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ORIGINAL RESEARCH

Biological response of broiler supplemented with varying dose of direct fed microbial

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Abstract

Aim: To study biological response of the isolated strain (Direct fed microbial- DFM) of *Enterococcus spp.* in broilers at different dose rates.

Materials and Methods: Various treatments consisted of T_0: Control (Culture medium); T_1- isolated direct fed microbial namely *Enterococcus faecium* (2.8 x 10^8 cfu/kg feed); T_2- *Enterococcus faecium* (4.8 x 10^8 cfu/kg feed), T_3- *Enterococcus faecium* (6.8 x 10^8 cfu/kg feed) and T_4- *Enterococcus faecium* (8.8 x 10^8 cfu/kg feed). Growth attributes and feed consumption was recorded at regular intervals. Randomly 6 birds per treatment were chosen for blood collection and for estimation of calcium, phosphorus and cholesterol concentration in blood. Representative sample of birds were slaughtered and caecal contents were collected under sterile conditions to study microbial flora.

Result: Dose specific results for growth attributes, FCR, plasma concentration of calcium, phosphorus and cholesterol, and microbiological evaluation of caeca were obtained after supplementation of isolated DFM. *Enterococcus spp.* supplementation at low doses (2.8 x 10^8 cfu) resulted in significant increase in weight gain and better FCR. Low dosage supplementation of *Enterococcus spp.* also increased calcium concentration and lowered cholesterol in blood. Birds in treatment T_4 supplemented with high dose of *Enterococcus spp*. performed poorly as compared to control. All treatments had high ratio of gram positive to gram negative bacteria.

Conclusion: It was concluded that isolated strain of *Enterococcus* acted as probiotics only at low dosage (2.8 x 10^8 cfu). Further it was found that overall biological performance was negatively correlated to higher dose (8.8 x 10^8 cfu) of supplementations of *Enterococcus spp*. Micro floral study of caecal content indicated that the strain was able to establish itself in the gut.

Keywords: broilers, direct fed microbial, dosage, *Enterococcus* spp., probiotics,

Introduction

The term Probiotics is defined by United Nation and WHO [1] expert panel as live organism which when administered in adequate amount confers a health benefit on host. In recent years, probiotics have emerged as major tool in improving the feed efficiency, growth promotion and disease control in animals. Probiotics could be useful in decreasing the cost of production, disease prevention and for better growth in food animals. Numerous studies till date have demonstrated increased weight gain and better feed conversion ratio as a result of probiotic supplementation in food animals [2-6]. Probiotics exert positive effect on host by improving the gut health [7,8]. Further it also affects mineral absorption [9] and cholesterol concentration in blood [2,10]. The action of probiotics varies with the dose rate [11], therefore it is very important to formulate dose for a particular probiotics microorganism so that suitable microbial balance is reached inside the gastrointestinal tract. The intestinal bacterium *Enterococcus faecium* has been used for more than a decade as a probiotic strain.

Hence, the aim of this study was to evaluate the use of *Enterococcus faecium* isolated from leopard faeces supplemented at different dose rates as a probiotics in poultry birds.

Materials and Methods

Experimental animals and ethical approval: Three hundred (300) commercial broiler chicks of Vancob strain were procured for the present investigation. Experiment was ethically approved in accordance with laws and regulations.

Study design: The broiler chicks were divided into 5 groups with three replicates consisting of 20 chicks each in a randomized block design. The feed was formulated and prepared as per the BIS standards [12]. Perusal of the proximate composition (Table-1) revealed that the per cent composition of the proximate principles in pre-starter, starter and finisher mash was as per the standards.

Experimental plan was comprised of 5 treatments

Table-1. Proximate composition of pre-starter, starter and finisher diet of broiler chicken.

Nutrients	Pre-starter mash	Starter mash	Finisher mash
Dry Matter (DM)	89.34	90.12	88.65
Crude Protein (CP)	22.72	21.46	19.77
Ether Extract (EE)	3.06	2.57	2.68
Crude Fibre (CF)	4.86	3.85	3.58
Total Ash (TA)	4.86	4.87	4.54
Nitrogen Free Extract (NFE)	64.5	67.25	69.43
Metabolizable Energy (ME) (Kcal/ Kg)	2800.45	2850.15	3115.57

Table-2. Growth performance of broiler chicks supplemented with Enterococcus strain

Parameter	Days	Treatments					
		T_0	T_1	T_2	T_3	T_4	PSE
Body weight gain (g/bird)	0-14	131.8	135.91	129.19	132.55	136.38	1.36
	15-28	480.06	468.3	449.06	453.05	481.95	10.44
	29-42	837.89^{ab}	1040.72^{c}	901.55^{b}	869.60^{ab}	792.16^{a}	12.19
	0-42	1450.42^{a}	1644.90^{b}	1477.18^{a}	1463.54^{a}	1407.54^{a}	11.07
FCR	0-14	1.81	1.68	1.8	1.79	1.73	0.05
	15-28	2.2	2.21	2.27	2.27	2.16	0.04
	29-42	3.15^{b}	2.40^{a}	2.82^{b}	3.05^{b}	3.51^{c}	0.1
	0-42	2.69^{bc}	2.27^{a}	2.55^{b}	2.52^{b}	2.81^{c}	0.05

PSE-Pooled standard error; Figures bearing different super scripts within a row differ significantly, ** *P<0.01*

i.e. T_0: Control (Culture medium); T_1- isolated direct fed microbial namely *Enterococcus faecium* (2.8×10^8 cfu/kg feed); T_2- *Enterococcus faecium* (4.8×10^8 cfu/kg feed), T_3- *Enterococcus faecium* (6.8×10^8 cfu/kg feed) and T_4- *Enterococcus faecium* (8.8×10^8 cfu/kg feed).

Isolation and characterization of probiotic: Direct fed microbial *Enterococcus faecium was* isolated from leopard (*Patheraleo*) faeces and characterized using morphological and biochemical characteristics as proposed by Cowan *et al.* [13]. Isolated probiotic strain *Enterococcus faecium* was grown in Elliker broth and subsequently stored at 4^{O}C. The viability and concentration of the *Enterococcus faecium* culture per ml of sample was obtained employing plate count method.

Method of administration of probiotics: Isolated microbial culture was mixed with feed quota of a particular treatment before feeding every day and it was continued during entire period of the feeding trial. Standard management practices were followed for rearing. Different parameters such as weight gain and feed consumed was recorded at regular interval.

Blood collection: Approximately 5 ml of blood was collected and plasma separated from randomly chosen 6 birds per treatment for estimation of calcium, phosphorus and cholesterol. Calcium was estimated by atomic absorption spectrophotometer (AAS) [Perkin Elmer 3100 (USA, 1982)] while inorganic phosphorus and cholesterol were estimated by photometer 5010 V 2.0 using analytical kits of Bayer diagnostics.

Microbial count in large intestine: Representative sample of birds were slaughtered and caecal contents were collected under sterile conditions to study microbial flora. Total bacterial count of the representative sample from caeca of birds was done on nutrient agar by plate count method. Serial dilutions were made and 100 µl of the final dilution was spread uniformly on nutrient agar plates which were incubated at 37^{O}C for 48 hrs. Different colonies obtained were stained using Gram's stain and subsequently count was obtained for different bacteria types.

Statistical analysis: The data was analyzed by using one-way analysis of variance technique (ANOVA) as per Snedecor and Cochran [14] by using SPSS version 12.

Results and Discussion

Gain in weight and feed conversion ration (FCR): The gain in live weight of different treatments during the pre-starter phase was almost similar to each other and there was no significant (*P>0.05*) difference between the treatments. The FCR value was better for T_1 and highest for control but difference was again non-significant (*P>0.05*) among different treatments.

The difference in live weight gain and FCR in different treatments during the starter phase was also found to be non-significant (*P >0.05*).

No distinct effect of probiotic supplementation was found during pre-starter and starter phase (Table- 2). During the finisher phase, highest live weight gain and better FCR was observed in treatment T_1 that was significant as compared to control and other treatments. The live weight gain in treatment T_2 was better (*P <0.01*) than T_4 but comparable to T_0 and T_3. The FCR value was highest in treatment T_4 and was highly significant (*P<0.01*) as compared to all other treatments including control. Only the birds in T_1 performed better (*P <0.01*) than control, while in other groups it was statistically comparable during the finisher phase.

Overall evaluation of results also revealed that birds in Treatment T_1 performed significantly better as compared to control and other treatments in terms of gain in weight and FCR whereas birds in treatment T_4,

Table-3. Blood plasma concentration of Ca, P and cholesterol in broiler chicks supplemented with *Enterococcus* strain

Parameter	Treatments					
	T_0	T_1	T_2	T_3	T_4	PSE
Ca** (mg/dl)	12.12[a]	14.60[b]	11.85[a]	10.20[a]	11.14[a]	0.38
P (mg/dl)	6.38	6.67	6.38	6.25	6.44	0.06
Cholesterol (mg/dl)	73.51	66.97	73.10	67.99	69.84	1.39

PSE-Pooled standard error; Figures bearing different super scripts within a row differ significantly, ** *P<0.01*

Table-4. Microbial count in large intestine of broilers chicks supplemented with *Enterococcus* strain

Parameter	Treatments					
	T_0	T_1	T_2	T_3	T_4	PSE
Gram negative bacilli ** (x 10^5cfu/ml)	11.50[b]	8.27[a]	7.33[a]	5.90[a]	7.40[a]	5.44
Gram positive cocci (x 10^5cfu/ml)	10.20	24.26	16.70	22.66	23.43	19.37
Ratio(G+/G-)	0.89	2.94	2.28	3.84	3.17	-

PSE-Pooled standard error; Figures bearing different super scripts within a row differ significantly, ** *P<0.01*

which was fed with high doses of probiotics, performed poor in terms of growth performance.

Thus, the perusal of results revealed that probiotics supplementation at different doses did not affect the performance of birds during pre-starter and starter phase but had significant effect during the finisher phase. Probiotics beneficially affects the performance of poultry [1, 5, 15] but suitable microbial balance be reached which positively affects the bird's performance at a particular dose of probiotics. The overall trend revealed that though probiotics supplementation at low doses was beneficial in terms of weight gain and FCR. higher doses may have detrimental effect on growth performance of broiler birds. Similar results were obtained by other workers who found a negative correlation of higher dose of probiotics with performance of broiler birds, supporting the above obtained results [16-19]. Karl *et al.*[20] stated negative growth owing to probiotic supplementation which may be due to inappropriate dosage.

Blood parameters: Blood parameters indicated significantly better calcium bioavailability in treatment T_1 (Table-3). Dose of probiotics did not have any significant effect on plasma concentration of phosphorus and cholesterol. However, cholesterol concentration was numerically lower in treatment T_2. Direct fed microbial affects bile metabolism and lowers cholesterol [10]. *Enterococcus* acted as probiotics at lower dose improving the availability of calcium and lowering the blood plasma cholesterol. Tang *et al*. [21] also stated that probiotics affects bioavailability of minerals related to lowering of gut pH associated with the production of organic acids which in turn leads to better mineral solubility especially of calcium. Many workers have reported decrease in the cholesterol content in blood as a result of probiotic supplementation [2, 3, 6, 21, 22].

Microbial count in large intestine: Microbiological evaluation of caeca contents revealed higher proportion of Gram +ve cocci in all the treatments supplemented with direct fed microbial, whereas the count of Gram –ve bacilli in large intestine was highest in control as compared to all other treatments (Table- 4). The above pattern probably indicates that the DFM was able to establish itself in the GIT of broiler birds as compared to control and excluded the dominance of Gram –ve bacteria in the large intestine of broiler birds. This may be probably due to the reason that *Enterococcus* strain possesses good colonization ability [23] also supporting the fact that probiotics are able to lower the colonization of coliforms and other gram –ve bacteria [24, 25].

Conclusion

Thus it may be concluded that feeding of isolated direct fed microbial (DFM) *Enterococcus faecium* from leopard faeces (*Pathera leo*) at a dose rate of 2.8 x 10^8 cfu as a DFM/probiotic to poultry is beneficial in terms of overall biological performance. Further it was found that biological performance was negatively correlated to dose of probiotics of *Enterococcus* strain.

Authors' contribution

All authors have contributed equally. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interest.

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