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EFFECT OF NOVEL PROBIOTIC PRODUCED FROM *PEDIOCOCCUS ACIDILACTICI* ON BROILER LIVE PERFORMANCE, CARCASS TRAITS AND NUTRIENT DIGESTIBILITY

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Abstract

The bacterial isolate, identified as *Pediococcus acidilactici* (NMCC-G, MK072824, 97.65% similarity) with the sequences of different lactic acid bacteria species in the NCBI genes database was used for the production of novel probiotics. A biological trial was conducted by using one-day-old Ross-308 broiler birds to determine the supplemental effects of novel indigenous probiotics. The broiler chicks were allocated at random to one of the five dietary treatment groups in a way that each treatment group was fed to 5 replicates with 15 birds per replicate. Dietary treatments include probiotic-free basal diet (Control; C), indigenous probiotic (2.01 x 109 CFU/g) with three different inclusion levels; 1 gm/10 kg of diet (IProb-1), 1.5 gm/10 kg of diet (IProb-2) and 2.0 gm/10 kg of diet (IProb-3). The broiler diets were formulated according to nutrient specifications for Ross-308. The results indicated that broilers fed diets supplemented with IProb-3 had lower feed intake, higher ($P<0.05$) body weight gain, and better FCR in comparison to broilers fed control (no probiotics). Carcass yield and breast meat yield increased with increasing levels of IProb. The highest values were noted in IProb-3 (@ 2 gm/10 kg of diet). The total tract apparent digestibility coefficient for crude protein and dry matter was higher in probiotic-supplemented groups compared to the control. In conclusion, the locally isolated *Pediococcus acidilactici* NMCC-G strain @ 2.0 gm/10 kg (having 2.01 $x10^9$ CFU/g) resulted in better growth and feed efficiency in broiler birds as compared to that of non-supplemented birds.

Keywords: *Pediococcus Acidilactici*, Novel Probiotic, Broiler, Performance, Nutrient Digestibility.

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INTRODUCTION

Antibiotics have been used to control pathogenic microorganisms in poultry. However, sub-therapeutic levels of antibiotics have also been used in some countries as a growth promoter in farm animals including poultry. The issue of antibiotics resistance in birds and humans led the European Union to ban its use as growth promoters in the year 2006 (Anadon, 2006). This has resulted both in increased disease incidences and decreased animal performance (Jha et al., 2020). The poultry sector is still facing the challenge of overcoming different diseases, including enteric diseases, due to the elimination of antibiotic feed additives. These enteric diseases are causing an increase in pathogenic bacterial load in the intestinal tract of birds, malabsorption of nutrients, and associated contamination of feed (Adhikari et al., 2017). Probiotics are considered as one of best alternative to antibiotics feed additives that may enhance the health and growth of broiler birds (Jha et al., 2020).

Probiotics are mono or mixed cultures of “live microorganisms” which when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2002). Probiotics generally improve the body weight gain, feed intake, feed: gain and intestinal health of chickens (Mookiah et al., 2014; Mountzouris et al., 2010).

Probiotics have the ability to positively affect the intestinal histomorphometry and improve intestinal digestion (Pelicano et al., 2005). Improvement in the microbial balance of chicken GIT has also been reported (Tarabees et al., 2019). Carcass characteristics have also been improved by supplementing probiotics in broiler diets (Soomro et al., 2019).

A number of microbes have been used as probiotic species in poultry feed during the past years. However, Lactic Acid Bacteria (LAB) species like *Leuconostoc, Lactococcus, Lactobacillus, Bifidobacterium, Lactosphaera, Melissococcus, Enterococcus, Pediococcus, and Streptococcus,* are commonly used in poultry as probiotics (Kerry et al., 2018). Recent studies have shown that LAB has mostly been used for preparing probiotics due to their status as “Generally Recognized as Safe” (GRAS) (Todorov et al., 2020).

Pakistan is producing more than 5.91 million tons of poultry feed per anum (PPA, 2022-23). Imported probiotics are the commonly used as microbial feed additives in both poultry and livestock diets, however, the imported probiotic products are not animal species specific and most of them have mixed species culture. The use of species-specific probiotics is thought to perform effectively owing to their presence in the same niche and compatibility with the gut environment (Dowarah et al., 2018).

Hence, it is hypothesized that a species-specific and indigenous probiotic, if made available will enhance poultry productivity in cost-effective manner. Keeping in view these issues and the possible benefits of indigenous probiotics, the present study was planned to determine the effect of indigenously produced probiotic product on the performance and blood parameters in broiler birds.

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MATERIALS AND METHODS

Dietary Treatments and Experimental Plan

This study was approved by the Institutional Ethic Committee (Approval No. PMAS-AAUR/IEC/89, PMAS-Arid Agriculture University Rawalpindi, Pakistan). A total of 300 one-day-old (Ross-308) male broiler chicks were used to determine the supplemental effects of indigenous probiotic produced from *Pediococcus acidilactici* NMCC-G strain. The broiler chicks were randomly allocated to one of four dietary treatment groups in a way that each treatment was fed to 5 replicates having 15 birds per replicate. Dietary treatment includes probiotic free basal diet (Control; C), novel probiotic (2.01×10^9 CFU/g) with three different inclusion levels; 1 gm/10 kg of diet (IProb-2), 1.5 gm/10 kg of diet (IProb-3) and 2.0 gm/10 kg of diet (IProb-4). Experimental diets were fed upto 35 days of broiler age. The novel probiotic was produced from *P. acidilactici* NMCC-G strain at the National Institute of Genomic and Advanced Biotechnology (NIGAB) laboratory, National Agriculture Research Council (NARC), Islamabad. Indigenous probiotic contained 2.01×10^9 CFU/g. The basal diet was formulated by following the nutrient guidelines recommended for Ross 308 (Aviagen, 2019). The formulation of experimental diets was made on the basis of the digestible amino acids (DAA), where lysine was used as a reference amino acid. The ingredients and nutrients composition of experimental diet is given in Table 1.

Table 1: Ingredient and Nutrient Composition of Experimental Broiler Diet

Ingredients composition	**(%)**
Maize	65.61
Soybean Meal 44%	23.49
Fish Meal	5.00
Poultry Fat	1.30
Marble Chips	0.87
Mono Calcium Phosphate 22.5%	0.62
NaCl	0.21
$NaHCO_3$	0.23
Lysine sulphate 70%	0.27
DL-Methionine	0.23
L-Threonine	0.07
Choline Chloride 70%	0.10
Broiler Vitamins premix[1]	1.00
Broiler Minerals premix[2]	1.00
Total	**100.00**
Nutrients composition Calculated values	**(%)**
Dry matter	89.65
Crude protein	19.50
ME (Kcal/kg)	3200
Ether extract	5.30
Crude fiber	4.50
Ca	0.79
Total P	0.75
Non phytate P	0.40

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Na	0.18
K	0.73
Cl	0.18
Digestible amino acids[3]	***(%)***
Dig. Lysine	1.03
Dig. Methionine	0.43
Dig. Met + Cyst	0.80
Dig. Tryptophan	0.16
Dig. Threonine	0.69
Dig. Isoleucine	0.71
Dig. Arginine	1.10
Dig. Leucine	1.13
Dig. Valine	0.78
Analyzed values	***(%)***
Dry matter	91.60
Crude protein	19.30
Ether extract	4.90
Crude fiber	4.70

[1]Broiler vitamins premix for starter phase = each kg premix contains vitamin A 15,000 IU, vitamin D_3 3,000 IU, vitamin E 80 mg, vitamin K_3 3.6 mg, vitamin B_1 3 mg, vitamin B_2 10 mg, vitamin B_3 60 mg, vitamin B_5 15 mg, vitamin B_6 4 mg, vitamin B_9 2 mg, vitamin B_{12} 1.2 mg, vitamin H 0.2 mg.

[2]Broiler minerals premix for starter Phase = each kg mineral premix contains Mn ($MnSO_4.H_20$) 80 mg, Zn ($ZnSO_4.H_20$) 80 mg, Cu ($CuSO_4.H_2O$) 10 mg, Fe ($FeSO_4.H_2O$) 60 mg, Iodine (KI) 1 mg, Se (Sodium Selenite) 0.2 mg.

[3]Digestible amino acids were calculated on the basis of DM and CP contents of the ingredients from Brazilian Tables of Poultry and Swine (Rostagno & Becker, 2005).

Animal Housing and Experimental Animals

One-day-old broiler chicks (Ross 308) were purchased from the local hatchery (K.K. Chicks Pvt. Ltd. Rawat, Pakistan) and reared for 35 days at Avian Research Station, Department of Poultry Sciences, PMAS Arid Agriculture University, Rawalpindi. The optimum environmental conditions *viz*., temperature, humidity, ventilation, and lighting at each stage of development were maintained as per guidelines recommended for Ross 308 (Aviagen, 2018).

Data Collection

The following parameters were recorded during the performance trial.

Growth Performance and Carcass Yield

Feed intake (FI) and body weight (BW) of birds were recorded on weekly basis to calculate weekly weight gain (BWG) and feed: gain. The data on mortality, if any, was also recorded.

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At the end of the experimental period (day 35), three birds from each replicate were randomly selected and slaughtered by severing the jugular vein. The carcass and breast meat yield were recorded as percentage.

Nutrients Digestibility Trial

The total tract nutrient digestibility coefficient was determined by using celite® as an external marker mixed @ 1 % of the experimental diet. These diets were fed to four experimental birds from day 35 to 38. The plastic sheets were spread in each pen to collect the fecal samples for 24 hours on the 38th day of the experimental period. It was made sure that fecal samples were not mixed with feed, feathers, and litter. Feces collected from 4 randomly selected birds from each replicate per treatment pen were dried and ground to pass through a 2 mm sieve of grinder (Mountzouris et al., 2010) for further nutrients analysis.

Chemical Analysis

The samples of experimental diets and feces were analyzed for dry matter, crude protein, crude fiber, ash and ether extract contents as described in AOAC, 2005.

Statistical Analysis

The data collected were analyzed by ANOVA following completely randomized design. The General Linear Model (GLM) procedures of Statistix software (version 10.0; Analytical Software, Tallahassee Inc., USA) was used for data analysis. The means, if significant were compared by using Tukey's Honestly Significant Test at $P \leq 0.05$ (Steel et al., 1997).

RESULTS

Growth Performance

The novel probiotics (IProb-3) supplemeted birds have shown better growth response as compared to other groups, during the whole grow-out-period (Table 2). The broiler birds during the whole grow-out period (1-35 d), broilers fed IProb-3 supplemented diets had higher (P<0.05) BWG and lower FI and FCR in comparison to broilers fed control (no probiotics) diet.

Table 2: Effect of novel probiotics on growth performance of broiler birds

Treatment/Diets*	FI[1]	BWG[1]	FCR[1]
	Day 1-35		
C	3103±0.6^{a}	2081±10^{c}	1.49±0.007^{c}
IProb-1	3095±2.6^{bc}	2127±14^{bc}	1.46±0.009^{bc}
IProb-2	3092±0.8^{c}	2175±18^{ab}	1.42±0.012^{ab}
IProb-3	3092±1.4^{c}	2193±12^{a}	1.41±0.008^{a}
P-value	0.01	0.01	0.01

^{a,b,c} Means in each column having different superscripts differ significantly (P< 0.05). Results are mean values from five replicates and presented as Mean ± S.E.

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[1]FI =Feed intake (g), BWG= Body weight gain (g), FCR= Feed conversion ratio

[*]C= Control diet without Probiotic; IProb-1, 2, 3 =Indigenous Probiotic containing diets having *Pediococcus acidilactici* (2.01 x10^9 CFU/g) added @ 1.0, 1.5 and 2.0 gm/10 kg of diet, respectively.

Slaughter Parameters

A non-significant (P>0.05) effect was noted on the weights of the liver, heart, bursa, spleen, and pancreas in all experimental groups (Table 3). However, the GIT length, carcass yield, and breast meat yield of broilers were significantly (P<0.05) affected by probiotics supplementation. The GIT length was significantly (P<0.05) higher in all IProb groups than control. Whereas no difference (P>0.05) was noted between all IProb groups. The carcass yield and breast meat yield increased with increasing supplementation of IProb and highest values were noted in IProb supplemented @ 2 gm/10 kg of diet (IProb-3).

Table 3: Effect of indigenous and commercial probiotics on slaughtering characteristics in broiler birds

Treatment/ Diets*	Liver (g)	Heart (g)	Bursa (g)	Spleen (g)	Pancreas (g)	[1]GIT (cm)	[1]CY (%)	[1]BMY (%)
C	40±0.8	11±0.3	3±0.2	1.6±0.1	4.7±0.3	200±3.9^{b}	61±0.6^{b}	21±0.3^{c}
IProb-1	44±0.7	10±0.2	3±0.3	2.0±0.2	4.7±0.2	216±5.2^{a}	64±1.0^{ab}	22±0.4^{bc}
IProb-2	44±1.3	10±0.3	3±0.1	2.1±0.1	4.7±0.3	214±2.4^{a}	65±0.9^{ab}	23±0.3^{ab}
IProb-3	44±1.5	10±0.4	3.±0.2	2.1±0.1	4.7±0.2	214±3.7^{a}	65±1.1^{a}	2±0.3^{a}
P-value	0.11	0.56	0.55	0.13	1	0.02	0.02	0.01

[a,b,c] Means in each column having different superscripts differ significantly (P< 0.05). Results are mean values from five replicates and presented as Mean ± S.E.

[1]BYM= Breast Meat Yield, CY= Carcass Yield, GITL= Gastrointestinal length

[*]C= Control diet without Probiotic; IProb-1, 2, 3 =Indigenous Probiotic containing diets having *Pediococcus acidilactici* (2.01 x10^9 CFU/g) added @ 1.0, 1.5 and 2.0 gm/10kg of diet, respectively.

Nutrients Digestibility in Broilers Birds

The total tract nutrient digestibility was significantly different (P< 0.05) between the control and probiotics supplemented groups (Table 4).

The dry matter (DM), CP and ash digestibility coefficient values, were higher (P< 0.05) in probiotic fed groups IProb-3 than the control. However, non-significant (P>0.05) differences were noted among all groups for ether extract and nitrogen free extract.

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Table 4: Effect of Indigenous and Commercial Probiotics on Nutrients Digestibility of Broiler

Treatment/Diets*	Total tract apparent digestibility coefficient (%)				
	DM[1]	Ash	CP[1]	EE[1]	NFE[1]
C	69±1.1[c]	35±2.0[c]	79±0.8[b]	64±1.7	78±1.7
IProb-1	73±0.6[a]	43±1.4[b]	83±1.4[ab]	71±1.4	80±0.9
IProb-2	73±0.7[a]	46±1.9[a]	84±0.8[ab]	71±1.2	81±0.6
IProb-3	74±1.2[a]	49±0.8[a]	85±0.9[a]	75±1.8	83±0.9
P-value	0.02	0.01	0.02	0.09	0.23

[abc] Means in each column having different superscripts differ significantly (P< 0.05)

[1]DM= Dry matter, CP= Crude protein, EE= Ether extract, NFE= Nitrogen free extract

*C= Control diet without Probiotic; IProb-1, 2, 3, =Indigenous Probiotic containing diets having *Pediococcus acidilactici* (2.01 x10^9 CFU/g) added @ 1.0, 1.5 and 2.0 gm/10kg of diet, respectively.

DISCUSSION

This study was conducted to evaluate the supplemental effects of indigenous (*Pediococcus acidilactici* NMCC-G strain) on the performance parameters of Ross 308 broiler. Probiotic fed-diets resulted in lower feed intake with higher body weight gain (BWG) and better feed: gain ratio (FCR). Results of FI were similar with the findings of Mookiah et al. (2014) who reported reduced FI in broilers supplemented with probiotics. In contrast, FI was increased by supplementing probiotics in the diet (Afsharmanesh & Sadaghi, 2014). The difference in findings could be due to multiple reasons *viz*., birds, sex, LAB strain and dose rate (Ferreira and Kussakawa, 1999). Results of BWG were similar with the findings of Sohail et al. (2012), who reported increased BWG with increasing levels of probiotics. In contrast, Yousefi and Karkoodi, (2007) has found that probiotics supplemented-diets had no affect BWG. The mechanism through which beneficial microbes improved weight gain is still not clear. However, it is presumed that probiotics' ability to produce multiple enzymes (*viz.,* protease, amylase and lipase) had enhanced the digestion of major feed nutrients (Jin et al., 1998) that led to enhanced BWG in broilers (Bedford, 2000). However, Afsharmanesh and Sadaghi (2014) observed that the feed: gain ratio did not improve when broilers were fed probiotic-supplemented diets. This proposes that the impact of various probiotics on the broiler growth can vary.

In the present study, visceral organs *viz*., bursa, heart, spleen, pancreas, and liver of broiler birds were not influenced by the group supplemented with probiotic compared to the control group. The findings agreed with Al-Khalaifa et al. (2019) who observed no change in the weight of visceral organs (*viz*., liver, heart, spleen, & bursa) of broiler birds. This may be due to the reason that effects of probiotics supplementation are equivocal in animals (Olnood et al., 2015). However, carcass yield was improved by probiotic supplementation which agreed with the findings of Mehr et al. (2007), whereas, Saiyed et al. (2015) observed that no effect of probiotic supplementation. Similarlry, BMY was improved with probiotic supplementation which was similar with the findings of Mehr et al.

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(2007), while, Abudabos et al. (2015) observed no improvement in BMY after supplementing with probiotics. Variations in the results could be attributed to numerous factors, including differences in breed/chicken line, probiotic species/strains, concentration, origin of microbial species, and route of administration of bacterial probiotics (Mountzouris et al., 2007).

The present study indicated a significant effect of probiotic supplementation on total tract apparent digestibility co-efficient of DM, ash and CP in broiler birds, whereas nitrogen free extract (NFE) and ether extract (EE) remained unaffected by experimental diets. Results of CP and DM were in accordance with the findings of Wu et al. (2019) who reported an improvement in the nutrients digestibility of CP and DM of broilers fed a probiotic (*Lactobacillus*) supplementation diet. Contrary to the present results, (Joysowal et al., 2018) noted that the TTADC of DM, EE, NFE, and OM (organic matter)/ash was not affected in pigs fed probiotic supplementation diet. The inconsistency in results could be attributed to the reason that LAB are the natural inhabitant of GIT and are capable of producing digestive enzymes and lactic acid, hence, stimulate peristaltic movements in GIT, and promote the nutrient digestibility (Wang et al., 2011).

CONCLUSIONS

In conclusion, the dietary supplementation of indigenous probiotics produced form *Pediococcus acidilactici* NMCC-G @ 2.0 gm/10 kg (having 2.01 x10^9 CFU/g) resulted in better growth and feed efficiency in broiler birds than non-supplemented birds. The overall performance of broiler birds supplemented with novel probiotic indicated its effectiveness in broiler birds.

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