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PROBIOTIC POTENTIAL OF LOCALLY ISOLATED STRAIN *Lactobacillus brevis* MF179529 AND ITS COMPARISON WITH COMMERCIAL PROBIOTICS IN CHICKEN MODEL

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Current study was designed to investigate and compare the effects of a locally isolated strain of *Lactobacillus brevis* MF179529 (LB) with commercial probiotic (CP), and yeast (Y) on growth performance and meat quality of chicken. One-day old Cobb broiler chicken (n=270) were randomly allocated into six groups (A-F) having 45 birds in each group. Groups, A-E were continuously given 1g of different probiotics (LB, CP, Y, LB+CP, LB+CP+Y, respectively) in 10 L of drinking water for 42 days except group F (Negative Control) which was given plain drinking water. Feed consumption and weight gain were recorded on weekly basis. Subsequently, at day 42, birds were slaughtered and breast meat samples were processed for meat quality parameters. FCR of groups A, D and E was significantly lower than group F. Similarly, group A displayed high body weight compared to B, C and F groups (p<0.05). Among color attributes, L^* (groups A-E) and b^* (Group A, C-E) were significantly higher than group F, while a^* of group A was higher (p<0.05) than groups C and F. The results indicate that supplementation of LB alone and in combination with, commercial probiotics lead to significant improvement (p<0.05) in growth rate, total proteins, mineral and fat contents as well as color attributes and tenderness of meat.
Keywords: Probiotics; *L. brevis* MF179529; feed conversion ratio; relative organ index; meat quality.

INTRODUCTION

Poultry industry is one of the most dynamic and expanding sectors of livestock that contributes a vital role in the economy of a country and provides low cost high quality protein to ever growing population. Since, the implementation of ban on the use of all types of chemical therapeutic growth promoters in livestock, the researchers are trying to find safer alternatives. Probiotics and medicinal plants are among these alternatives. Probiotics are live microorganisms that improve the vigor of animals by maintaining the healthy gut microenvironment (Hill *et al*., 2014). These also provide diverse health benefits through numerous types of interactions *viz*., competitive exclusion, pathogen antagonism and modulation of host immune system (Ohimain and Ofongo, 2012). Furthermore, the efficacy of probiotics can be improved by selection of more efficient strains and blending of different probiotic strains (McFarland *et al*., 2018) *Lactobacillus, Streptococcus, Bacillus, Bifidobacterium, Enterococcus* and *Saccharomyces* are among the wide variety of microbial species that have been used extensively as probiotics in broiler (Vieco-Saiz *et al*., 2019).Yeast (*Saccharomyces cerevisea*) has also been reported to affect growth performance, carcass yield, meat quality parameters and antioxidant ability in chicken (Jayasena *et al*., 2017; Adhikari *et al*., 2017; Chen *et al*., 2017; Wei *et al*., 2017).

Different probiotic formulations are in use, as feed additives in farm animals, with variable efficacy. In addition, new strains are being explored globally with the intentions of improving/launching new probiotic formulation. Lb-MF179529 is a locally isolated strain that is known to possess probiotic properties (bile salt, pH, temperature and NaCl tolerance). In addition, it produces antioxidant and antimicrobial metabolites (Riaz *et al*., 2018). It is hypothesized that Lb-MF179529 may work as an effective probiotic by improving gut environment and competing with pathogens. In current study the efficacy of Lb-MF179529 was explored in independent application and by blending with other probiotics. It is assumed that addition of Lb-MF179529 will improve efficacy of commercially available probiotic preparation.

Additionally, it may provide considerable economic impact if applied at commercial level. Probiotics that are used commercially in Pakistan to improve livestock (Protexin, Floramix Plus) generally are imported from Korean company (Hanpoong industry) that contains multi-strain probiotics *viz*., *Saccharomyces cerevisiae*, *Lactobacillous acidophilus*, *Bacillus subtilis, Bacillus licheniformis* and *Aspergillous oryzae* (Anjum *et al*., 2005; Kim *et al*., 2010).

The objective of present study was to evaluate and compare the growth performance and meat quality of broiler chicks following administration of locally isolated dietary probiotics

strain Lb-MF179529. The strain has accession number MF-175929 and has been deposited with no. FCBP-692 in First Fungal Culture Bank of Pakistan for use of other researchers. The strain was administered alone and in combination with Floramix Plus (*B. licheniformis, B. subtilis*) and Yeast (*Saccharomyces cerevisea*).

***Statement of Novelty*:** Effects of probiotics on growth performance and meat quality are variable. The controversial findings can be linked with strain specificity. Moreover, least information is available on locally isolated probiotic strains. Pakistan spends substantial amount of foreign exchange on the import of probiotics for use in poultry. *Lactobacillus brevis* MF179529 is one of the locally isolated strains. It has already been reported to possess antimicrobial and antioxidant potential. In present study, the probiotic potential of *Lactobacillus brevis* MF179529 has been investigated alone and in combination with commercial strains and yeast on growth and meat quality of broiler chicks.

MATERIALS AND METHODS

***Experimental design*:** The study design was approved by Ethical Committee of the Department of Zoology, University of the Punjab, Lahore, Pakistan. One day-old chicks (n= 270) of Cobb breed with an average weight of 37±02 g were procured from hatchery. The experimental birds were kept in cages fitted with feeders and drinking system, and maintained under standard poultry practices. During the whole period of experimental work, birds were vaccinated as per recommended schedule and examined physically for injuries or abrasions.

Experimental work was conducted at Department of Zoology, University of Punjab, Lahore, Pakistan. The chicks were divided randomly into six groups (A-F) with 45 chicks per group and each group consisted of three replicates of 15 chicks. The groups were treated with different probiotics for six weeks. Details of experimental groups and treatments are shown in Table 1.

***Dose of Probiotics and Mode of Application*:** The probiotics were offered to birds in drinking water at a dose of 1g/10 L. The drinking water was replaced thrice a day. In case of combination, different probiotics were mixed in equal ratio. Drinking water was provided *ad libitum*.

***Experimental studies*:** Animals of each group were given a weighed amount of food daily and leftover feed was weighed and removed from cage. Weight of all animals was recorded on weekly basis. Growth performance of broilers was analyzed in terms of feed consumed, weight gain, food conversion ratio, live body weight and relative organ index. Meat quality was assessed using color attributes, cooking and thawing loss, dry matter, crude fat and crude protein.

***Food Conversion Ratio (FCR)*:** FCR was calculated on day 7, 14, 21, 28, 35 and 42 day of the experiment by dividing feed consumed by total weight gain (Singh and Panda, 1992).

***Slaughtering and sampling*:** Live body weight was recorded using Electronic Compact Scale SF-400A before slaughtering. Birds were slaughtered according to the ethical standards approved by the Ethical Committee of the Department of Zoology, University of the Punjab, Lahore, Pakistan. Internal organs including gizzard, liver, spleen, heart, kidney, gallbladder and lungs were collected without fat and their weight was measured using digital balance (Scalenet Precision Balance GL-01) for calculating organ index. The organ index was calculated by dividing organ weight with live body weight. Samples of breast meat were quickly frozen for meat quality analysis.

***Meat quality*:** Sensory color attributes (L^*, a^*, b^*, chroma value and hue angle) were analyzed to determine the quality of meat. Meat color was measured with a colorimeter (Minolta CR-410 Langenhagen, Germany). TA/TX Plus-Texture analyzer apparatus (UK) was used for determination of tenderness of meat including Warner Bratzler shear force (WBSF) for cooked breast meat. Physical characteristics of

Table1. Experimental grouping, treatments and measurements.

Treatment/Measurements*	Age (days)	Groups					
		A	B	C	D	E	F
LB	1-42	+	-	-	-	-	-
CP	1-42	-	+	-	-	-	-
Y	1-42	-	-	+	-	-	-
LB + CP	1-42	-	-	-	+	-	-
LB + CP + Y	1-42	-	-	-	-	+	-
No Probiotic	1-42	-	-	-	-	-	+
Measurements							
FCR	Weekly	+	+	+	+	+	+
Sampling	42	+	+	+	+	+	+
Relative organ index	42	+	+	+	+	+	+
Meat quality	-	+	+	+	+	+	+

*LB= *Lactobacillus brevis* MF179529; CP= Commercial probiotic, Floramix®, containing *B. licheniformis, B. subtilis*; Y= Yeast (*Saccharomyces cerevisiae*): FCR= Feed conversion ratio.

meat including thawing loss and cooking loss were determined (Bailey *et al*., 1974). Biochemical characteristics (Dry matter, crude protein, crude fat and ash content) of meat were determined following Warriss, 2000.

***Statistical analysis*:** Data were analyzed by using repeated measures ANOVA followed by Bonferroni test for body weight and FCR and oneway ANOVA followed by Tuckey's test for meat quality in SPSS software version 21.0. Values are expressed as Mean ± SEM. Differences were considered significant at $p \leq 0.05$.

RESULTS

***Food conversion ratio (FCR)*:** After one week of feeding all treatment groups displayed lower FCR as compared to the negative control group. From 2nd-4th week, no difference could be observed whereas during 5th-6th week differences in FCR were noticed among different groups. The groups A, D and E displayed significantly ($p<0.05$) lower FCR than negative control group in 6th week. Groups B and C did not show significant difference in FCR from the control group (Figure 1).

In this trial no significant difference was observed in feed consumption among treated groups but live body weight showed statistically significant differences ($p<0.05$). The group, fed on locally isolated strain (group A), showed highest live body weight compared to all other experimental groups (Figure 2). No difference in relative organ weight was observed in this study (Table 2).

***Meat quality attributes*:** Among the meat color attributes the lightness (L^*) of all treated group was higher ($p<0.05$) as compared to negative control group while difference among groups was not statistically significant ($p>0.05$). Whereas, the value of redness (a^*) was higher in group A as compared groups C and F.

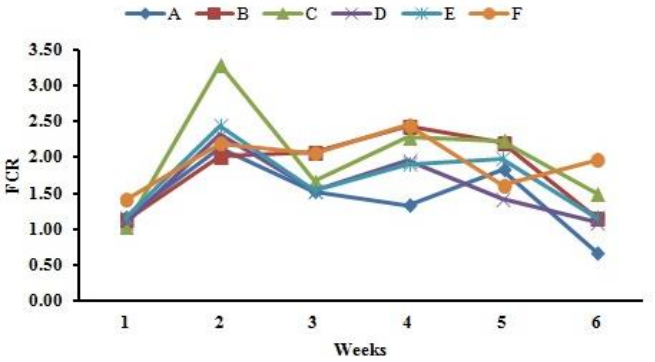

Figure 1. Comparison of different probiotic treatments on feed conversion ratio (FCR) of broiler chicken. [A: *Lactobacillus brevis* MF179529, B: Commercial probiotics Floramix® (*B.licheniformis, B.subtilis*), C: Yeast (*Saccharomyces cerevisiae*), D: *L. brevis* MF179529+ Commercial probiotics, E: *L. brevis* MF179529+ Commercial probiotics+ Yeast and F: Negative Control (without any probiotic)].

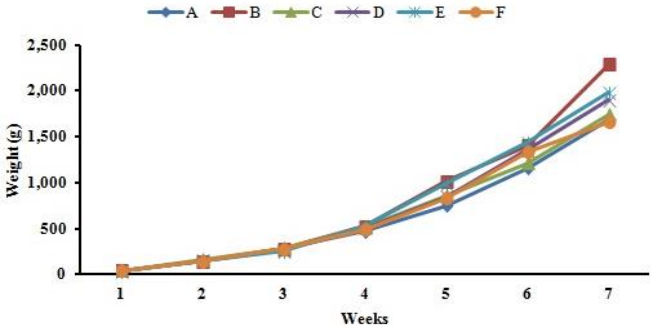

Figure 2. Growth response of chicks having different probiotic treatments. [A: *Lactobacillus brevis* MF179529, B: Commercial probiotics (*B.licheniformis, B.subtilis*), C: Yeast (*Saccharomyces cerevisiae*), D: *L. brevis* MF179529+ Commercial probiotics, E: *L. brevis* MF179529 + Commercial probiotics + Yeast and F: Negative Control (without any probiotic)].

Table 2. Effects of different probiotic treatments on feed consumption, live body weight and organ index of chicken.

Parameter		Treatment Groups*					
		A	B	C	D	E	F
Feed consumed (g)		2728.7±46.25	2977.2±161.92	3138.7±268.62	2700.9±71.98	3203.1±171.37	3131.4±169.29
Live body weight (g)		2290.7±83.65^{c}	1692.9±16.78^{ab}	1742.8±44.15 ^{ab}	1898.4±63.03^{ab}	1985.9±106.96^{bc}	1665.4±41.01^{a}
Organ index (%)	Gizzard	1.76±0.28	1.43±0.26	2.10±0.62	1.41±0.05	1.27±0.13	1.49±0.22
	Liver	3.13±0.29	3.30±0.21	2.21±0.14	1.87±0.09	2.43±0.27	3.14±0.55
	Spleen	0.20±0.02	0.20±0.06	0.11±0.02	0.13±0.01	0.17±0.03	0.18±0.04
	Heart	0.63±0.07	0.73±0.06	0.69±0.01	0.54±0.03	0.63±0.05	1.01±0.11
	Kidney	0.42±0.04	0.43±0.02	0.43±0.19	0.25±0.05	0.26±0.03	0.54±0.19
	Gall bladder	0.16±0.04	0.17±0.04	0.11±0.01	0.09±0.01	0.11±0.01	0.08±0.02
	Lungs	0.62±0.10	0.47±0.04	0.65±0.04	0.60±0.04	0.57±0.07	0.93±0.12

*Treatment groups: A: *Lactobacillus brevis* MF179529, B: Commercial probioticsFloramix®(*B. licheniformis, B. subtilis*), C: Yeast (*Saccharomyces cerevisiae*), D: *L. brevis* MF179529 + Commercial probiotics, E: *L. brevis* MF179529 + Commercial probiotics + Yeast and F: Negative Control (without any probiotic)]. The data was analyzed using repeated measures ANOVA followed by Bonferroni test. The treatments of groups having no common letter (in a row) are significantly different from each other.

Table 3. Comparison of physical and biochemical attributes of meat quality following treatment with different probiotics.

Meat quality		Treatment Groups[#]					
		A	**B**	**C**	**D**	**E**	**F**
Thawing loss (%)		9.33±1.45	15.33±2.60	6.33±0.67	10.67±3.18	8.00±2.08	15.33±1.76
Cooking loss (%)		12.67±0.88^{a}	17.67±0.88^{ab}	28.67±0.88^{bc}	33.00±7.37^{bc}	37.33±5.24^{c}	21.33±2.03^{abc}
WBSF(N/cm^2)		10.92±0.51^{a}	11.04±1.03^{a}	12.24±0.30^{ab}	11.83±0.75^{ab}	14.43±0.29^{b}	13.07±0.69^{ab}
Dry Matter (%)		27.95±0.58	27.85±0.96	27.34±0.26	27.05±0.17	28.89±0.44	27.90±0.49
Crude Protein (%)		85.57±0.26^{c}	84.63±0.53^{bc}	83.26±0.28^{ab}	84.08±0.79^{ab}	83.75±0.21^{ab}	82.50±0.28^{a}
Crude Fat (%)		6.53±0.19^{a}	6.75±0.32^{abc}	7.85±0.08^{bc}	6.67±0.34^{ab}	7.20±0.36^{abc}	7.94±0.20^{c}
Ash (%)		5.13±0.10^{c}	4.21±0.31^{ab}	4.23±0.14^{abc}	4.40±0.28^{abc}	4.93±0.11^{bc}	3.87±0.02^{a}
Meat	Lightness(L*)	55.20±0.21^{b}	51.56±1.24^{b}	52.89±2.10^{b}	51.46±0.64^{b}	56.35±0.19^{b}	41.33±0.07^{a}
sensory	Redness(a*)	20.35±0.32^{b}	18.07±0.64^{ab}	14.57±1.44^{a}	17.16±1.28^{ab}	16.59±0.80^{ab}	14.02±0.15^{a}
attributes	Yellowness(b*)	16.09±0.49^{b}	15.34±1.38^{ab}	17.48±0.75^{b}	16.41±0.30^{b}	17.49±0.34^{b}	12.54±0.24^{a}
	Chroma Value	21.33±0.28	23.75±0.98	22.82±0.49	23.79±0.83	24.11±0.75	23.91±0.40
	Hue Angle	31.07±0.44^{a}	40.19±2.76^{b}	48.98±1.11^{b}	43.87±2.47^{b}	46.80±1.00^{b}	48.98±1.11^{b}

[#]Treatment groups: A: *Lactobacillus brevis* MF179529, B: Commercial probiotics Floramix® (*B.licheniformis, B.subtilis*), C: Yeast (*Saccharomyces cerevisiae*), D: *L. brevis* MF179529 + Commercial probiotics, E: *L. brevis* MF179529 + Commercial probiotics + Yeast and F: Negative Control (without any probiotic). The data was analyzed using one-way ANOVA followed by Tuckeys test. The treatments of groups having no common letter (in a row) are significantly different from each other

Similarly, the value of yellowness (b*) in group A was found higher as compared to group B fed with commercial probiotics strains and negative control group (F). The Chroma values showed no statistically significant difference (p>0.05) among groups. A low hue angle value was found in group A as compared to all other groups. The study also showed that the value of WBSF (indicator of tenderness) was lower in group A and B as compared to C, E and F. No significant difference (p>0.05) was observed in thawing loss among treatment groups. However, group A displayed lower value (p<0.05) of cooking loss as compared to group C, D and E (Table 3).

No significant difference was found in dry matter (p>0.05) among treatment groups. The value of crude protein was significantly high (p<0.05) in group A as compared to group C, D, E and F. Conversely, a significantly (p<0.05) lower value of crude fat was observed in group A as compared to treatment groups C and F. A statistically significant difference (p<0.05) was observed in ash content among experimental groups. The value of ash content was higher in group A when compared with other groups (Table 3).

DISCUSSION

The use of antibiotic feed additives in poultry has been banned due to emergence of resistance in pathogenic microbes and its impact on meat quality. This in turn opened new avenues of research to find alternative growth promoters that could help in supply of quality protein to ever growing human population. The search of probiotics is one of the emerging fields in this context. A number of probiotic strains are being assessed and reported globally with variable efficacy (Kothari *et al*., 2019; Kerry *et al*., 2019).

The current study was intended to check and compare the efficiency of a locally isolated probiotic strain *L. brevis* MF179529, Floramix Plus (a commercial probiotic containing *B. licheniformis* and *B. subtilis*) and yeast (*Saccharomyces cerevisiae*) on growth performance and meat quality of broiler chicken.

***Feed conversion ratio (FCR*)**: Feed conversion ratio is the measurement of animal's efficiency with which they convert consumed feed into body weight. A high value of FCR depicts low efficiency of animals and vice versa (Cottle and Pitchford, 2014). Our data indicated that administration of Lb-MF179529 can result in lowering of FCR in experimental birds. While, supplementation of local strain with commercial probiotic displayed lower FCR as compared to commercial probiotics alone. On the other hand, yeast alone and commercial probiotic did not affect FCR. These findings indicated that Lb- MF179529 can improve FCR alone or in combination with other probiotics. The improvement in FCR in current study might be due to better digestion and absorption of nutrients (Liu *et al*., 2018). Rajput (2012) reported variation in digestive enzymes following treatment with probiotics. In contrast, Sarangi (2016) reported that probiotics do not affect FCR. The discrepancy in this study might be explained on the basis of strain specificity.

The birds of all treatment groups were offered similar quantity of feed. However, consistent with findings of FCR, the body weight of birds per treatment was significantly different. The birds receiving strain Lb-MF179529 alone and in combination with commercial probiotics and yeast displayed higher body weight. While, the birds receiving yeast only showed lower body weight. In recent trial Lb-MF179529 showed overall better results (p<0.05) with reference to weight gain. Our results are in agreement with Khan *et al*.

(2019) who reported weight gain in probiotic treated groups. This weight gain might be due to better digestion and assimilation of feed (Palmidi *et al*., 2016; Ahmad *et al*., 2019). Contrary to our results Salehimanesh (2016) reported no significant difference in weight gain among experimental birds in his study which may also be linked with probiotic strain specificity, dose and mode of administration.

***Relative Organ Index*:** Relative weight of different organs (gizzard, liver, spleen, heart, kidney, gallbladder and lungs) was compared to evaluate any adverse influence of treatments. No significant difference in relative weights of any organ could be observed in this study that points towards safe nature of the probiotics used in the study. Data on safety of probiotics and yeast are already available (Di Gioia and Biavati, 2018) while, current study provided evidence on safe nature of locally isolated strain Lb- MF179529. In consistent with current findings, Yun *et al*. (2017) also reported no significant differences ($p>0.05$) in the organ index (heart, liver, pancreas and spleen) following probiotic treatments.

***Meat Quality*:** Meat quality was assessed using physical and biochemical attributes. In present study, physical attributes including color, cooking loss, thawing loss and texture were analyzed. Among color attributes we analyzed lightness, redness, yellowness, chroma value and hue angle. The color of broiler meat is important because of consumer's preference for fresh and high-quality products. The sensory color attributes *L**, *a** and *b** indicating lightness, redness and yellowness, respectively were higher in Lb-MF179529 treated groups. On the other hand, no statistically significant difference ($p>0.05$) was observed in chroma values. The small hue angle indicates more redness of meat is favorably accepted by consumers. The Lb-MF179529 treated group exhibited low hue angle value as compared to groups fed with yeast and negative control. Contrasting reports are available regarding influence of probiotics on color attributes of meat. Our results are in accordance with the findings of Abdulla *et al*. (2017) who reported higher values of *L**, *a**and *b** in chicken meat supplemented with dietary probiotic as compared to its control. In contrast, Froning (1995) reported low values of color attributes of meat in probiotic fed group. This difference might be due to the growth rate in broiler chicken (Chen *et al*., 2013). The data of *L**, *a**, *b**, chroma value and hue angle indicate that Lb-MF179529 favorably influences meat quality.

Thawing loss, cooking loss and tenderness of meat was evaluated as physical indicators of meat quality after treatment with probiotics in recent trial. The degree of shrinkage upon cooking is directly associated with loss of juiciness while water loss generally is associated with decrease in tenderness and nutritional contents (Al-Owaimer *et al*., 2014). The values of thawing loss and cooking loss were statistically lower in Lb-MF179529 treated groups as compared to negative control group. Data indicates that Lb-MF179529 have positive effect on cooking quality of meat. Our results are in accordance with Abdulla *et al*. (2017) who reported lower cooking loss values following probiotic treatment.

WBSF is an inverse indicator of tenderness of meat (Alfaig *et al*., 2013). The group fed on Lb-MF179529 displayed lower value of WBSF as compared to negative control, yeast and commercial probiotic fed groups. It further strengthens the view that Lb- MF179529 leaves positive impact on meat quality.

In this trial biochemical attributes like dry matter, crude protein, fat and ash contents were analyzed. No significant difference in dry matter in different treatment groups was observed but the crude protein was higher in Lb- MF179529 treated group. The protein contents were also significantly higher in group fed with combination of commercial strains and Lb-MF179529. These findings indicate that Lb-MF179529 affect protein contents of meat. The increase in FCR, growth and meat quality may be due to influence of Lb-MF179529 on digestibility and absorption of food or due to provision of essential amino acids by local probiotic strain. Consistent with our findings Hossain *et al*. (2016) reported higher protein contents in broilers fed on probiotics. Amount of crude fat was lowest in Lb-MF179529 treated group. In addition, ash analysis is an indicator of amount of minerals present in test sample. The ash contents in the group treated with Lb-MF179529 was also significantly better than negative control group. Administration of Lb-MF179529 with commercial probiotic and yeast also resulted in improvement in ash contents. In conclusion, the data of crude protein, crude fat and ash contents clearly indicate that Lb-MF179529 favorably influences meat quality.

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