

**Research Article****Lactic Acid Bacteria as a Substitute for Antibiotics in Broiler Chickens Raised Under Colored Environment**Majid Shakeri^{1*} ¹ U.S. National Poultry Research Center, Agricultural Research Service, USDA-ARS, Athens, USA* **Corresponding author:** Majid Shakeri, U.S. National Poultry Research Center, Agricultural Research Service, USDA-ARS, Athens, USA; Email: majid.shakeri.phd@gmail.com**ARTICLE INFO****Article History:**

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ABSTRACT

Introduction: Antibiotics have been used to enhance the growth performance of broiler chickens; however, significant concerns about antibiotic resistance are driving interest in finding new alternatives to support growth performance. One promising additive is probiotics, which have shown positive impacts on broiler performance. The present study aimed to investigate the effects of probiotics (Lactic acid bacteria) on growth, feed intake, feed conversion ratio, and immunity parameters such as corticosterone, compared to an antibiotic (virginiamycin) when chickens were raised in different colored environments.

Materials and methods: A total of 120 one-day-old male Cobb-500 chickens, with an average weight of 45 ± 2 grams, were randomly distributed into 12 white and 12 red pens (five chickens in each pen). Three dietary treatments were assigned, each containing four pens from each color, for a total of eight pens per treatment. Each treatment group consisted of 40 chickens, and four replicates. The dietary treatments included a control diet, the control diet supplemented with 4 cm^3 of lactic acid-based probiotic bacteria (10^9 CFU/kg) per kilogram of feed, and the control diet supplemented with 0.4 grams of virginiamycin per kilogram of feed. On day 42, the chickens were sampled for evaluation of performance factors, corticosterone levels, foot-pad dermatitis, and villi length of the Jejunum.

Results: The data showed that both probiotic and virginiamycin improved body weight and villi length compared to the control. Additionally, chickens supplemented with the probiotic in the white pens exhibited a better growth rate than those supplemented with virginiamycin or the control. Corticosterone levels were reduced for both probiotic and virginiamycin compared to the control group, with more potent effects observed in the white pens. Chickens supplemented with either probiotic or virginiamycin demonstrated a lower incidence of foot-pad dermatitis in both pen color environments compared to the control group.

Conclusion: Supplementation of chickens with probiotic ($4 \text{ cm}^3/\text{kg}$, lactic acid bacteria; 10^9 CFU/kg) improved body weight similarly to virginiamycin, while a white environment can reduce chickens' stress, leading to better growth performance.

1. Introduction

Antibiotics are growth promoters that have been used in broiler diets to improve the performance and health of chickens¹. Antibiotics such as virginiamycin play a crucial role in enhancing broiler performance by regulating the population of microorganisms² and promoting gut health by facilitating villi development³. Despite the positive effects of antibiotics on performance, the harmful aspects of using antibiotics on human safety, such as increasing microbial resistance, have caused many countries to ban the use of antibiotics in the diet of broiler

chickens. Therefore, there is a need to find alternatives for antibiotics to avoid potential threats to human health. One of the potential alternatives that has shown a positive impact on broiler growth performance is probiotics⁴.

Probiotics are a group of microorganisms that enhance performance and immunity in the animal body by improving gut health⁴ and stimulate the immune system⁵. Probiotics, such as lactic acid bacteria, protect the body against pathogens and enhance the immune system⁶. Additionally, probiotics are effective supplements



when chickens are under stress conditions⁷. One of the stresses that chickens experience during their growing period is the condition of their house/cage. Previous studies have shown that chickens' growth performance is affected by lighting⁸, the complexity of the house⁹, and colored feed¹⁰. Furthermore, red-colored feed has been shown to increase aggression among chickens compared to normal feed¹¹, which could cause stress among chickens in the same cage/pen. The present study evaluated how virginiamycin and lactic acid bacteria supplementation influence broiler performance, gut health, and stress response under different colored housing environments

2. Materials and Methods

2.1. Ethical approval

All procedures used in this experiment followed the guidelines of the Institutional Animal Care Committee of Universiti Putra Malaysia (2015).

2.2. Diets and housing

A total of 120 one-day-old Cobb-500 male broiler chickens were randomly divided into 24 equally sized pens (Five chickens/pen), including 12 white pens and 12 red pens. The pens were separated by 1m-high walls and painted from the inside (either red or white). Moreover, drinkers and feeders were painted the same color as the walls. Body weight, feed intake, and feed conversion ratio were calculated at the end of the study on day 42. The treatments consisted of a control diet, a control diet supplemented with 4 cm³/kg probiotic (Lactic acid bacteria; 10⁹ CFU/kg), and a control diet supplemented with 0.4 g/kg virginiamycin. The water and feed were provided *ad libitum*. The light was provided continuously during the experiment. The feed was provided as starter (Crude protein: 24.5%, metabolized energy: 12 kcal/kg), grower (Crude protein: 23.5%, metabolized energy: 12.6 kcal/kg), and finisher (Crude protein: 21.6%, metabolized energy: 12.9 kcal/kg).

2.3. Sampling

On day 42, chickens were weighed and then slaughtered by cutting their jugular veins. Twenty-four chickens per treatment were randomly selected (12 from red and 12 from white pens) to collect blood from the jugular vein and the Jejunum tissues. Blood samples, 2 mL in EDTA tubes, were centrifuged to obtain serum, then stored at -80°C for further analysis.

2.4. Intestinal morphology

The collected tissues were fixed in paraffin. Sections were stained by H&E (10 villi per slide). The villi length was measured by using a light microscope (20x). The distance from the tip of the villus to the villus crypt junction represents villus height. A total of 10 villi per slide were measured¹.

2.5. Corticosterone

The Corticosterone level was measured by a commercial kit (IDS, Boldon, UK). Serial standards were prepared according to the manufacturer's protocol (500- 6.17 ng/mL), and the absorbance was measured at 450 nm.

2.6. Foot pad dermatitis

The chickens were grown in wood-shape litter in pens. At the end of the experiment, all chickens were scored for foot pad dermatitis according to Nagaraj et al.¹². The scoring system ranged from 0 to 3, where 0 indicated no signs and 3 indicated the most severe lesion.

2.7. Statistical analysis

The current data were subjected to ANOVA using SAS (SAS Institute Inc., Cary, NC). Analysis was conducted using diet, colored environments, and their interactions, employing the GLM procedure. Results were considered significant at $p < 0.05$. Mean values are given as mean \pm standard error.

3. Results

The effects of diets and environments on growth, feed intake, and feed conversion ratio are presented in Table 1. Probiotic and Virginiamycin improved weight gain under both conditions, with the effects being more substantial in the white environment ($p < 0.05$). There were significant interactions between diets and environmental conditions ($p < 0.05$).

Both diets reduced corticosterone levels, with the effect being more substantial in a white environment ($p < 0.05$; Table 2). Food pad dermatitis scores and villi length were improved with both diets compared to the control ($p < 0.05$). There was no interaction between the diets and the conditions.

Table 1. Body weight, feed intake, and feed conversion ratio by different treatments under two colored environments in broiler chickens aged 42 days

	Final body weight (g)	Feed intake (g)	Feed conversion ratio
Red environment			
Control*	2104 \pm 80 ^e	4418 \pm 95 ^{bc}	2.09 \pm 0.06 ^a
Probiotic (Lactic acid bacteria 10 ⁹ CFU/kg)	2275 \pm 99 ^b	4402 \pm 76 ^{bc}	1.93 \pm 0.03 ^b
Virginiamycin (0.4 g/kg)	2240 \pm 78 ^c	4203 \pm 32 ^{bc}	1.93 \pm 0.03 ^b
White environment			
Control	2167 \pm 96 ^d	4647 \pm 101 ^a	2.14 \pm 0.01 ^a
Probiotic (Lactic acid bacteria 10 ⁹ CFU/kg)	2387 \pm 87 ^a	4213 \pm 46 ^c	1.76 \pm 0.08 ^c
Virginiamycin (0.4 g/kg)	2390 \pm 54 ^a	4445 \pm 105 ^{ab}	1.85 \pm 0.03 ^c
P-values			
	Diet	Environment	Diet*environment
Weight gain	0.0001	0.0001	0.01
Feed intake	0.02	0.35	0.03
Feed conversion ratio	0.0001	0.01	0.01

Data are presented as mean \pm SEM. ^{abcde} within a column-subgroup with no common superscript letters are different ($p < 0.05$). *: Interaction of diet and environment

Table 2. Corticosterone, villi length, and foot pad dermatitis by treatments under two colored environments in broiler chickens aged 42 days

	Corticosterone (ng/ml)	Foot pad dermatitis	Villi length (µm)
Red environment			
Control*	2.37 ± 0.11 ^a	1.88 ± 0.13 ^a	953 ± 41 ^b
Probiotic (Lactic acid bacteria 10 ⁹ CFU/kg)	1.89 ± 0.13 ^b	1.38 ± 0.12 ^b	1150 ± 53 ^a
Virginiamycin (0.4 g/kg)	1.68 ± 0.15 ^{bc}	1.54 ± 0.12 ^b	1185 ± 25 ^a
White environment			
Control	1.79 ± 0.08 ^b	1.79 ± 0.14 ^a	944 ± 38 ^b
Probiotic (Lactic acid bacteria 10 ⁹ CFU/kg)	1.32 ± 0.14 ^d	1.44 ± 0.11 ^b	1192 ± 32 ^a
Virginiamycin (0.4 g/kg)	1.46 ± 0.18 ^{dc}	1.49 ± 0.13 ^b	1199 ± 18 ^a
P-values			
	Diet	Environment	Diet*environment
Corticosterone	0.0001	0.0001	0.07
Foot pad dermatitis	0.0002	0.65	0.57
Villi length	0.0001	0.07	0.17

Data are presented as mean ± SEM. ^{abcd} within a column-subgroup with no common superscript letters are different (p < 0.05). *: Interaction of diet and environment

4. Discussion

The current results are in agreement with studies that have shown probiotics^{5,13,14} and virginiamycin³ to improve chicken growth performance. The reason might be related to the positive impacts of both additives on gut health^{2,13}. Both additives potentially enhanced absorption in the gut as evidenced by increased villi length. Chickens kept in the red environment had the worst growth performance. It has been shown that the color red can increase chickens' physical activity, which means that the chickens spend some of their energy on activities such as walking, rather than growth¹⁵.

As mentioned earlier, some colors, such as red, could increase chickens' activities and aggressive behaviors¹¹. Aggressive behaviors might increase general stress among chickens in the cage/pen¹⁶ growing type. The current results indicated that the corticosterone level increased for chickens in the red pens, while probiotic and virginiamycin groups reduced the level of this hormone. Previous studies have suggested that probiotics⁷ and virginiamycin³ can improve chickens' welfare under stress conditions by modulating hormone activity and corticosterone levels. Corticosterone (glucocorticoid hormone) levels are released into the bloodstream in response to psychological stress¹⁷.

5. Conclusion

It can be concluded that probiotics (Lactic acid bacteria, 4 cm³/kg; 10⁹ CFU/kg) could be an alternative to antibiotics (virginiamycin) under normal or stressful conditions. The color of the environment could play a significant role in chickens' performance, as it may impact their behavior during rearing. Providing a lighter environment might help chickens allocate their energy from feed more efficiently towards producing meat rather than engaging in other physical activities. More studies should be conducted for different dosages of probiotics containing lactic acid bacteria in broiler chickens reared in pen conditions.

Declarations

Competing interests

There is no conflict of interest.

Ethical considerations

The author confirmed that the manuscript has been reviewed

and submitted to this journal for the first time.

Authors' contributions

The study was conducted by Majid Shakeri. The author approved the final edition of the manuscript.

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Availability of data and materials

All data are presented in the text and upon reasonable request from the corresponding author.

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