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Effect of Lactic Acid bacteria as Feed Additive on Growth Performance, Intestinal Flora and Serum Parameters in Broilers

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ABSTRACT

This research aimed to study the effect of supplementation of *Lactobacillus brevis* and a combination of *Lactobacillus brevis* and *Enterococus faecalis* on growth performance, bacterial population and serum biochemical parameters in broilers. A total of 200 one-day-old chicks were obtained from a local hatchery. They were divided into four groups: group 1 served as a control, group 2 received commercial probiotics, group 3 received *Lactobacillus brevis,* and group 4 received the combination. The main parameters measured through 6 weeks were body weight, food intake, food conversion ratio (FCR), blood total protein, albumin and cholesterol. In addition to enumeration of bacteria from cloaca and caecum. The results showed a statistically significant increase in body weight (26%) for the group receiving the combined supplementation compared to the control. Similarly, there was a significant increase in FCR for this group compared to the control. The results also showed an increase in the Lactic acid bacteria (LAB) count in the caecum and a decrease in the total late count (TPC) and coliform count, showing the inhibitory effect of LAB. Also, there was a significant decrease in cholesterol and albumin level, while there was no difference between the different groups regarding protein level. The results showed that *Lactobacillus brevis* and *Enterococus faecalis* have a good potential to be used as probiotics in poultry.

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INTRODUCTION

Broiler production is a rapidly expanding sector in the animal industry. To mitigate economic losses and enhance production, producers often resort to using antibiotics to combat diseases and promote growth. However, the imprudent use of antibiotics can result in their appearance in food products and also led to the emergence of multidrug-resistant bacteria, both of which pose significant challenges to public health and food safety **(Ogbuewu *et al.*, 2022).** Thus, there has been a growing interest in alternative approaches to improve animal health and performance without the drawbacks associated with antibiotic use. Probiotics have emerged as a possible alternative, with their benefits for immune function and growth rates in animals. The term probiotics is defined as "living microorganisms which, when administered in an adequate amount confer a health benefit on the host **(Hotel and Cordoba, 2001).**

Among the commonly used probiotics in the poultry industry are Lactic Acid Bacteria (LAB), including genera such as *Lactobacillus, Streptococcus, and Enterococcus*. Other beneficial microbes used include *Leuconostoc, Bifidobacterium, Weissella, Bacillus, Pediococcus, Aspergillus,* Candida, and Saccharomyces, with *Bacillus licheniformis* and *Bacillus subtilis* being notable for their positive impact on nutrient digestion, absorption, and overall broiler growth and performance **(Sögaard and Suhr-Jessen 1990; Aliakbarpour *et al.*, 2012; Sathishkumar *et al.*, 2017).**

LAB probiotics are believed to exert their beneficial effects through various mechanisms. These include: production of antimicrobial compounds such as bacteriocins and organic acids, which suppress pathogens and promote a healthy gut microbiome **(Vernocchi *et al.*, 2020);** adherence to the intestinal epithelium leading to modulation of the intestinal microflora and inhibition of **pathogens (Corcionivoschi *et al.*, 2010); competitive exclusion and antagonism, maintaining normal intestinal microflora (Kizerwetter-Swida and Binek 2009)**;

altering metabolism by enhancing digestive enzyme activity and reducing bacterial enzyme activity and ammonia production **(Yoon *et al*., 2004)** and improving feed intake, digestion, and stimulating the immune system **(Awad *et al*., 2009).**

Administration of probiotics has been shown to influence various health indices in poultry, such as growth performance, intestinal microflora, and blood components, including total proteins, lipids, and cholesterol, which are crucial indicators of health status **(Filipovic *et al*., 2007).** Given these considerations, this study aimed to investigate the effects of LAB strains on growth performance, intestinal microflora, blood proteins, and cholesterol levels in broilers.

MATERIALS AND METHODS

1. Source of Lactic Acid Bacteria

In this study, two strains of LAB namely Lactobacillus *brevis* and a combination of *Lactobacillus brevis* and *Enterococcus faecalis* were used. These strains were previously isolated and identified from fermented camel milk **(Amel *et al*., 2021).**

2. Broilers and Experimental Design

Two hundred one-day-old broiler chicks (Ross 208) were obtained from a local hatchery in Sudan. They were allocated into four groups as follows:

- Group 1: Control Group (CG), which received a standard diet and drinking water without any supplements.
- Group 2: (PGc): Group receiving commercially available probiotics used as poultry feed supplements. This group received a standard diet and water supplemented with probiotics containing *Bacillus subtilis* (1.6×10^9 CFU/g).
- Group 3: PG1, which received a standard diet and water supplemented with *Lactobacillus brevis* (1.6×10^6 CFU/ml).
- Group 4: PG2, which received a standard diet and water supplemented with a combination of Brevis and *Enterococcus faecalis.*

The chicks were housed in clean battery cages for the 42-day duration of the study. Upon arrival, all chicks were found to be healthy and were subsequently vaccinated against Newcastle Disease (ND) after one week, followed by an infectious bursitis (IBD) vaccine administered via drinking water on day 14. Booster doses for IBD and ND vaccines were given on days 14 and 30, respectively. Daily observations were recorded for health status and mortality.

3. Measurements

3.1 Growth Performance

Growth performance metrics included body weight, feed intake (FI), and feed conversion ratio (FCR). Body weight and feed intake were recorded weekly from day 1 to determine changes in body weight and FCR. Feed intake was measured on a per pen basis, with uneaten feed discarded at the end of every day and replaced with fresh feed. FCR was calculated as the amount of feed consumed per unit of body weight, adjusted for the weight of dead birds.

3.2. Intestinal Microflora

3.2.1. Collection and Analysis of Cloacal Swab Samples

Cloacal swabs were collected (five samples/treatment) and microbiologically analyzed for viable bacterial counts. Swabs were placed in 50 ml of sterile buffered peptone water, followed by serial dilution as needed. Plate Count Agar (PCA), MacConkey agar, XLD (Xylose Lysine Deoxycholate Agar), EMB (Eosin Methylene Blue Agar), and De Man-Rogosa-Sharpe Agar (MRS) were used for bacterial counting and identification. Plates were incubated at 37 °C for 24 hours and anaerobically for 2 days, with colonies between 25 and 250 counted and expressed in CFU/ml (Cunniff, 1996).

3.2.2. Collection and Analysis of Caeca

Post-slaughter, the gastrointestinal tract was aseptically removed, segmented, and the ceca samples were collected in sterile bags containing 50 ml broth, then stored at -20°C. Samples were homogenized, and 10-fold serial dilutions were made in buffered peptone water, streaked in duplicate on PCA, MacConkey agar, XLD, MRS agar, and EMB agar for enumeration and identification of target bacterial groups.

3.3 Blood Analysis

Blood samples were collected from the wing vein on a weekly basis. Samples were then centrifuged at 3000 rpm for 15 minutes, and serum was used for analyzing total protein, albumin, and cholesterol concentrations using the enzymatic colorimetric method. Commercially available kits (Spinreact, Spain) were used. All procedures were done following the manufacturer's instructions.

4. Statistical Analysis

Data were analyzed using the Kruskal-Walli’s test, with P values < 0.05 considered statistically significant. Data is expressed as Mean ±SD.

RESULTS

Growth performance

The impact of LAB supplementation on the growth performance of broilers is detailed in **Table 1 and Fig. 1.** The average body weight of chickens in the control group (CG) was 1.391 gm, and there was a 26%

increase when compared to PG2 (1.756 gm), with a statistically significant difference ($p = 0.009$) between the groups. Groups receiving LAB supplementation (PG1 and PG2) showed the highest body weight gain among all groups.

Feed intake among the study groups ranged from 2.11 g/bird in the control group (CG) to 2.99 g/bird in the PG1 group; no significant difference was observed in the daily feed in take across all four groups (Table 1). However, the feed conversion ratio (FCR) exhibited statistically significant differences ($p<0.001$), with the highest efficiency observed in the PG2 group, as detailed in **Table 1.** Concerning mortality rates, the highest was observed in the group receiving the commercial probiotic (1.6%). The relative risk (RR) indicated that the probability of death in the PGC group was 1.6 times higher than in the control group.

Table 1: Effect of probiotic supplementations on total feed intake, body weight, body weight gain and FCR.

Component	CG	PGc	PG1	PG2	P-value
Body weight (gm/bird)	1.391 ± 23.97	1.456±48.155	1.618±71.33	1.756 ±59.38	0.009
Feed intake (gm/bird)	2.108 ± 17.35	2.208 ± 19.20	2.458±16.07	2.992±17.95	0.52
FCR (gm/gm)	1.546 ± 0.06	1.517 ±0.08	1.519 ± 0.04	1.703±0.05	0.000
Mortality (%)	0.1	1.6	0.00	0.04	
RR mortality		(RR=1.6)	(RR= 0.00)	(RR=0.4)	

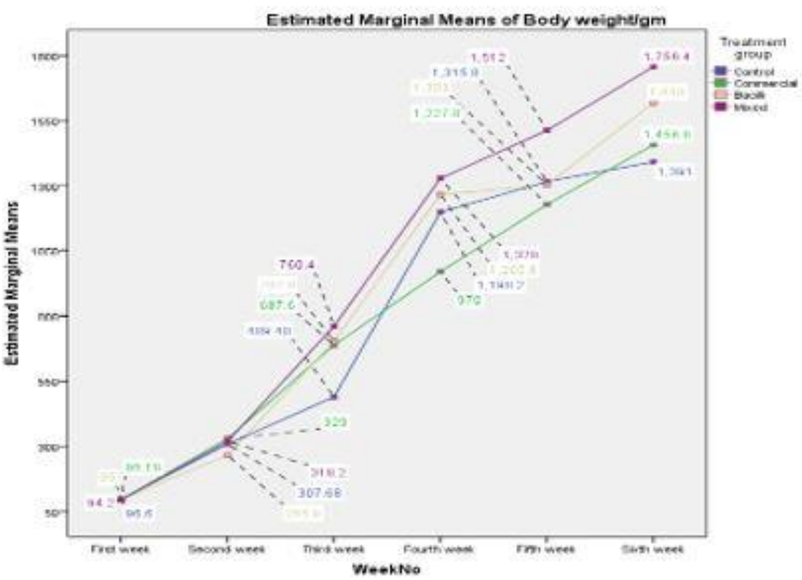

Fig.1 Changes of mean body weight

Bacterial Growth in the Cloaca and Caecum

In the present study, the enumeration of various bacteria isolated from the cloaca and caecum samples is presented in **Table 2.** In this study, both treatment groups exhibited lower total plate count (TPC) and Coliform counts compared to the control group, while the populations of LAB were higher.

Table 2: Count of different bacteria from the cloaca and caecum on the 6th week.

Treatment	TPC (cloaca) (cfu /ml)	LAB count caecum (cfu/ gm)	Coliform (caecum) (cfu/gm)
CG	$2.4x10^{6}$	$1 x10^{4}$	$2.3x10^{7}$
PGc	$3 x 10^{6}$	$2.3 x10^{4}$	$1.2 x10^{7}$
PG1	$1.2x 10^{2}$	$1.8 x10^{9}$	$1.5x10^{2}$
PG2	$1.1x10^{2}$	$2.1x10^{9}$	$1.2x10^{2}$

Effect of LAB on serum parameters

The impact of LAB supplementation on serum total protein, albumin, and cholesterol levels is presented in **Table 3 and Fig. 2a, 2b, and 2c.** By week 6, significant differences were observed among the various treatment groups in terms of albumin and cholesterol levels, while no significant difference was noticed regarding total protein levels.

Table 3: Overall Effect of LAB supplementation on Total protein, albumin and cholesterol level

Component	CG	PGc	PG1	PG2	P-value
Total protein level(g/l)	34.4 ± 0.71	31.9± 1.61	33.5±0.84	26.5±0.06	0.212
Albumin level(g/l)	13.7 ± 0.76	15.3 ± 1.31	10.6 ± 0.84	10.4 ±1.03	0.010
Cholesterol level(g/dl)	173.3 ± 25.25	150.0±9.64	133.3± 29.4	81.47±24.30	0.002

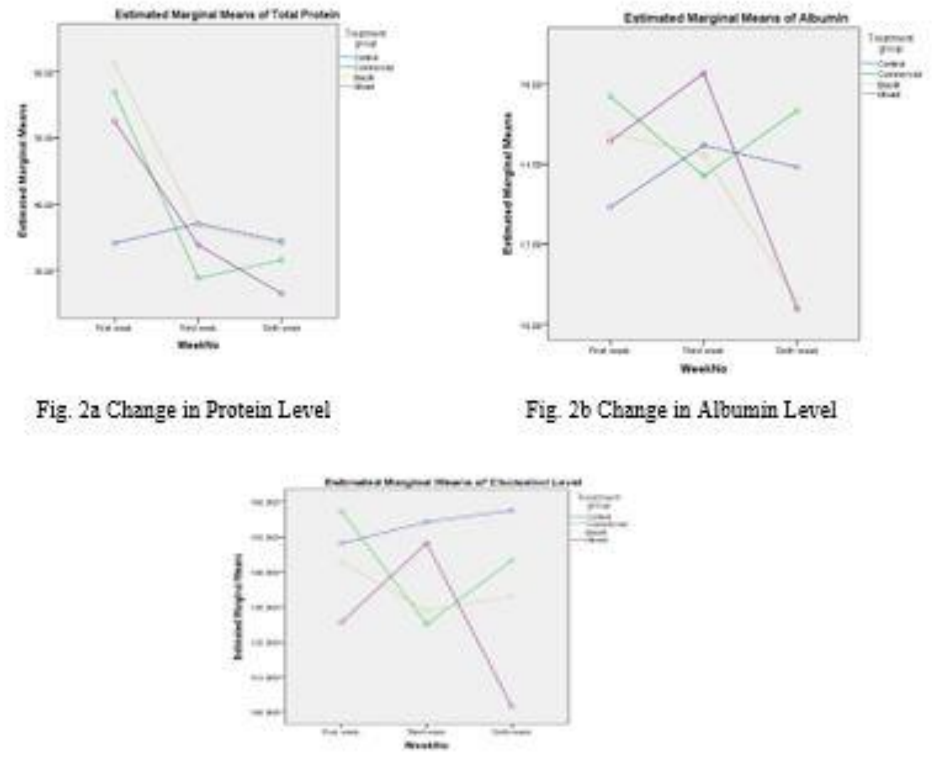

Fig. 2a Change in Protein Level

Fig. 2b Change in Albumin Level

Fig. 2c Change in Cholesterol Level

Fig. 2: The impact of LAB supplementation on serum total protein, albumin, and cholesterol levels

DISCUSSION

This study aimed to investigate the effect of administration of LAB on growth performance, intestinal bacteria and some blood parameters in poultry. The results showed an increase in body weight, which aligns with previous research indicating that probiotic or postbiotic supplementation in broiler diets can enhance growth performance **(Mountzouris *et al.*, 2007; Awad *et al.*, 2009; Houshmand *et al.*, 2011; Mahmoud and Kareem, 2024).** However, other studies have reported no significant impact of probiotics on broiler performance **(Mutus *et al.*, 2006; Knap *et al.*, 2011).** This result is consistent with various studies that support the positive effects of LAB on body weight gain in broilers in different countries **(Kalavathy *et al.*, 2003; Islam *et al.*, 2004; Ashayerizadeh *et al.*, 2009; Abdel-Hafeez *et al.*, 2017; Shokryazdan *et al.*, 2017; Park *et al.*, 2018; Mandana *et al.*, 2019 and Hati *et al.*, 2023).** These findings underscore the potential of LAB supplementation as a beneficial strategy for improving the growth performance of broilers, although variations in results across different studies suggest that the effects may depend on other factors such as specific strains of LAB, the host species, and other dietary and environmental factors **(Zhang *et al.*, 2021).** This is in line with the findings of **Mandana *et al., (2019*; Tsega *et al.*, 2024)** who reported no significant differences in feed intake among broilers supplemented with various Lactobacillus strains and a commercial probiotic blend. On the contrary, the study of **Hati *et al., (*2023)** found an improvement of FCR using different probiotic strains.

Other growth performance characteristics, such as feed intake and FCR, were also investigated in this study. The effect of LAB supplementation on the various parameters may be due to the beneficial impacts of LAB strains on intestinal microflora, along with improved nutrient digestibility and absorption **(Shokryazdan *et al.*, 2017).** The specific strains of probiotics used can significantly influence body weight gain responses **(Khan *et al.*, 2013)** and potentially affect chicken appetite **(Ferket and Gernat, 2006).**

From an industrial point of view, the mortality rate is of major concern. As shown in the current study, the highest mortality rate was 1.6%, although the study of **O'dea *et al.* (2006)** reported no effect of probiotic treatment on cumulative mortality of broilers from 0 to 35 days of age.

Lactic acid bacteria are known for their capability to produce lactic acid. Lactic acid has been reported to be effective in removing pathogenic bacteria such as Shiga toxin-producing *E. coli* and *Salmonella* spp. from meat and poultry **(Norasak *et al.*, 2016; Yuhui *et al.*, 2022; Israa and Omar, 2024).** In addition, the ability of LAB to modulate the intestinal microflora in broilers has been previously documented **(Mountzouris *et al.*, 2007; Chim-anage *et al.*, 2008; Beski and Al-Sardary, 2015; Pourakbari *et al.*, 2016).** The intestinal microbiota plays a crucial role in the growth performance of broilers and enhances their defense against various pathogens **(Mandana *et al.*, 2019).**

As shown in this study, the increased LAB count and decreased TPC and Coliform count suggest the effectiveness of LAB supplementation in reducing other bacterial groups and enriching the lactic acid bacteria population in the caecum. This finding aligns with previous studies **(Mandana *et al.*, 2019; Quinto *et al.*, 2014; Tsega *et al.*, 2024)** which demonstrated that LAB supplementation increased *Lactobacillus spp*. populations and decreased *E. coli* in the ileum and intestine of birds on probiotic diets compared to control. These effects can be attributed to the decreased intestinal pH, the acid tolerance ability of LAB, and the antibacterial activity of LAB strains **(Quinto *et al.*, 2014).**

Among other parameters widely investigated as an effect of LAB administration are the blood constituents, as some of them are considered health indicators. In this study, blood proteins and cholesterol were studied. The primary blood proteins, albumin, globulin, and fibrinogen, play crucial roles in the body's physiological processes, including protein deposition, osmotic pressure regulation, and transport of small molecules within the blood **(Mushawwir and Latipudin, 2011).** In this study, while total protein levels decreased by week 6, no significant differences were observed between the groups, and albumin levels showed a significant decrease. Similar studies also reported no significant effect of probiotics in serum total protein **(Mandana *et al.*, 2019; Lovita *et al.*, 2021)** or total protein, albumin, and globulin concentrations in chickens **(Abdel-Hafeez *et al.*, 2017; Al-Khalaifa *et al.*, 2019).**

On the contrary, **Dev *et al*. (2020)** reported increased serum total protein and globulin levels in birds fed Mannan-oligosaccharides and *Lactobacillus acidophilus*. Albumin and blood protein play a major role in the deposition of protein into the meat. The high protein level in blood indicates that protein deposition in meat is also high, while the low levels below standard indicate a lack of nutrition **(Lovita *et al.*, 2021).** Low protein consumption causes a reduced albumin level, and thus protein deposition into the meat will also decrease **(Liu *et al.*, 2015).**

Some probiotic species, such as Bacillus, may improve blood proteins through the ability of Bacillus to improve dietary protein digestion and utilization by increasing the absorptive efficiency of the small intestine **(Lee *et al.*, 2013; Kadaikunnan *et al.*, 2015 and Abdel-Moneim *et al.*, 2020).**

Cholesterol is an important component of lipid membranes and a precursor for the synthesis of steroid hormones and bile acids. It is synthesized by all animal cells, particularly hepatocytes **(Howles, 2016).** Given that meat and eggs are significant dietary sources of cholesterol **(Ahn *et al.*, 1999),** managing dietary and hepatic cholesterol synthesis is crucial to prevent health problems such as hypercholesterolemia and cardiovascular disease **(Cha and Park, 2019).** As shown in this study, there was a significant decrease in cholesterol levels by week 6, especially among the PG2 group. This aligns with findings from similar studies **(Rahimi and Khaksefidi, 2006; Mansoub, 2010; Mandana *et al*., 2019).** Other studies showed decreases in cholesterol and LDL **(Kalavathy *et al*., 2003; Ghasemi and Taherpour, 2013)** cholesterol and triglycerides **(Mandana *et al*., 2019).** The cholesterol-lowering mechanisms of probiotics remain partly elucidated. One hypothesis suggests that the mechanisms may involve the incorporation of cholesterol into bacterial cells by Lactobacillus, de-conjugation of bile salts, and inhibition of key enzymes in cholesterol biosynthesis like acetyl-CoA carboxylase and 3-hydroxy-3-methylglutaryl coenzyme A reductase. These actions potentially promote cholesterol catabolism during enterohepatic circulation **(DeBose-Boyd, 2008; Mandana *et al.*, 2019; Deng *et al*., 2020).**

CONCLUSION

This study revealed the positive effects of supplementing broiler diets with *Lactobacillus brevis* alone or in combination with *Enterococcus faecalis* on growth performance and serum biochemical parameters. The combined supplementation notably increased body weight and increased lactic acid bacteria count in the caecum, suggesting improved gut health. In addition, reductions in enterococci count and cholesterol level indicate potential health benefits of probiotic supplementation in broilers.

Conflict of Interest

The authors declare no competing of interest.

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