

ABSTRACT

This study was conducted for the production of probiotics for poultry birds from spore forming *Bacillus* species and its efficacy on poultry birds. For this purpose soil samples were collected from all over Pakistan to isolate spore forming *Bacillus* bacteria by heat shock method. Total 120 soil samples were collected. On the basis of morphological characteristics colonies were considered. Further, various biochemical tests were carried out and 120 colonies were confirmed as *Bacillus* species. Some physical tests were also conducted like growth of isolates on different pH, time period, different concentrations of NaCl and different temperatures. The isolates showing more growth and colony forming unit CFU/ml at same parameters like pH, time, temperature, inoculums size and age, were selected. Thus 3 isolates were selected on priority basis which gave more CFU and activity against *Micrococcus leuteus* but three other strains which gave nearest growth to first 3 selected isolates were also selected for safe side and future use. Species level determination was carried out by ribotyping in which 16S rDNA was amplified, sequenced and verified through BLAST. Out of 6 isolates, strain Zii12 *Bacillus licheniformis* accession No. KT443923 was finally selected for probiotics production on the basis of biochemical tests, physiological tests, molecular identification, antibacterial activity against bacteria (*Micrococcus leuteus*) and number of CFU/ml. In shake flask method 1 (g) cell mass per liter of *Bacillus licheniformis* KT443923 with antibiotic activity (22.37 mm zone of inhibition and CFU 5.1×10^{10}) was obtained but in scale up studies where we used batch fermentation method, it was 4 gm per liter, 3 times enhancement. The bacterial culture was centrifuged and lyophilized; the product in dried powdered form was indicated in grams. The activity and CFU/g were re checked the results were same as before. Now the product was supplemented in the poultry feed to check its efficacy, for this purpose two different types of poultry trials were conducted.

For rural poultry trials, aseel birds were used and growth parameters and mortality were studied. The encouraging results with increased body weights (g) for birds fed with feed supplemented with probiotics in comparison to antibiotic and control group. After six weeks the results obtained in three groups 197.48 ± 5.11 , 172.48 ± 5.81 , 146.98 ± 4.86 ,

weight gain (g) 156.15 ± 4.96 , 132.25 ± 5.56 , 106.69 ± 4.80 , folds of weight gain 4.82 ± 0.14 , 4.34 ± 0.16 , 3.72 ± 0.14 , Feed intake (g) 711.46 ± 22.58 , 653.92 ± 29.74 , 595.79 ± 28.94 , feed conversion ratio FCR 4.57 ± 0.25 , 4.92 ± 0.24 , 5.55 ± 0.34 , mortality 0.28, 0.84, 1.25% respectively.

In another trial of commercial broiler (Cobb 500) strain was used. Growth performance, mortality, slaughtering characteristics, histology, blood parameters and prevalence of pathogens in broiler meat were studied. In commercial broiler, best results obtained in birds that were fed with feed supplemented with probiotic. At the age of 5th weeks, body weight (g) of broiler birds fed with feed supplemented with probiotic, antibiotic and control were 2155.11 ± 30.79 , 1978.40 ± 57.02 , 1840 ± 0.71 ; total weight gain 2133.98 ± 36.90 , 1946.12 ± 63.00 , 1805.23 ± 24.50 feed intake (g) 3305.96 ± 11.44 , 3370.05 ± 44.15 , 3496.67 ± 8.17 maximum folds of weight gain 4.50 ± 0.02 , 4.23 ± 0.14 , 4.32 ± 0.10 FCR 1.47 ± 0.03 , 1.64 ± 0.03 , 1.86 ± 0.01 mortality 0, 0.74, 1.48% respectively. After a successful study, 54 birds were slaughtered according to their body size and sex wise. Live weight, slaughtered weight, dressed weight, pancreas weight and heart weight of high body size was high in males that used feed supplemented with probiotic. No difference was found in bursa weight of all the slaughtered birds. Gizzard, liver, spleen and weight and intestinal among all groups was approximately not significant. Less abdominal fat (17.50 ± 0.84 g) was observed in probiotic group. pH of small intestine was almost not significant in birds of all groups. Blood parameters results were also satisfactory. Cholesterol level in probiotic group was very low (119.07 ± 0.44 mg/dl) in comparison with other groups. Pathogens like *E.coli*, *Campylobacter* and *Salmonella* were not found in the meat of probiotic groups but low or high percentage was found in other groups. High villus length of ileum (1439.11 ± 22.83 mm) was also found in probiotic group. Similarly, maximum quantity of goblet cells (167.55 ± 4.55) was found in the ileum of probiotic group. The manure of all type of treated birds was applied separately to lentil crop to check the efficacy of every group. Maximum growth of plant and high seed yield (1243.93 ± 8.99 kg ha⁻¹) was found in probiotic treated plots. Less or nil pathogen prevalence was found in probiotic group manure and soil. *Bacillus*