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EVALUATIING THE *LACTISAR* EFFECTS ON GROWTH PERFORMANCE AND IMMUNE RESPONSE IN BROILER CHICKENS

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ABSTRACT

The use of multiple strains of probiotics and multi-enzymes has been shown to enhance growth and immune function in poultry production. This study evaluated the effects of a *LACTISAR* (LACT), which contains probiotics and enzymes, compared to antibiotics. A total of 180 healthy one-day-old male chicks were randomly allocated into three dietary groups: (i) basal diet (BSD) with no feed additive, (ii) LACT (BSD + 100g *LACTISAR*/100 kg feed), and (iii) AGP (BSD + 200 g lincomycin/100 kg feed). Each group was subdivided into six replicates, with 10 birds per replicate. Results indicated that during the starter period, the LACT group showed significant improvements in live weight (LW), average daily gain (ADG), and feed conversion ratio (FCR) compared to the BSD group. During the finisher and overall periods, the LACT group exhibited significant (P< 0.01) improvements in LW, ADG, and FCR when compared to both the BSD and AGP groups. No significant differences in average daily feed intake (ADFI) were observed among all groups (P> 0.05). Furthermore, the weight of immune organs (thymus, spleen, and bursa) was significantly higher (P< 0.01) in the LACT group compared to the BSD group. Additionally, the LACT group demonstrated significantly higher levels of immunoglobulins (IgA, IgG, and IgM) in serum (P< 0.01) compared to the BSD group. Overall, dietary supplementation with *LACTISAR* (a multi-probiotic and enzyme mixture) enhances both the growth performance and immune response of broiler chickens.

Keywords: *LACTISAR,* growth, immunity, lymphoid organs, broilers

INTRODUCTION

Over the past few decades, antibiotics have been routinely incorporated into poultry diets to function as growth enhancers, as well as for the prophylaxis and therapeutic management of infectious diseases. Antibiotics have also contributed significantly to improving the success of animal husbandry (Yaqoob *et al.,* 2022; Aslam *et al.,* 2024). However, in current situations use of antibiotics are permanent or partially banned almost globally due to antibiotic resistance, therefore, there is an acute need to explore possible substitutes so growers and farmers can sustain or enhance production (Adewole *et al.,* 2021).

Probiotics are innovative feed supplements that have gained widespread popularity in the poultry feed industry following the prohibition of antibiotics in animal diets. Probiotics play a vital role in supporting animal performance and welfare by improving gut health (Yu *et al.,* 2022; Qureshi *et al.,* 2024). Probiotics have been reported to inhibit harmful pathogenic bacteria and decrease enhances stomach pH, increasing the digestive enzyme activity and enhancing the nutrient digestibility (Mohamed *et al.,* 2022; Soomro *et al.,* 2024). They have also been reported to decrease the bacterial production of toxic elements and change the morphology of the intestinal wall in that way decreasing colonization of harmful microbes, like inhibiting epithelial cell damage and described the mechanism of action of probiotics on pathogenic bacteria as penetrating the cell wall of

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pathogenic bacteria and disrupting their normal physiology, by preventing enzymatic reactions and denaturing protein and DNA (Saleh *et al.,* 2020).

Probiotics *species* are being studied to improve production performance, immunity, health status and intestinal morphometry of broilers (Purba *et al.,* 2022). *Enterococcus, Saccharomyces,* and *Bacillus spp.* Based probiotics have potential to high resistant temperatures, shigh acid and bile salt actions, multiply in the intestine to make biofilms and secrete antimicrobials (Larsen *et al.,* 2014). Though various novel multi-probiotics have been used in animal diets, specifically in poultry diet is very limited (Abrioue *et al.,* 2011). Multi probiotics are one of the most important probiotics currently used in fisheries, aquaculture, and poultry feed industries. It is generally accepted that multi probiotics and various enzymes improve growth performance, feed efficiency, specific growth rate, intestinal morphology and health of fishes and other poultry species (Zbikowski *et al.,* 2020). Moreover, combined probiotics also enhanced production performance, significantly reduced ammonia nitrogen emission and provide economic benefits in laying hens (Ding *et al.,* 2016).

Enzymes are well known as biological catalysts and increase the essential biochemical processes for life, and also help the breakdown of macromolecules i.e; protein, fats, and carbohydrates into simple forms (amino acid, fatty acids, and glucose), enhance digestibility and absorption in the gut of animals (Velazquez-De Lucio *et al.,* 2021). The enzymes have an indirect capacity to prevent the proliferation of deleterious bacteria by keeping a suitable environment in the GI tract for the digestibility and absorbability of nutrients (Khatun *et al.,* 2022). Therefore, supplementation of multi-probiotics with enzymes or manganese increases the daily weight gain, nutrient digestibility, and immunity of broilers (Babaei *et al.,* 2021; Bughio *et al.,* 2021).

The use of probiotics, beneficial fungi and exogenous enzymes like protease, cellulase and lipase as possible substitutes for antibiotics and health boosters is gaining strong interest in poultry industry (Gao *et al.,* 2022). Thus, the symbiotic effect of these novel supplement's probiotics, and enzymes might be more effective in improving the growth performance, intestinal morphology, and nutrient utilization by modulation of gut health (Khatun *et al.,* 2022). A few recent research studies reported that the positive effects of multi probiotics and multi enzymes in broilers had improved live body weight and carcasses, the activity of GIT enzymes, intestinal morphology, immunity and integrity and barrier functions (Chowdhry *et al.,* 2020). To the best of our knowledge, very limited studies have examined the effect of the combination of probiotics and exogenous enzymes on the production performance and immunity of broilers.

The research findings on poultry and livestock have prompted new insights into the potential use of *LACTISAR* in poultry feed as an alternative to antibiotic growth promoters (AGPs). However, limited literature exists on the impact of dietary supplementation with *LACTISAR* (multi probiotics and multi enzymes) on broiler production. Thus, we aimed to explore the effect of dietary supplementation of *LACTISAR* on growth performance and immunity of broiler chickens.

MATERIALS AND METHODS

Birds' management and experimental design

A total of 180 broiler chicks (day old) were randomly allotted into three dietary groups, with six replicates of ten chicks in each. The treatment groups are as follows: (i) basal diet (BSD) group was fed with a normal diet (Table 1) (ii) LACT (BSD + 100g *LACTISAR*/ 100 kg feed) and (iii) AGP (BSD + 20g Lincomycin/ 100 kg feed. Before the beginning of trial, the house was cleaned and disinfected properly. The wood shaving (2 inches) was used as bedding material for comfort of birds. Twenty-four hours lighting was provided for birds during the first three days of age, and then it gradually decreased by one hour each week until it reached 18 hours. The initial temperature of broiler house was set at 32°C in the 1st week and gradually reduced to 2C per week till it reached 22°C last week. The humidity was maintained at 55-60% throughout the experiment. The current study was carried out at Poultry Multiplication Centre (PMC), Poultry Production and Research, Hyderabad, Sindh.

Products (*LACTISAR*) and (Antibiotic Lincomycin) sources

LACTISAR is a commercial product containing various probiotics, fungi, and enzymes. It is used as a feed additive in the feed industry. The product for this experiment was purchased from Al- Asar Enterprises, Pvt, Ltd, Pakistan. The main ingredients of (*LACTISAR)* and Lincomycin (AGP) are given in the following (Table 2).

Table 1. Feed ingredients (per kg of diet) and chemical composition of basal diet: Vitamin A, 12,000 I.U; Vitamin D_3, 5000 I.U; Vitamin E, 130.0mg; Vitamin K_3, 3.605mg; Vitamin B_1 (thiamin), 3.0mg; Vitamin B_2 (riboflavin), 8.0mg; Vitamin B_6, 4.950mg; Vitamin B_{12}, 17.0mg; Niacin, 60.0 mg; D-Biotin, 200.0mg; Calcium D-pantothenate, 18.333mg; Folic acid, 2.083mg; manganese, 100.0mg; iron, 80.0mg; zinc, 80.0mg; copper, 8.0mg; iodine, 2.0mg; cobalt, 500.0mg; and selenium, 150.0mg

Components	Starter phase	Finisher phase
Maize	60	72.5
Soyabean meal	36	24.43
Vegetable Oil	0	0.5
L-Lysine Sulphate	0.46	0.4
DL-Methionine	0.35	0.24
L-Threonine	0.18	0.07
MCP	1.06	0.15
Limestone	1.15	0.97
Salt	0.25	0.25
Soda	0.25	0.25
NSP	0.01	0.01
Phytase	0.01	0.01
Toxin Binder	0.05	0.05
Vitamin Premix	0.05	0.05
Mineral Premix	0.05	0.05
L- Valine	0.07	0.01
L-Isoleucine	0	0
Total	100	100
Calculated nutritive composition (%)		
ME (kcal/kg)	2905.00	3004.00
CP	21.53	19.56
Ca	0.96	0.72
Ph	0.58	0.36
Na	0.22	0.20
Cl	0.20	0.20
D. Lysine	1.26	0.98
D.M+C	0.94	0.74
D. Threonine	0.86	0.62
D. Valine	0.96	0.74
D. Isoleucine	0.85	0.67

Table 2. Composition of *LACTISAR*

Sr. No	Name probiotics/ Enzymes	Concentration/ kg
1.	*Bacillus subtilis*	1.0×10^{11} CFU
2.	*Streptococcus faecium*	1.0×10^{10} CFU
3.	*Lactobacillus casei*	1.0×10^{10} CFU
4.	*Saccharomyces cerevisiae*	5.0×10^{10} CFU
5.	Protease	125,000 IU
6.	α Amylase	250,000 IU
7.	β Amylase	10,000 IU
8.	Cellulase	3000 IU
Composition of Lincomycin		
9.	Lincomycin HCL (Soybean base)	44 gms

Growth performance

The weight of birds at 1, 21 and 42 days was measured on a replicate basis. The feed intake was measured daily (replicate basis) throughout the period. However, FCR was calculated at 1-21, 22-42, and 1-42 days of age. Mortality was noted every day. FCR and growth performance were rectified by dead birds. The FCR and ADG were calculated using the following formula.

$$ADG = \frac{\text{Final body weight - Initial body weight}}{\text{Number of days}}$$

FCR= replicate feed consumption/ replicate weight gain (alive + dead birds)

Immune organs

On the 42 days, six birds from each group were slaughtered by Halal method for collection of immune organs. The bursa of *Fabricius*, spleen and thymus were removed from the body and weighed individually and expressed in grams.

Immunoglobulins

Six blood samples from each treatment on a haphazard basis were collected into red cap vacutainers and left for four hours at room temperature to collect the serum. The remaining clotted blood was further centrifuged at 2500 rpm for 15 minutes to collect serum. The serum was put in the Eppendorf tubes and stored at-20°C for further investigation. The immunoglobulins (Ig A, Ig G, and Ig M) were evaluated using an automated analyzer (BTS-350, Biosystem pvt, LTD, France) with Thermo Fisher Scientific company, according to the manufacturer protocol.

Statistical analysis

The recorded data was input into Excel sheets and subjected to analysis using IBM SPSS-24 software. The statistical method employed for the study was one-way ANOVA. The comparison of means between different groups and standard errors were presented. The Duncan multiple range test determined significance levels (P< 0.05).

RESULTS

Growth performance

The impact of *LACTISAR* diet on growth performance is illustrated in (Table 3). The LW and ADG in the AGP and LACT treatment group were significantly boosted than that in the BSD group (P< 0.01). The ADFI showed no change between all groups, and improved FCR was recorded in LACT and AGP groups during starter phase (P< 0.01). Moreover, the birds received a diet with LACT supplementation increased LW and ADG followed by AGP and BSD during finisher and overall phases (P< 0.01). No difference was noted on ADFI during finisher and overall phases among all groups. The FCR recorded better in LACT in comparison with AGP and BSD during finisher and overall periods (P< 0.01).

Weight of immune organs

The impact of dietary supplementation of *LACISAR* is shown in (Table 4). Adding LACT in broiler feed significantly (*P*< 0.01) higher thymus weight compared with BSD treatment, but no difference was noticed with AGP treated group (*P*> 0.01). Moreover, birds fed diets containing LACT showed higher weight of spleen and bursa than BSD (*P*< 0.01). However, similar results were recorded with AGP supplemented group.

Serum immunoglobulins

The influence of dietary supplementation of multi probiotics is shown in (Table 5). Results indicated that the inclusion of LACT in birds diet exhibits a significant (*P*< 0.01) increase in serum Ig A (191.96) level to BDS (119.3) and AGP (158.38) treatment groups. In addition, Ig G and Ig M serum levels were highest (*P*< 0.01) in the LACT compared to AGP and BSD treatments.

DISCUSSION

Nowadays multi- probiotics and multienzymes are getting more attention in the poultry industry for their growth-promoting and immune boosting activity. Moreover, multi probiotics and multienzymes for farm animals have positive impacts on gut health, immune response, and feed utilization (Zhang *et al.,* 2022; Soisuwan *et al.,* 2023). In the current study, LACT treated groups augmented the production performance of broilers. Following previous findings Biswas, *et al.* (2022) clarified that better LBW, ADG and FCR were found in broilers fed a diet containing MP compared to the BSD group. Similarly, Awad *et al.* (2009) reported that feed supplemented with MP heightened the LBW, ADG, and improved feed efficiency in broiler chickens. Yu, *et al.* (2022) implied that MP (*Lactobacillus plantarum* and *Bacillus coagulans*) addition was effective in augmenting the growth and improving the FCR of broilers. Peng *et al.* (2016) demonstrated that feeding Lactobacillus plantarum B1 has significantly improved broilers' LBW, ADG and FCR. Chen *et al.* (2020) asserted that the addition of *Bacillus Lichniformis* fermented feed in broiler feed significantly increased the LBW, ADG as well as improved FCR significantly. Conversely, Zhou *et al.* (2022) directed that body weight and feed efficiency were significantly improved when broilers were supplemented with MP compared to control. Supplementing 5mg MP/kg feed enhanced the LBW (Zhang *et al.,* 2014). Increasing the MP Level from 10^{10} to 10^{11} cfu/kg feed enhanced body weight and improved the FCR (Zheng *et al.,* 2021). Mahdi *et al.* (2022) pointed out that broiler fed diets treated with MP and multienzymes had better LBW, ADG and FCR. The approbative impact of MP and multienzymes could be owing to its antioxidant activity which improves the nutrient digestibility and absorbability (Wang *et al.,* 2021; Zhang *et al.,* 2022). The improvement in growth performance in MP supplemented groups may highlight the essential role of probiotics in enhancing the activity of deiodinase which converts the thyroxin to the active triiodothyronine, which may increase the protein absorption, energy levels, muscle metabolism and comprehensive body growth regulation in birds (El-kelawy *et al.,* 2018). Singh, (2018) stated that the reason for increased weight gain may be due to enhancing the protein utilization of body. Additionally, probiotics and enzymes regulating the somatic cell growth increase muscle mass formation, by enhancing the T_3, T_4, and IGF-I activity (Saleh *et al.,* 2020). The improvement in feed efficiency may result from higher utilization of probiotics due to their distinctive properties for bioavailability, improved extraordinary cellular absorption, significant surface activity, solubility, and mobility (Li *et al.,* 2023). Immunological attributes are the most significant indicators of animals' health and immune status (Heckert *et al.,* 2002). The poultry birds' lymphoid (immune) organs may be responsible for their immune status. The weight and development of the spleen, bursa Fabricius and thymus organs directly determine the overall immune status enhanced of birds and adjustment in the response of stressors present in their living environments. Moreover, these immune organs mature and produce B and T cells, strengthening the immune system. In current experimental trial feeding with a diet containing multi probiotics and enzymes (*LACTISAR*) improves the immune organ indexes. Correspondingly, other studies found the positive impact of various probiotics and enzyme addition on bursa, spleen, and thymus weight of broilers (Attia *et al.,* 2020). Awad *et al.* (2009) revealed that multi strain probiotics significantly impact broiler chickens' immune organ weight. Moreover, Chen *et al.* (2013) described that dietary addition of various probiotics in combination significantly influences the immune organ in broilers. Teo and Tan (2007) highlighted that significant difference found in the weight of lymphoid organs with the addition of various probiotics in broiler feed.

In our study serum immunoglobulins (Ig A, Ig G, and Ig M) were positively boosted by dietary *LACTISAR*. The addition of various probiotics in combination enhanced the immunoglobulins level in serum. Elkelawy *et al.,* (2018) revealed a significant increase in the levels of immunoglobulins in broiler fed diets containing different probiotics and enzymes. Additionally, Gong *et al.* (2018) highlighted that the dietary addition of probiotics augments the concentration of immunoglobulins in the serum of broilers. Amer *et al.* (2021) confirmed that the immunoglobulins were higher in birds diets containing different *lactobacillus* probiotics and protease addition than control. The improvement of immunological attributes may be due to the strong impact of probiotics and their mode of action, primarily as they can elevate the lymphatic function and stimulate the immune responses, increasing disease resistance (Yang *et al.,* 2012). Enhanced immunoglobulin concentration in the serum may be recognized as the significant role of probiotics in increasing T helper cells, B cell lymphocytes, stimulating cytokinin secretions that are very important for humoral immunity and synthesis of immunoglobulins (Arif *et al.,* 2020).

The inclusion of probiotics and enzymes in the broiler diet positively impacted the growth of production and immunological attributes in the current study. These advantages were endorsed by the LACT to regulate enzyme activity and modulate the gut microbiome. By enhancing the growth and immune functions, this study offers a promising solution to the challenges of broilers health.

Table 3. Effect of dietary supplementation of *LACTISAR* on growth performance of broilers

Parameters	**BSD**	**LACT**	**AGP**	**SE**	**P. Value**
Starter phase					
LW	728.19 ^{b}	843.59 ^{a}	854.40 ^{a}	20.17	0.006
ADG	34.67 ^{b}	40.17 ^{a}	40.68 ^{a}	0.96	0.006
ADFI	52.16 ^{a}	52.44 ^{a}	55.24 ^{a}	0.62	0.071
FCR	1.50 ^{a}	1.31 ^{b}	1.35 ^{b}	0.12	0.001
Finisher phase					
LW	1621.64 ^{c}	1881.26 ^{a}	1733.89 ^{b}	31.69	0.0001
ADG	73.71^{c}	85.51 ^{a}	78.81 ^{b}	1.44	0.0001
ADFI	134.11 ^{a}	134.92 ^{a}	136.08 ^{a}	2.42	0.870
FCR	1.81 ^{a}	1.57 ^{c}	1.72 ^{b}	0.49	0.0001
Overall phase					
LW	2276 ^{c}	2639.35 ^{a}	2594.8 ^{b}	47.42	0.001
ADG	54.19 ^{c}	62.84 ^{a}	59.74 ^{b}	1.12	0.001
ADFI	93.14 ^{a}	93.68 ^{a}	95.66 ^{a}	1.05	0.477
FCR	1.71 ^{a}	1.49 ^{c}	1.60 ^{b}	0.26	0.0001

^{a,b,c} in the similar line with dissimilar letters change significantly. SE= standard error; P= (probability <0.05); BSD= basal diet; LACT= *LACTISAR* (multi probiotics +enzymes); AGP= Anitibiotics; LW=live weight; ADG; average daily gain; ADFI= average daily feed intake.

Table 4. Effect of dietary supplementation of *LACTISAR* on immune organs of broiler chickens (g)

Parameters	BSD	LACT	AGP	SE	P. Value
Thymus	2.55^{b}	3.92^{a}	3.76^{a}	0.18	0.0001
Spleen	2.04^{b}	3.10^{a}	2.89^{a}	0.12	0.0001
Bursa	3.22^{b}	6.14^{a}	5.96^{a}	0.23	0.0001

^{a,b,c} in the similar line with dissimilar letters change significantly. SE= standard error; P= (probability <0.05); BSD= basal diet; LACT= *LACTISAR* (multi probiotics +enzymes); AGP= Antibiotics.

Table 5. Effect of dietary supplementation of *LACTISAR* on serum immunoglobulins of broiler chickens

Parameters	BSD	LACT	AGP	SE	P. Value
Ig A (µm/mL)	119.3^{c}	191.96^{a}	158.38^{b}	8.08	0.0001
Ig G (µm/mL)	519.59^{c}	600.74^{a}	551.50^{b}	9.71	0.001
Ig M (µm/mL)	1162.07^{c}	1422.71^{a}	1262.22^{b}	30.07	0.0001

^{a,b,c} in the similar line with dissimilar letters change significantly. SE= standard error; P= (probability <0.05); BSD= basal diet; LACT= *LACTISAR* (multi probiotics + enzymes); AGP= Antibiotics; Ig= immunoglobulins.

CONCLUSION

In conclusion, the inclusion of *LACTISAR* (LACT) in broiler diets led to significant improvements in live weight, average daily gain, and feed conversion ratio (FCR). Furthermore, LACT positively impacted the immune status of broiler chickens. These findings suggest that LACT could serve as a viable alternative to antibiotics, providing an effective strategy to reduce reliance on antibiotics in broiler production.

CONFLICT OF INTEREST

We certify that there is no conflict of interest regarding anything associated with this manuscript.

AUTHOR'S CONTRIBUTION

A. H. Rajput: Conducted experimental trial and laboratorial analysis.
S. A. Pirzado: Conceptualize, supervised Research and final proof reading of the manuscript.
M. S. Bughio: Assisted in Feed formulation and experimental trial.
M. Zakria: Wrote the first manuscript draft.
M. B. Peerzado: Statistical data analysis.

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