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PROBIOTIC PRODUCTION FROM BACILLUS SUBTILIS AND ITS EFFECT ON BROILER GROWTH PERFORMANCE

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Abstract

The production of probiotic from Bacillus subtilis bearing accession number KT427377 was carried out by shake flask method. 1 liter of Luria Bertani Agar (LB) charged in 2.5L flasks, the flask was tighten with cotton plug and autoclaved for 15 min (at 121°C and 15 lbs to sterilize . Media was inoculated with fresh prepared inoculum and incubated for 24 hours at 37⁰ C using a rotatory shaker (at 159 rpm). Cultured media was centrifuged (at 10,000 rpm) for 15min to obtain the pellet. Pellet lyophilized. The dried/powdered of Bacillus was filled in a safe container. Colony Forming Units (CFU) was determined in the cell mass of probiotic with the help of serial dilution method.

Anti-bacterial activity was observed. Here the effects on broiler were also checked like Feed Conversion Ratio (FCR), growth performance and carcass parameters. The experiment was conducted on 270 day-old broiler chicks purchased from local market The experimental chicks were arranged randomly in three experimental group's i.e G1, G2 and G3. 90 birds were kept in each group having six replicates having 15 birds. Controlled shed was chosen for the trial. Group 1 was negative control with birds fed with diet having no feed additive. G2 and G3 were positive control groups of birds fed with diet containing probiotic 1% and 1.5% in the starter and finisher diet respectively. Feeders and drinkers were kept for feed and water.

The Feed Conversion Ratio (FCR) in fed on probiotic-supplemented chicks was found significantly ($p<0.05$) higher as compared to control group. Whereas, the chicks of G3 showed a significant ($p<0.05$) heavier weight gain than chicks of G2. Furthermore, the other parameters viz; feed intake, composition of meat, empty organ weights and dressing percentage was found non-significant amongst the all diets. However, supplemented groups showed significantly ($p<0.05$) reduced abdominal fat content. So this experiment shows that the probiotic produce from Bacillus subtilis accession No KT427377 has beneficial effect on broiler health, weight gain and feed efficient.

Keywords: *Probiotic, Bacillus subtilis, Broiler growth, Feed conversion ratio*

INTRODUCTION

The poultry business in Pakistan is an old and traditional practice, as an important part of agriculture sector contributing to national GDP (1.3%). From 1960 the commercial poultry production has been started in Pakistan (1).

Before 1960 the average meat production of a poultry bird was 0.769 kg in 4 months of age (2). But from 2000 to 2010 poultry production increased by introducing using highly productive cross breed Lyallpur silver black (LSB) (3).

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To improve poultry production it is necessary to increase poultry meat production in this way balanced and effective feeding is the most important factor (5). To improve growth performance in broilers the different feed additives like probiotics are generally used (6, 7).

Probiotics are beneficial live microorganisms which improve the host health (8). Use of probiotics is an alternative to antibiotics for poultry production that has the ability to reduce diseases in chicks and contamination of its products (9). Introducing the feed additives like probiotics as growth promoters can enhance the production of birds. Its increase growth performance through various mechanisms which includes; i- Nutrients absorption increased more efficiently, ii- intestinal micro-flora are reduced which increase more nutrients availability to animal, iii- harmful gut bacteria are decreased, iv- reduced the production of growth suppressing toxins or metabolites, v- decreased the microbial de-conjugation of bile acids (10).

On the basis of different modes of action of *Bacillus subtilis* is used as probiotics in poultry feed. The different enzymes like subtilizing and catalase enzymes are produced by *Bacillus subtilis* consuming oxygen in GIT. In response to this the environmental improvement for a beneficial bacterium, like *Lactobacilli* takes place. These beneficial bacteria cause a mechanism known as competitive inhibition through which the gut wall is colonized and block the binding sites of *Salmonella, E.Coli, Campylobacterum* and *Clostridium* which are pathogenic (11).

Probiotics can be prepared by many genera of bacteria but the probiotics prepared by genus *Bacillus* are considered good because of its characteristics like aerobic and spore forming which resist the environment during transportation, storage and feed palliating process (12-14). Many other researchers reported that the use of *B. subtilus* as probiotic in poultry feed reduce the pathogenic contamination with *salmonella* and *clostridium* during slaughtering (15) or with salmonella and campylobacter by the use of *Bacillus subtilis* (16).

In this study probiotics was produced to obtain the above mentioned beneficial effects for poultry birds. *Bacillus subtilis* KT427377 was used as a source having good antimicrobial properties in the lab as well as in poultry bird's intestines.

METHODOLOGY

SELECTION OF BACTERIA

In order to produce the probiotic by using the shake flask method we selected *Bacillus subtitles* bearing the accession number KT427377 received from Institute of Industrial Biotechnology, G C University Lahore.

CELL CULTURE IN BULK BY SHAKE FLASK METHOD

Cells in bulk were obtained to follow Zia PhD thesis in 2017. Sterilized equipment was used in the procedure. Molasses a cheap and easily available medium was used in the process. For optimization the LB media was used. The molasses as fermentation medium was clarified because the molasses having ash and other compounds in high amount freshly prepared inoculums was always used because of the high number of live bacteria present in fresh culture.

During fermentation process the shake flasks method was used, 2.5 liter volume flask was filled with one liter of 6% molasses. The flask was tighten with cotton wool plug and then the fermented medium was kept in an autoclave for sterilization (at 121°C temperature and 15 lbs/in2 pressure) for the time of 15 minutes with 7 pH, cooling process took place after sterilization. After cooling freshly prepared bacterial inoculums inoculated in flasks then incubated it at 37°C on a rotator shaker at 159rpm for 24hrs. 5 L cell suspended media 1 L in each flask of culture broth was then centrifuged at 10 thousand rounds per minute for 15minuts to separate supernatant are discarded but the pellet washed for 3 times with distilled water, centrifuged and stored for lyophilization, this procedure carried out in lyophilized until to get the product in pure powder. The dried/powdered permitted *Bacillus* filled in a safe container.

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COLONY FORMING UNITS (CFU) AFTER LYOPHILIZATION

The method used to count the CFU after freeze drying was serial dilution (17). For spore suspension the 90°C heat for 15 minutes was applied as a heat shake method. To get spore suspensions between 10^{-5} and 10^{-10} spores per ml, the serial dilution was prepared. From each dilution 100 µl aliquots were plated on LB agar plate in triplicate. Then incubated at 37°C for 24 hours. At last counted the colony number and in each sample calculated the mean number of CFU per ml.

BIRD HUSBANDRY

At this stage of trail preparation of housing, purchasing, vaccination, distribution in different groups, rearing and slaughtering of broiler chicks took place.

HOUSE MANAGEMENT FOR TRAIL BIRDS AND MANAGEMENT

From the local market at Quetta 270 one-day old broiler chicks were purchased. Each bird had an average body weight 41g by weighing individually. The vaccination against diseases, like New Castle Disease, Infectious Bronchitis, Infectious Bursal Disease and Hydro-pericardium Syndrome was done for the prevention measures. The selected birds were randomly distributed in three treatment groups in six replicates. There were 15 chicks per replicate. There were separate pens measuring 6*6 areas for each replicate, before starting the trail the area was insured to clean with disinfectant and fumigated to avoid diseases. The litter was of saw dust made in each pen. During the trial the precautionary measures were followed as the recommended practices for broiler rearing. Temperature was noted daily, at morning, afternoon and evening relative humidity by agro thermometer.

DIET PREPARATION AND FEEDING

Commercial starter and finisher diets supplemented with probiotics in feed as follows:
Group 1: negative control diets containing no probiotics.
Group 2: diets containing 1% probiotics.
Group3: diets containing 1.5% probiotics.
The composition of starter and finisher diets is given in Table 1.

Table I. Composition of feed distributed to each group of birds equally

Ingredient	Starter (0-3weeks)	Finisher (4-6 weeks)
Soyabin cack	12%	12%
Maiz	50%	45%
Rice bran	10%	15%
Ground net cake	25%	25%
Minral mixer	2%	2%
Naftin	25gm	25gm
Vitamin A,B2,D3+k	20gm	20gm
Maridot	50gm	50gm

The birds were weighed and the FCR of each group was calculated at weekly intervals to calculate the Feed Consumption Ratio (FCR). Therefore, the calculation of FCR and feed consumption were taken from the average value of each group in pens. Six chicks from each replicate were selected for slaughtering purposes on the basis of body size on completion of the experiment. Immediately after slaughtering, the chicks eviscerated for abdominal fat content, intestinal length, and intestinal weight and edible offal's i.e. liver, heart and gizzard collection. The carcass weight was recorded after skin removal and non-edible organs. The results of the experiment were economically analyzed by the total weight gain cost minus the total expenditure on per chick during the trial.

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Table II. Experimental birds arranged for group1, group2 and group3 dietary treatments as such

Treatment	G1	G2	G3	Total birds
Replecate1	15	15	15	45
Replecate2	15	15	15	45
Replecate3	15	15	15	45
Replecate4	15	15	15	45
Replecate5	15	15	15	45
Replecate6	15	15	15	45
Total	90	90	90	270

Table II shows three different groups of treatment. Each of the G1, G2 and G3 replicate has 15 birds. Thus, with 45 birds, each group has 6 replicates, whereas, total number of birds were 270 in the whole experiment.

STATISTICAL ANALYSIS

Complete Randomized Design (CRD) was used for statistical analysis. And analysis of variance (ANOVA) technique was used to analyze the collected data (18) a portable software SAS, 9.1 (2002-03) used. And least significant used to compare the means.

RESULTS AND DISCUSSION

Bacillus subtillis obtained had CFU 4.9*10/gm. Our results, as shown in the Table I, experimental chicks fed a commercial diet without any probiotic addition (group1), while an addition of probiotic diets were given at the ratio of 1.0% (group 2) and 1.5% (group 3) in the starter and finisher feeds respectively. The average live weight gain of the group fed with probiotic supplement was found significantly ($p<0.05$) higher during overall growing period Table IV. Whereas, the chicks of group 3 showed significantly ($p<0.05$) higher weight gains as that of the chicks of group 2 (Table III). However, during overall growing period Feed Conversion Ratio (FCR) was observed significantly ($p<0.05$) lower in the groups 2 and 3 given the probiotic additive feeds comparing with the controlled one, similarly, significant difference was found between the supplemented groups 2 and 3 (Table III).

Table III. Growth Performance Summary of Broiler Fed Probiotics supplemented feed and without probiotic (five weeks study).

Calculated Parameters	Group1	Group 2	Group 3
Average Body Weight (g)	1.84 ± 0.03^{c}	2.08 ± 0.07^{b}	2.12 ± 0.04^{a}
Total Weight Gain (g)	1.78 ± 0.03^{b}	2.07 ± 0.04^{b}	2.13 ± 0.05^{a}
Total Feed Intake (g)	3.48 ± 0.04^{a}	3.33 ± 0.05^{b}	3.31 ± 0.02^{b}
Average Feed Consumption Ratio (FCR)	1.78 ± 0.03^{a}	1.55 ± 0.04^{b}	1.39 ± 0.04^{c}

Table III shows the growth performance of the experimental birds of three different groups where the best FCR was obtained in group 3 where probiotic was added in the feed in high quality as compare to other 2 groups. Table IV shows the carcass parameters in different groups after slaughtering the birds

Many species of the bacteria belonging to the genus *Bacillus* present in nature are diverse and commercially useful (19). Now a day, the use of probiotics has good results in feeding practice of animals to maintain the health and support gut micro- flora. Spore forming probiotics of the genus *Bacillus subtilis* have been referred (DGS MAGZIN 2014). Therefore, the probiotics prepared by genus *Bacillus,* bacteria have its characteristics like aerobic and spore forming which helps in transportation, storage, feed palliating process, to maintain animals' health and help GIT micro-flora (20).

In our study the shake flask method for cell culture was used and obtained a very huge amount of colony forming unit (CFU) 4.9*10 10cfu/gm.

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Table IV. Carcass Characteristics Observed in Experimental Broiler Birds in G1 G2 and G3

Parameters	G1	G2	G3
Average live body weight (BW, g/bird)	1952 ± 72a	1965 ± 44a	1975 ± 55a
Average dressed carcass weight (g/bird)	1171 ± 50a	1179 ± 69a	1205 ± 49a
Average liver weight	43	44	44
Average heart weight	10	10.1	10.3
Average gizzard weight	39.11±0.32[b]	40.95±0.33[a]	40.89±0.21[a]
Average abdominal fat	21.09±1.70[a]	18.97±1.14[a]	18.10±0.84[b]

Many researchers proved that the use of probiotics via feed has a good effect on broiler growth performance, feed efficiency, enhance nutrient and increase birds' average daily weight gain which was proven in our study. Our study follows the arguments of Jin et al., 1996 (21). When we compared a negative controlled group with probiotics delivered group via feed as a feed supplement, it had a significant effect on growth performance and feed conversion in positive controlled groups. The main focus in this study was the production of probiotics through the fermentation process by the shake flask method for cell culture. Here we obtained colony forming unit (CFU) 4.9*10 10cfu/gm. Furthermore, to determine the benefit of addition of a probiotic strain *Bacillus subtilis* KT427377 having good antimicrobial activities (particularly against *Salmonella)*, showed remarkable results to improve the parameters viz; feed intake, growth performance, intestinal morphology and gut micro-flora in the experimented broiler chicken groups.

CONCLUSION

Supplementation of probiotics in the feed can boost the broiler health and growth performance, through contributions to gut health and nutrient use. *Bacillus subtilis* KT427377 has been a popular bacterium used as brobiotic within the poultry industry and was shown to improve feed intake, growth performance, intestinal morphology and gut micro-flora.

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References:

1. Hussain J, Rabbani I, Aslam S, Ahmad HA. An overview of poultry industry in Pakistan. World's poultry science journal. 2015;71(4):689-700.
2. Sahotaaw BB. Production performance of classified chicken as affected under deep litter stem. Pakistan journal of veterinary research. 2003:35-8.
3. Sadiq M. Pakistan poultry sector still on an upward swing. Poultry World. 2004;20(8):10-1.
4. Memon NA. Poultry: Country's second-largest industry. Exclusive on Poultry. 2012 Nov.
5. Bidarkar VK, Swain PS, Ray S, Dominic G. Probiotics: Potential alternative to antibiotics in ruminant feeding. Trends in Veterinary and Animal Sciences. 2014;1(1):1-4.
6. Abolfotouh MA, Al-Alwan IA, Al-Rowaily MA. Prevalence of Metabolic Abnormalities and Association with Obesity among Saudi College Students. International journal of hypertension. 2012;2012:819726.
7. Fadlalla IM, B.H. & Bakhiet, Amel. Effect of Feeding Garlic on the Performance and Immunity of Broilers. Asian Journal of Poultry Science. 2010;4:182-9.
8. Manafi M. Comparison Study of a Natural Non-Antibiotic Growth Promoter and a Commercial Probiotic on Growth Performance, Immune Response and Biochemical Parameters of Broiler Chicks. The Journal of Poultry Science. 2015;52.

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9. Patterson JAB, Kristin. Application of prebiotics and probiotics in poultry production. Poultry science. 2003;82:627-31.
10. Ohimain EI, Sylvester & Jenakumo, Nimi. Physicochemical and Microbial Screening of Palm Oil Mill Effluents for Amylase Production. Greener Journal of Biological Sciences. 2013;3:307-18.
11. Hosoi T, Ametani, A., Kiuchi, K., Kaminogawa, S. Improved growth and viability of lactobacilli in the presence of Bacillus subitlis (natto), catalase or subtilisin. Canadian Journal of Microbiology. 2000;46:892-7.
12. Setlow P. Spores of Bacillus subtilis: Their resistance to and killing by radiation, heat and chemicals. J App Microbiol. 2006;101:514-25.
13. Cartman ST, La Ragione, R.M. &Woodward, M.J. Bacillus subtilis spores germinate in the chicken gastrointestinal tract. Appl Environ Microbiol 2008;74(16)):5254-8.
14. Wu BQ, Zhang, T., Guo, L.Q. & Lin, J.F. Effects of Bacillus subtilis KD1 on broiler intestinal flora. Poult sci. 2011;90(11):2493-9.
15. La Ragione R, Woodward, M. Competitive exclusion by Bacillus subtilis spores of Salmonella enterica serotype enteritidis and Clostridium perfringens in young chickens. Veterinary microbiology. 2003;94:245-56.
16. Fritts C, Kersey, J., Motl, M., Kroger, E. Yan, F., Jiang, J., Campos, M., Waldroup, L., Waldroup P. Bacillus subtilis C-3102 (Calsporin) improves live performance and microbial status of broiler chickens. Journal of Applied Poultry Research. 2000;9:149-55.
17. Turnbull AT, H. & Kyzar, Kathleen. Family-Professional Partnerships as Catalysts for Successful Inclusion: A United States of America Perspective. Revista de Educacion. 2009;349.
18. Steel RGD, Torrie, J.H., Dickey, D.A. Principles and procedures of statistics. A biochemical approach McGraw Hill Book Co Inc, New York, USA. 1997.
19. HARWOOD CR. Introduction to the biotechnology of Bacillus. Harwood (Ed)Biotechnology Handbooks, 1989;2:1-4.
20. Setlow P. Spores of Bacillus subtilis: Their resistance to and killing by radiation, heat and chemicals. Journal of applied microbiology. 2006; 101:514-25.
21. Jin LZ, Ho YW, Abdullah N, Jalaudin S. Influence of dried Bacillus subtilis and lactobacilli cultures on intestinal microflora and performance in broilers. Asian-Australia's J Anim Sci. 1996;9:397-403.

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