see that this may have occurred because there werea little data in the group of antibiotics that showed greater weight gain, but without higher consumption.

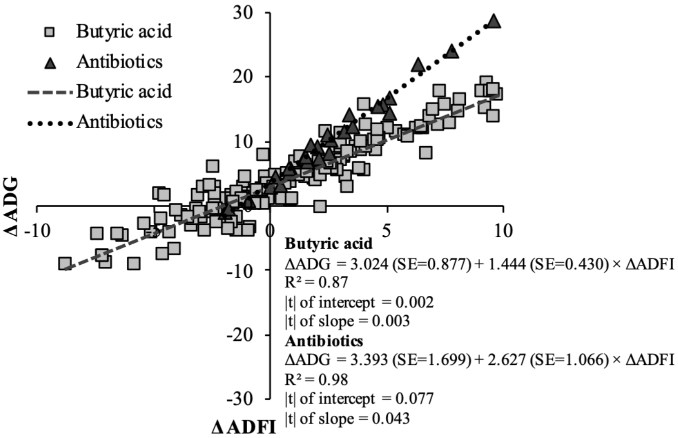

Figure 4 – Relationship between the variation in the average daily gain (ΔADG) in function of variation in average daily feed intake (ΔADFI) of broilers. The data were obtained in relation to control group (Δ). Observations were adjusted for the other variables in the model calculating the predicted value of ΔADG + residual, according to St-Pierre (2001).

The slope was significant for chickens fed butyric acid (p=0.003) and for those fed antibiotics (p=0.043). The slope coefficient demonstrates that for each 1% increase in ADFI as compared to control, ΔADG increased by 1.44% and 2.63% for butyric acid and antibiotics, respectively. That is, the stimulation of intake of supplemented feed enhances the effect of both additives. The rate of 1.44% greater gain with 1% more feed intake for butyric acid in relation to the control group is very interesting from a zootechnical point of view, because the relationship represents 0.69 of feed:gain efficiency, a FCR that clearly demonstrates the biological superiority caused by butyric acid in broiler physiology, as observed in the interpretation of the intercept.

Recently, Deepa *et al.* (2018) published a review paper on the use of butyric acid as an antibiotic substitute for broilers, listing some beneficial effects that are beyond the antimicrobial effect: a) immunity, b) positive effects on intestinal integrity, c) pH effect on gastrointestinal digestion, d) mineral absorption, e) anti-catabolic effect, f) antioxidant effect (catalase), among others. Thus, actions that stimulate an increase in the consumption of poultry feed supplemented with butyric acid tend to benefit broiler performance.

CONCLUSION

The microbiological challenge has a strong influence on the evaluation of butyric acid as a performance-enhancing additive, evidencing the importance of the health challenge for an adequate evaluation. In conditions with microbiological challenge, butyric acid improves ADG and FCR, promoting performance similar to antibiotics.

We conclude that butyric acid increase growth performance of broiler chickens and can replace growth-promoting antibiotics.

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