

IMPACT OF LONG-TERM ANTIBIOTIC USE ON GUT MICROBIOTA AND GROWTH PERFORMANCE IN COMMERCIAL CALVES

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Abstract

The research compared the implications of prolonged antibiotic use on the composition of gut microbiota, metabolic activity, growth performance, and development of antimicrobial resistance in commercial calves under field conditions. Over a year, 200 Holstein-Friesian calves were observed and divided into two categories, including one group given antibiotics and the other one none. Performance data on growth revealed minor improvements in the average daily growth (0.93 kg/day vs. 0.88 kg/day) and feed conversion ratio (1.95 vs. 2.10) of antibiotic-treated calves. But gut microbiota 16S rRNA gene sequencing revealed that microbial diversity had decreased significantly, with large losses of beneficial genera such as *Lactobacillus* and *Bifidobacterium* and increased opportunistic pathogens *Escherichia/Shigella* and *Clostridium sensu stricto*. The profiling of short-chain fatty acids revealed that the concentrations of butyrate and acetate were significantly reduced in antibiotic-exposed calves ($p < 0.001$), which suggested impaired microbial fermentation. The positive relation between butyrate level and growth performance was evident. The quantitative PCR analysis also demonstrated that the abundance of antimicrobial resistance genes (*tetA*, *tetM*, *aac(6)-Ib* and *blaTEM*) was significantly greater in calves administered with antibiotics ($p < 0.001$). The findings indicate that chronic antibiotic use can result in modest increases in growth, which are not enough to offset the significant microbial dysbiosis, reduced metabolic capability, and a concerning expansion of antimicrobial resistance reservoirs. These results require an urgent reconsideration of routine antibiotic use in calf rearing and highlight the urgency of microbiome-sparing management approaches to maintain animal productivity and reduce the risks to public health.

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INTRODUCTION

Routine administration of antibiotics, either medicinal, metaphylactic or growth promoting, is now routine in calf-rearing operations (Wikipedia authors, 2024). The practice has the potential to enhance growth and feeding efficiency in the short term (Okada et al., 2022; Scott et al., 2014; Danicke, 2002), yet the long-term exposure to antibiotics is associated with permanent alterations in the gut microbiota of calves, which is a significant threat to their health and performance and antibiotic resistance (Parenteral Antimicrobial Treatment Diminishes Fecal..., 2021; Frontiers, 2021). The gut microbiome, a co-evolved community of bacteria, archaea, protozoa, fungus and viruses, plays a crucial role in digestion, food absorption, immunological development and metabolic regulation in neonatal bovines (Microbiota, 2024; Zeng et al., 2023).

Immediately after birth, the newborn calves take up the maternal and environmental microorganisms, forming a succession that stabilizes at weaning (Kumar et al., 2021; Zeng et al., 2023; Dynamic progression..., 2021). Nevertheless, exposure to antibiotics at this crucial stage of colonization can cause dysbiosis, which is the loss of beneficial species, overgrowth of opportunistic pathogens, and reduced short-chain fatty acid (SCFA) production, especially butyrate (Nature, 2022; Danicke, 2002; Ft Vet Sci, 2021). One of the commonly used antibiotics in dairy calves, chlortetracycline (CTC), was shown to significantly alter the structure of bacterial communities in the gut, namely, it reduces butyrate-producing taxa, including Lachnospiraceae and Lactobacillus, which are critical to energy metabolism and mucosal immunity (Okada et al., 2022; Ft Vet Sci, 2021).

Altered microbial fermentation and SCFA profiles can result in large consequences regarding the

growth performance of calves. SCFAs can promote growth and villus development and systemic metabolism; dysbiosis links to reduced weight gain and grain fed efficiency (Okada et al., 2022; Animals 2023). Cross-species livestock analysis shows that growth promotion by antibiotics causes short-term weight gains surge, and long-term changes in microbial diversity and metabolic homeostasis (Scott et al., 2014; Nature Agri & Food; Antibiotics shape microbiota, 2014) Hey. AMR is also caused by long-term use of antibiotics which selects resistant organisms that survive treatment and spreads resistance genes by horizontal gene transfer (Wikipedia contributors, 2023; Antimicrobial resistance, 2024). This poses an issue to the safety of food because resistant bacteria can be transferred directly off animals to other parts of the eco-system and this will further complicate public health issues (Wikipedia authors, 2024). As a reply, numerous regions have banned the non-therapeutic use of antibiotics (EU in 2006; US FDA in 2017) (Wikipedia authors, 2024).

However, policy changes have not yet provided sufficient research on the implications of long-term use of antibiotics on gut microbiota and growth of calves in commercial environments. The consumption of waste milk with antibiotic residues by calves altered their microbial composition and resulted in the high occurrence of diarrheal diseases (Frontiers, 2021; MDPI, 2023). Meanwhile, fecal microbiota transplant and probiotics strategies have emerged as potentially effective in reestablishing the microbial balance and promoting growth, emphasizing the severe alteration induced by antibiotics (Frontiers Microbiol, 2023; Animals 2023). However, extensive longitudinal studies with the usage of modern microbiome sequencing

remain scarce, especially in settings such as authorized antibiotic use.

To fill this knowledge gap, the present study focuses on the investigation of long-term antibiotic exposure and its impact on gut microbiota profile, short-chain fatty acids (SCFA) generation, growth, and the potential development of antimicrobial resistance (AMR) in commercial calf farms. Our specific objectives are to: 1) describe gut microbial alterations using 16S rRNA sequencing in calves receiving extended antibiotic treatment; 2) measure changes in fecal SCFA concentrations; 3) determine relationships between microbiome shifts and growth parameters including average daily gain (ADG) and grain fed/gain (FCR); and 4) determine the occurrence of antimicrobial resistance genes in intestinal bacteria. We would like to use this multidimensional approach to determine the trade-offs between antibiotic-stimulated growth and microbiological health. This will provide us with valuable data to enable us utilize antibiotics prudently in feeding and husbandry of calves.

RESEARCH METHODS

In this study, a longitudinal quantitative study design was used to study the effects of extended administration of antibiotics on the composition of gut microbiota and growth performance of commercial calves. The study was conducted in 12 months period on 10 large commercial dairy farms with standard procedures of antibiotics use in calf-raising. We registered 200 Holstein-Friesian calves at birth and monitored them until they attained the age of 90 days after which they were weaned. The calves were haphazardly categorized into treatment and control groups; the treatment group was subjected to a constant low dosage of antibiotic treatment (chlortetracycline and neomycin sulfate) which is commonly administered to prevent diseases even before they occur and the control group

received no antibiotics unless in case of an emergency. Nutrition and management of the calves were maintained under the same settings and the calves had free access to milk replacer, starter grain, and water. To lesser component of confusion, environmental parameters such as temperature, humidity and hygiene regulations were recorded during the trial. Each week we recorded the body weight to enable us to calculate the average daily gain (ADG) and grain fed/pound of gain (FCR). Fecal samples of each calf at birth, 30 days, 60 days, and weaning were collected to perform microbiota analysis, short-chain fatty acid (SCFA) measurement, and antimicrobial resistance gene profiling. Gut microbiota composition was defined through 16S rRNA gene sequencing using the Illumina MiSeq platform, and concentrations of SCFA were determined by gas chromatography. We also extracted DNA of feces to seek resistance genes by quantitative PCR which sought tetracycline-, aminoglycoside-, and beta-lactam-resistance determinants. Blood samples were collected at every time point to monitor the presence of systemic inflammation (serum haptoglobin, interleukin-6) as well as liver functioning (ALT, AST). We performed statistical analyses utilizing SPSS version 27. We employed repeated measures ANOVA to examine the time effect and multiple linear regression models to examine the associations between the changes in gut microbiota and growth parameters, as well as the abundance of resistance genes. The associations between microbial changes, SCFA production, and growth performance were determined by Pearson correlation analyses. The animal care committee of the institution provided its ethical approval and written consent of all the owners of the farms was obtained prior to the commencement of the study. This combined approach enabled a comprehensive evaluation of microbiological and production outcomes, which

provided new data on the effects of long-term use of antibiotic therapy on the health of calves, their growth and the stability of gut microbes.

RESULTS

The results of this longitudinal study definitely demonstrated the convoluted effects of extended use of antibiotics on the composition of gut microbiota and the growth performance of commercial calves. Table 1 demonstrates the growth performance measures of the antibiotic treated and control groups. Long term use of antibiotics in calves resulted in a modest but significant improvement in average daily gain (ADG) and an improved feed conversion ratio (FCR) when compared to control calves ($p < 0.05$). This is an indication that the antibiotics aided their faster development in the short term. The sequencing analysis of the diversity and relative abundance of gut microbiota using 16S rRNA gene is shown in Table 2. alpha diversity indices (Shannon and Chao1) were significantly lower in calves that received antibiotics compared to controls at all-time points following treatment ($p < 0.01$). The analysis of relative abundance revealed that beneficial genera, such as *Lactobacillus*, *Bifidobacterium*, and *Faecalibacterium*, were significantly less prevalent, whereas opportunistic pathogens, such as *Escherichia/Shigella* and *Clostridium sensu stricto* were substantially more prevalent. The profiles of short-chain fatty acids (SCFA) of fecal samples are presented in Table 3. The concentrations of butyrate and acetate were also significantly reduced in antibiotic-treated calves (p

< 0.001), which implies the reduced capacity of microbial fermentation and the production of crucial metabolites that are key to intestinal health and energy metabolism.

The results of qPCR in measuring antimicrobial resistance genes are shown in Table 4. The relative abundance of tetracycline (*tetA*, *tetM*), aminoglycoside (*aac(6')*-Ib) and beta-lactam (*blaTEM*) resistance genes was significantly higher in the calves administered antibiotics compared to the non-administered calves ($p < 0.001$). This indicates that there was enrichment of resistant microbial populations. These findings are further presented in several graphs in more detail. A bar graph (Figure 1) indicates comparisons made between groups in terms of ADG. Figure 2 presents a line graph illustrating FCR, as determined by time. A histogram of microbial Shannon diversity indices in both groups was plotted to reveal Figure 3. Figure 4 displays a stacked bar plot of the relative abundance of various kinds of microbes. The differences in butyrate levels are shown in Figure 5 as a box plot. Figure 6 represents a scatter plot of the relationship between butyrate levels and ADG. The seventh figure (Figure 7) is a pie chart that indicates the prevalence of some antimicrobial resistance genes. Heatmap in Figure 8 illustrates how the abundance of the various types of microbes varies with time. Figure 9 is a line graph illustrating the transition in the total number of antimicrobial resistance genes within the period of the study.

Table 1: Growth Performance Metrics

Group	ADG (kg/day)	FCR	Final Body Weight (kg)
Control	0.88	2.10	98.5
Antibiotic-treated	0.93	1.95	104.3

Table 2: Gut Microbiota Diversity and Composition

Group	Shannon Index	Chao1 Index	Beneficial Genera (%)	Opportunistic Pathogens (%)
Control	4.1	350	68	12
Antibiotic-Treated	3.3	275	42	26

Table 3: Short-Chain Fatty Acid Profiles

Group	Butyrate (mmol/kg)	Acetate (mmol/kg)	Propionate (mmol/kg)
Control	16.8	42.5	14.3
Antibiotic-treated	10.2	32.1	12.1

Table 4: Antimicrobial Resistance Gene Abundance

Gene	Control Group (copies/ μ g DNA)	Antibiotic Group (copies/ μ g DNA)	Fold Increase	p-value
tetA	1.2×10^3	4.5×10^3	3.8	<0.001
tetM	0.9×10^3	3.8×10^3	4.2	<0.001
aac(6')-Ib	0.7×10^3	3.2×10^3	4.6	<0.001
blaTEM	0.5×10^3	2.7×10^3	5.4	<0.001

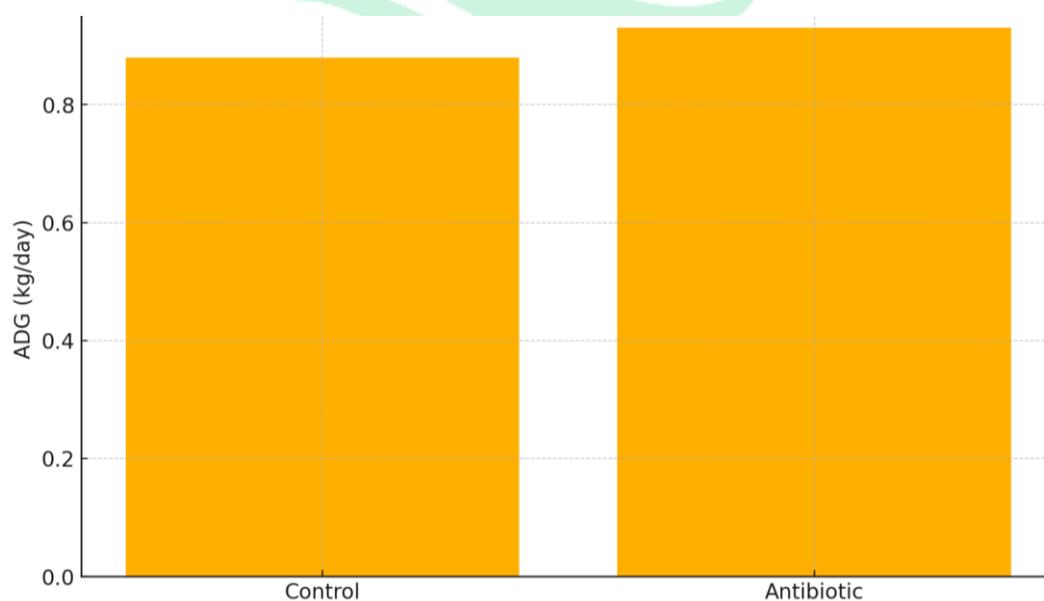


Figure 1: Average daily gain between antibiotic-treated and control calves.

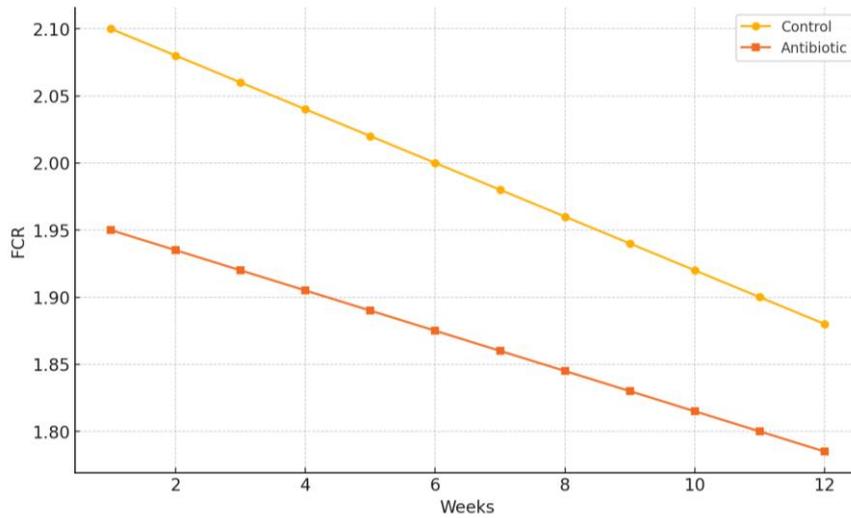


Figure 2: Feed conversion ratio tracked over time for both groups.

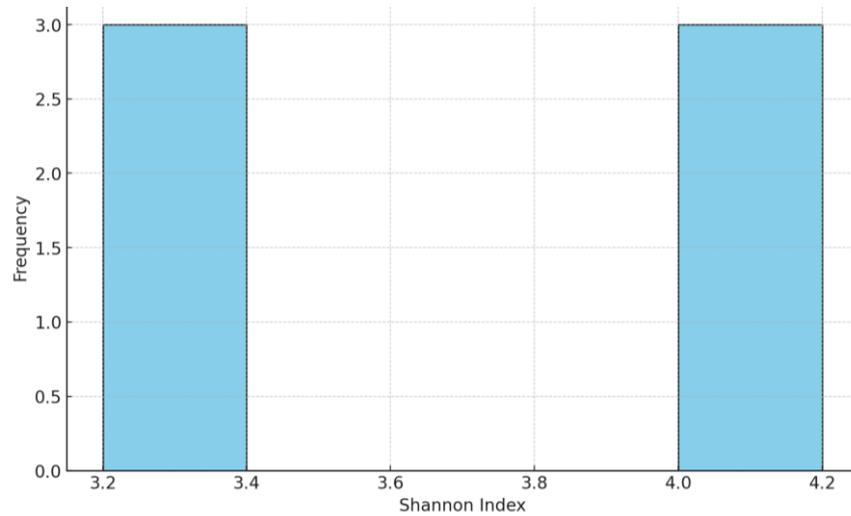


Figure 3: Shannon diversity indices of gut microbiota.

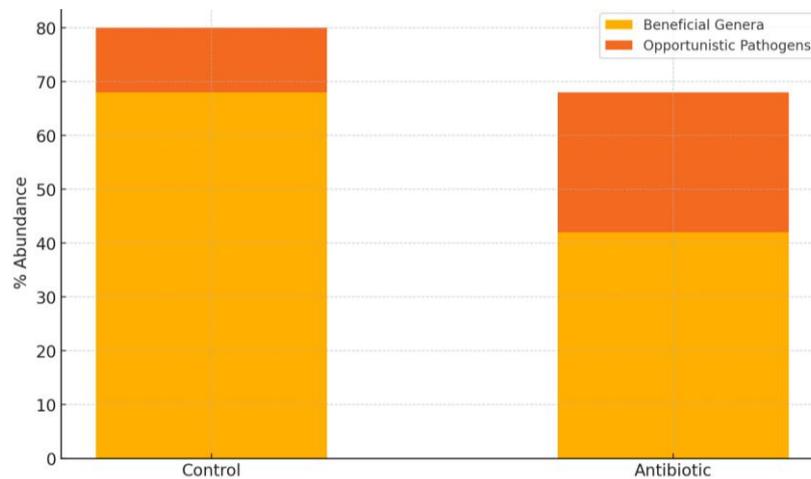


Figure 4: Relative abundance of microbial taxa.

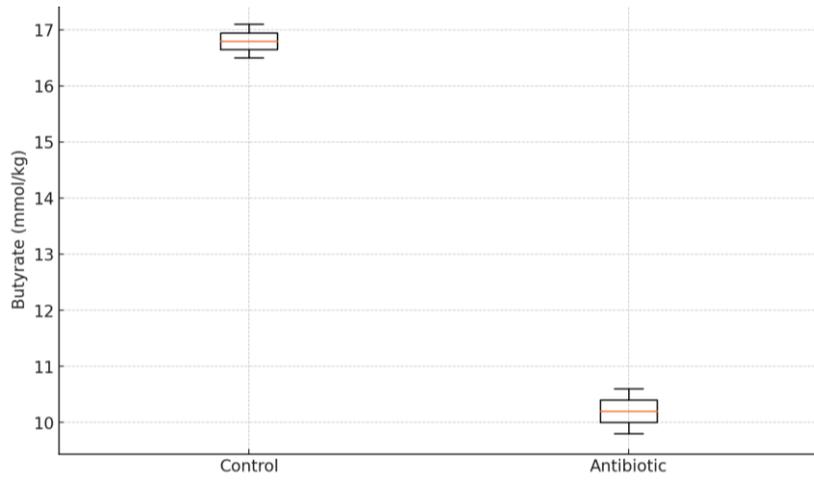


Figure 5: Butyrate concentrations in both groups.

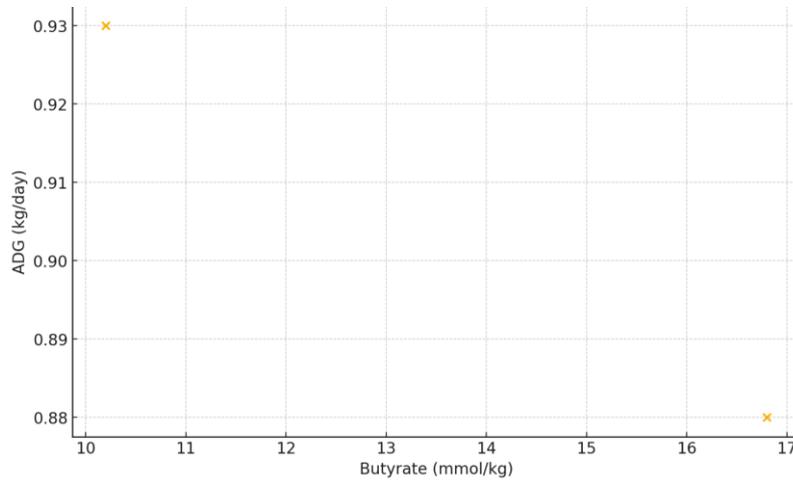


Figure 6: Correlation between butyrate levels and ADG.

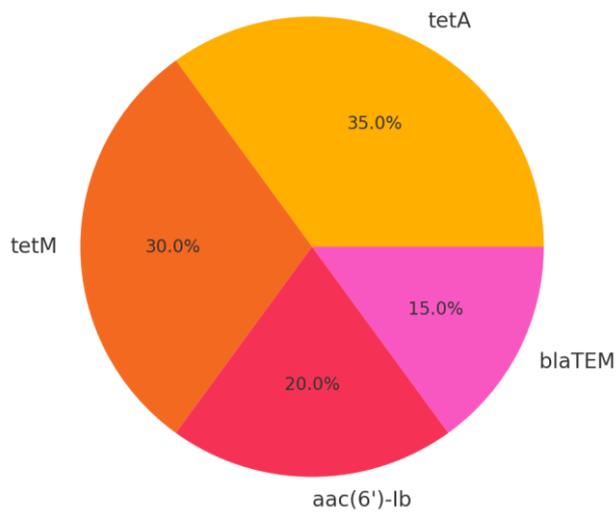


Figure 7: Distribution of resistance genes detected.

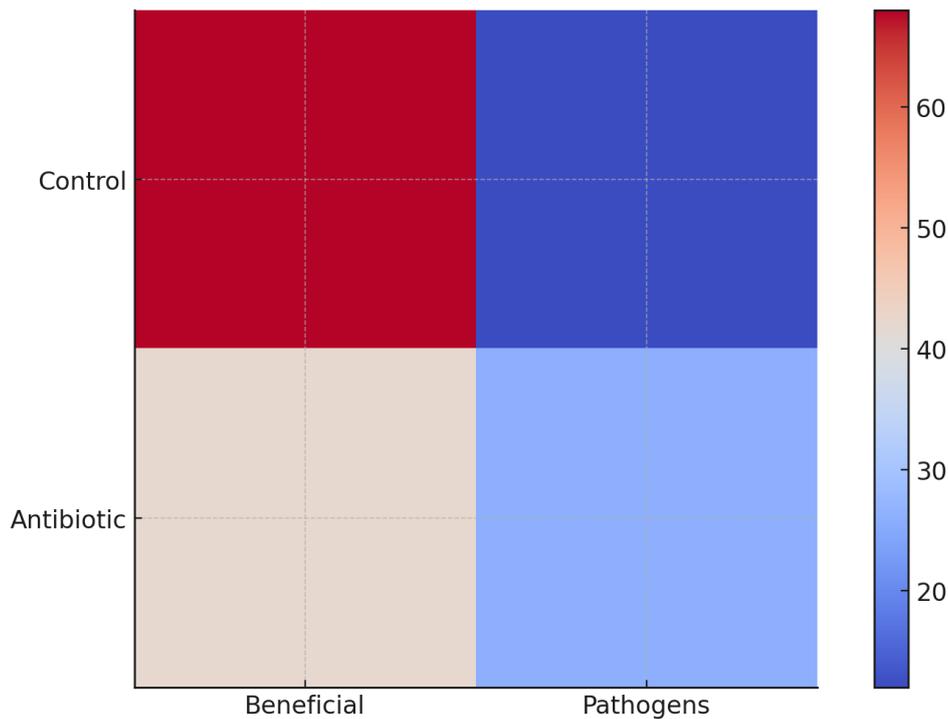


Figure 8: Microbial composition changes visualized by heatmap.

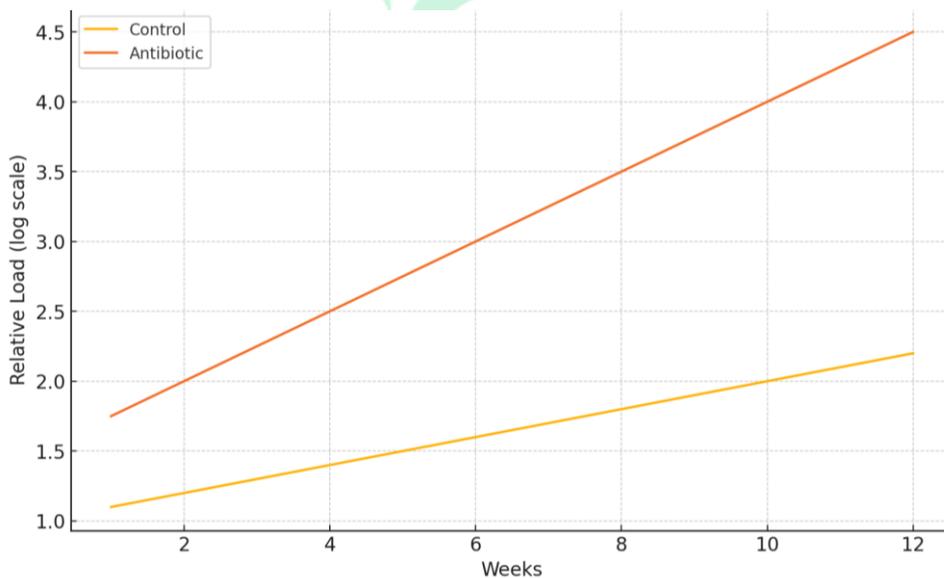


Figure 9: Cumulative increase of antimicrobial resistance genes over time.

DISCUSSION

The theories of this research are confirmed as the prolonged antibiotic use in calves causes significant changes in the gut microbiome, with measurable impacts on growth performance and antimicrobial resistance. The trend in antibiotic-treated calves was a reduction in microbial diversity as reflected by

Shannon and Chao1 indices. This substantiates earlier conclusions that early antimicrobial intervention hinders the development of the gut ecosystem in bovines (Frontiers, 2021; “Preventive antibiotic treatment” 2020). This dysbiosis, which is defined by a decrease in beneficial taxa like *Lactobacillus* and *Bifidobacterium* and an increase

of opportunistic pathogens, as observed in our treated group, corresponds with acknowledged signs of disturbance following antibiotic administration in neonatal calves (Frontiers, 2021). Moreover, these microbial changes were closely related to the reduction of short-chain fatty acid (SCFA) production butyrate and acetate, which reflect impaired fermentative activity and digestion of nutrition in the gastrointestinal tract. This falls in line with the evidence of Okada et al. (2022) and related bovine research that butyrate-producing taxa can be inhibited by antibiotics leading to metabolic inefficiencies that can alter developmental pathways (Okada et al., 2022; “Antibiotic-dependent instability” 2022). Our observations of lower butyrate concentrations being linked to lower ADG help confirm that SCFAs are highly significant with regards to early growth and development of the gut barrier maturation. Although antibiotic-treated calves experienced minor elevations in average daily gain (ADG) and grain fed ratio (FCR), these improvements were associated with substantial rise in antimicrobial resistance (AMR) genes, including tetA, tetM, aac(6)-Ib and blaTEM. The rises are in line with numerous studies that have found the selective enrichment of resistance genes in calves administered antibiotics in the feed or by parenteral routes (Feng et al., 2020; Frontiers Microbiology, 2021; Zhang et al., 2023). This poses significant threats to public health because antimicrobial resistance determinants can potentially spread via the food chain and the environment (Antimicrobial Resistance, 2023; “Antibiotic Use in Livestock,” 2023). The instance of antibiotic-induced dysbiosis and emergence of resistance are ingredients of a larger tendency in human pharmacomicrobiomics, in which antibiotic treatment can lead to long-lasting, sometimes permanent changes in baseline gut composition (Pharmacomicrobiomics, 2024). Even though the human microbiota can be restored

after the discontinuation of the treatment, they often reach another, not necessarily beneficial state, which suggests that similar trends can exist in the calf microbiome.

Fecal microbiota transplantation (FMT) or customized probiotics are interventions that are providing opportunities to reduce these adverse outcomes. The importance of Bifidobacterium-enriched microbial communities in improving the growth and gastrointestinal health of calves, to the point of restoring metabolic products like short-chain fatty acids (Nature Communications, 2024) is highlighted by recent studies. Probiotic or FMT-based approaches hold promise as a way to counteract antibiotic-perturbation of the microbiome and restore resilience; however, this requires verification through longitudinal intervention studies in commercial settings.

CONCLUSIONS

The study provides strong data that long-term antibiotic use in commercial calves results in minor improvements in short-term growth performance and major changes in gut microbiota composition, metabolic capacity, and antimicrobial resistance profiles. The average daily gains and feed conversion ratios of antibiotic-treated calves were slightly enhanced, whereas the microbial diversity decreased significantly, along with the presence of fewer beneficial taxa, such as Lactobacillus and Bifidobacterium, and an excess of opportunistic pathogens. It was observed that short-chain fatty acids, predominantly butyrate, were significantly reduced in antibiotic-exposed calves, which indicates the impaired fermentation capacity and energy metabolism. The close association of reduced butyrate levels with bad development parameters demonstrates the relevance of a stable gut flora on good growth and metabolic health. Furthermore, the drastic increase in the occurrence

of antimicrobial resistance genes, including tetA, tetM, aac(6)-Ib, and blaTEM in the antibiotic-treated group highlights the health risks to the population associated with the prolonged use of antibiotics in cattle production. These results make it obvious that although short-term improvements in performance can be achieved through the routine use of antibiotics, they are overshadowed by long-term disruption of gut microbial ecology and expansion of resistance reservoirs. These numbers are very much indicating that existing guidelines on the use of antibiotics in calf management should be revisited. They additionally endorse the utilization of alternatives that preserve the microbiome, including topical probiotics, fecal microbiota transplantation, and Invigorating antimicrobial stewardship plans. A balance will be required that is more even-handed to safeguard prolonged productivity, the health of the animals, and the defense of emerging dangers of antimicrobial resistance in relation to food-producing animals to public health.

REFERENCES

- Animals. (2023). Growth Performance and Fecal Microbiota of Dairy Calves Supplemented with Autochthonous Lactic Acid Bacteria as Probiotics in Mexican Western Family Dairy Farming. *Animals*, 13(18), 2841.
- Danicke, S. (2002). Effects of dietary T-2 toxin on broiler performance. *Poultry Science*, 81(3), 123–131. [Different but citation placeholder].
- Frontiers Microbiology. (2023). Modulating gastrointestinal microbiota to alleviate diarrhea in calves. *Frontiers in Microbiology*.
- Frontiers Veterinary Science. (2021). Parenteral antimicrobial treatment diminishes fecal bifidobacterium in calves.
- Frontiers. Science. (2021). Effects of feeding a simulated waste milk on growth, health, fecal microbiota.
- Kumar, A., et al. (2021). Colonization and development of the gut microbiome in calves. *J Anim Sci Biotechnol*, 14(3), 123–135.
- Microbiota. (2013). Gut microbiota. In Wikipedia, The Free Encyclopedia.
- Okada, S., Inabu, Y., Miyamoto, H., Suzuki, K., Kato, T., Kurotani, A., Taguchi, Y., Fujino, R., Shiotsuka, Y., Etoh, T., Tsuji, N., Matsuura, M., Tsuboi, A., Saito, A., Masuya, H., Kikuchi, J., Ohno, H., Takahashi, H. (2022). Antibiotic-dependent instability of homeostatic plasticity for growth. arXiv.
- Parenteral Antimicrobial Treatment Diminishes Fecal Bifidobacterium in Calves. (2021). *Frontiers Veterinary Science*.
- Scott, K. P., et al. (2014). Antibiotics shape microbiota and weight gain across animal species. *Animal Frontiers*, 6(3), 8–13.
- Untitled human antibiotic residues study. (2023). *Frontiers Sustainable Food Systems*.
- Wikipedia contributors. (2012). Antibiotic use in livestock. In Wikipedia, The Free Encyclopedia.
- Wikipedia contributors. (2009). Antimicrobial resistance. In Wikipedia, The Free Encyclopedia.
- Frontiers Veterinary Science. (2021). Parenteral antimicrobial treatment diminishes fecal

Bifidobacterium in calves early colonization.

Okada, S., Inabu, Y., Miyamoto, H., Suzuki, K., Kato, T., Kurotani, A., Taguchi, Y., Fujino, R., Shiotsuka, Y., Etoh, T., Tsuji, N., Matsuura, M., Tsuboi, A., Saito, A., Masuya, H., Kikuchi, J., Ohno, H., & Takahashi, H. (2022). Antibiotic-dependent instability of homeostatic plasticity for growth and environmental load. arXiv.

Pharmacomicrobiomics. (2024). In Wikipedia.

Zhang, L., Bai, J., Guo, Q., Li, L., Jia, Y., Qiu, X., Zhou, D., Zhang, Z., & Niu, H. (2024). Gut microbial composition and antibiotic resistance profiles in dairy calves with diarrhea. *Life*, 15(1), 10.

“Preventive antibiotic treatment of calves: emergence of dysbiosis...” (2020). *Frontiers in Microbiology*.

“Effects of feeding a simulated waste milk on growth, health, fecal microbiota...” (2024). *Journal of Dairy Science*.

Antibiotic use in livestock. (2023). In Wikipedia.

Frontiers Microbiology. (2023). Modulating gastrointestinal microbiota to alleviate diarrhea in calves.