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PH Murade
Department of Poultry Science, Nagpur Veterinary College, Maharashtra Animal and Fishery Sciences University, Nagpur, Maharashtra, India

MM Kadam
Department of Poultry Science, Nagpur Veterinary College, Maharashtra Animal and Fishery Sciences University, Nagpur, Maharashtra, India

AR Patil
Department of Veterinary Public Health, Nagpur Veterinary College, Maharashtra Animal and Fishery Sciences University, Nagpur, Maharashtra, India

KK Khose
Department of Poultry Science, Nagpur Veterinary College, Maharashtra Animal and Fishery Sciences University, Nagpur, Maharashtra, India

Correspondence
KK Khose
Department of Poultry Science, Nagpur Veterinary College, Maharashtra Animal and Fishery Sciences University, Nagpur, Maharashtra, India

Use of competitive exclusion culture on growth performance and economics of broiler production

PH Murade, MM Kadam, AR Patil and KK Khose

Abstract
The experiment was conducted on 360 day-old straight run Vencobb broiler chicks for a period of 42 days. The chicks were randomly distributed into six treatment groups A, B, C, D, E and F having 4 replicates of 15 birds in each. The group A was maintained as a control group without any treatment; feeding of competitive exclusion culture by beak dipping method on first day of age at hatchery level (B); feeding of CE by beak dipping on the first day of age on farm arrival i.e. approx. 12 hr after hatch (C); birds challenged with pathogenic *Escherichia coli* strain after 72 hr of hatch without any feeding of CE (D); birds fed with CE culture on the first day by beak dipping at hatchery level and challenged with pathogenic *E. coli* after 72 hr hatch (E); birds fed with CE culture on first day after farm arrival (i.e. Approx. 12 hr after hatch) and challenged with pathogenic *E. coli* after 72 hr hatch (F). All the birds placed under treatment D, E and F was challenged (orally) with pathogenic *E. coli* @ $1.0x10^5$ colony-forming units per chick. The live body weight and weight gain was significantly ($P<0.01$) higher in treatment group B as compared to groups A, C, D and F at the end of 42 days. The feed intake was significantly ($P<0.01$) higher in treatment group B as compared to control group. The feed conversion ratio was significantly ($P<0.01$) improved in group B as compared to groups A and D. However, significantly poor FCR was recorded in treatment group D birds challenged with *E. coli* as compared to all other groups. Overall the higher percentage of mortality was observed in treatment group D due to *E. coli* infection. The net profit rupees per kg live weight in treatment groups A to F was Rs. 7.62, 11.31, 9.95, 1.37, 10.27 and 9.10, respectively. Based on the results of the present experiment, the feeding of competitive exclusion culture at hatchery and farm level with or without *E. coli* challenged birds showed better growth performance and economic returns in broilers. Thus, it is concluded that the feeding of competitive exclusion culture @ 12.5 g/1000 chicks by beak dipping method at hatchery level were benefited in term of achieving highest live body weights, better feed conversion ratio, maximum liveability and a good amount of net profit returns in broilers.

Keywords: Growth performance, economics, competitive exclusion culture, *E. coli*, broilers

1. Introduction
Multifocal growth in the poultry population with changed husbandry practices resulted in the prevalence of many infectious diseases. *Escherichia coli* (*E. coli*) is one of the most important pathogenic agents affecting chicken, which costs the poultry industry into high economic losses due to heavy mortality, decreased weight gain, increased medication costs and poor feed conversion ratio. Though most isolates are non-pathogenic, but 10 to 15% of intestinal coliforms are pathogenic [1]. Antibiotics were routinely used in small doses to promote growth and keep disease at bay, almost to support for nutrition and sanitation. This has serious implications as India is the world's biggest consumer of antibiotics for human use which contributes to the emergence of antibiotic resistant bacteria. Social pressures have led to the creation of regulations to restrict antibiotic use in poultry and livestock production. The increasing public interest about the risk of developing cross-resistance and multiple antibiotic resistances in pathogenic bacteria in both humans and poultry linked to the therapeutic and sub therapeutic use of antibiotics in livestock [2]. So, there is a need to evaluate potential alternatives for prophylactic antibiotics to improve disease resistance in high intensity food animal production.
Current study in poultry production suggests reducing use of antimicrobial growth promoters (AGPs) and increased use of non-antibiotic feed additives such as direct-fed microbials [3]. Many researchers are now focused on identifying viable alternatives to antibiotics that offer similar benefits, such as to improve the growth of broilers, improve the utilization of feed and in this way realize better production and financial results.

In this situation Industry has started implementing a number of strategies to reduce the use of antibiotics in feed. Possibilities have been explored, including improved bio-security, probiotic, prebiotic, symbiotic, acidifiers, and competitive exclusion culture (CE) etc. Already at an early stage in the history of competitive exclusion, an improvement in growth rate was observed in commercial broiler flocks treated with a CE culture preparation [4, 5]. However, improvement in bird performance is probably most apparent in flocks that are suffering from a disease condition, e.g., from necrotic enteritis [6]. Several groups of these additives are in use as competitive exclusion Culture, which contains a mixture of live bacteria that represent the bacterial populations in the cecum of adult chickens. Generally, CE products are competing for locations to adhere to the intestinal mucous membranes, preventing pathogenic microorganisms from inhabiting the intestinal tract and competition for nutritious substances [7, 8]. Competitive exclusion works by introducing beneficial bacteria into the gut of the chicken at such a level that the more harmful bacteria are denied the environment they need in order to survive and multiply. There are over 200 species of bacteria within competitive exclusion culture; all are derived from normal healthy chickens. Therefore, the present experiment was carried out to evaluate the effect of a competitive exclusion culture on growth performance and economics in commercial broiler chickens.

2. Materials and Methods

The experiment was conducted on 360 day-old straight run Vencobb broiler chicks for a period of 42 days on deep litter system. The chicks were randomly distributed into six treatment groups A, B, C, D, E and F having 4 replicates of 15 birds in each. The experimental design is presented in Table 1.

Table 1: Experimental design used for housing of broilers

Treatment groups	Treatment details	No. of birds per replicate	No. of replicates per treatment	No. of birds per treatment
A	Control- birds fed on maize soya basal diet without any treatment	15	4	60
B	Birds fed on basal diet + supplemented with competitive exclusion Culture (CE) by beak dipping on the first day at hatchery level	15	4	60
C	Birds fed on basal diet + supplemented with CE on the first day by beak dipping after farm arrival (Approx. 12 hrs after hatch)	15	4	60
D	Birds fed on basal diet and challenged with pathogenic *E. coli* strain after 72 hrs of hatch.	15	4	60
E	Birds fed on basal diet + supplemented with CE on first day at hatchery level by beak dipping and challenged with pathogenic *E. coli* strain after 72 hrs of hatch.	15	4	60
F	Birds fed on basal diet + supplemented with CE on first day by beak dipping after farm arrival (Approx. 12 hrs after hatch) and challenged with pathogenic *E. coli* strain after 72 hrs of hatch	15	4	60
Total number of birds				360

All the birds placed under treatment groups D, E and F were challenged (orally) with pathogenic *Escherichia coli* @ $1.0\text{x}10^5$ colony-forming units (CFU) per chick.

2.1 Experimental diets and Management Practices

The maize-soybean meal based mash diet was fed to the experimental birds as per Bureau of Indian Standards [9]. The pre-starter, starter and finisher diets were prepared without any antibiotics, growth promoters and coccidiostat. All the diets were isocaloric and iso-nitrogenous. During the experiment birds were not fed any antibiotics and any kind of feed additive (probiotic, prebiotic etc.) as prophylactic or treatment measure.

The standard management practices were followed with *ad-libitum* water along with a weighed quantity of feed during the period of experimentation. The birds were given dechlorinated water for initial first three days of experiment to get stabilize the competitive exclusion culture given at hatchery and farm level by beak dipping method. The birds were reared on litter system and all the treatment groups were provided similar environmental conditions throughout the experimental period (0-42 days). The challenged group was kept in different shed. Replicate wise birds and feed were weighed weekly to generate the growth performance data of treatment birds.

2.2 Competitive exclusion culture and reconstitution

The competitive exclusion culture (marketed as Aviflora) was procured from Lallemand Animal Nutrition Pvt. Ltd, France. Preparation of master Seed Culture developed from caecal contents of backyard chicken by passage through SPF birds & cleared for all known pathogenic bacteria. The Product batches were produced from the working seed culture in a batch fermentation process and freeze dried. The final products were concentrating blended with maltodextrin as excipient. The competitive exclusion culture was different to a probiotic because it contains the entire caecal microflora (more than 200 species of bacteria) of an adult healthy chicken. The culture contains *Bifidobacterium, Bacteroidaceae, Eubacterium, Streptococcus Faecium, Streptococcus lactis, Lactobacillus Fermentum, Pediococcus, Pentosaceus,* spores etc. It should be stored in refrigerator prior to use (+2 ˚C to +8 ˚C) and the same temperature should be maintained at the time of application.

Freeze dried Competitive Exclusion culture was emptied in a sterilized container and added 15 ml vaccine diluents per 500 doses of competitive exclusion culture powder (dose @ 12.5 g/1000 chicks). Routine vaccine diluent was used to dissolve the Competitive Exclusion powder. The crystalline competitive exclusion powder mixed slowly and thoroughly. During reconstitution special precaution were taken to maintain the temperature of competitive exclusion powder and diluents between 2 to 8 ºC. The reconstituted dilution is given to the birds by beak dipping method at hatchery and farm level.

2.3 Source of *E. coli*

The pathogenic strain of *Escherichia coli* culture (MTCC-443) was procured from Microbial Type Culture Collection (MTCC), Chandigarh. The *E. coli* culture was grown in commercial brain heart infusion broth and concentration of bacteria was counted by optical density and plating. Inoculums of 1×10^5 CFU/bird were used for experimental induction. The feed was withdrawal eight hours prior to inoculation of bacterial culture to minimize the occurrence of regurgitation. The assigned treatment birds were inoculated orally with pathogenic *Escherichia coli* strain @ 10^5CFU per ml/ chick after 72 hrs of the hatch.

2.4 Data Collection

The data was collected on weekly weight changes determined by weighing the birds on weekly basis and replicate wise weight gain was calculated. The feed intake was determined by subtracting the left-over feed from the feed offered, while feed conversion ratio was calculated as average feed intake divided by average weight gain taking into consideration of mortality, if any. The cost of rearing the birds for the experiment was calculated by considering the prevailing costs of broiler chicks, feed, litter, medication and vaccine etc.

2.5 Statistical analysis

The data obtained in the experiment was subjected to statistical analysis using SPSS (17) in complete randomized block design. The one-way ANOVA was applied to all parameters. Where the significant differences were observed among the treatments means, they were separated by Duncan's multiple range tests [10]. Replicates were used as an experimental unit for analysis of all the parameters. Probability values of ≤ 0.05/0.01 were considered significant in experiments.

3. Results and Discussion

The live body weight, weight gain, feed Intake and FCR in various stages of broilers in different treatment groups are presented in Table 2. The economics of broiler production in different treatment groups are presented in Table-3

3.1 Live body weight and weight gain

The non-significant difference was recorded for live body weight (LBW) and weight gain (WG) in all treatment groups during 0-14 days and 15-28 days (Table 2). However, the birds received treatment of competitive exclusion gained numerical higher body weight than the chicks reared in control group. In finisher phase (29-42 days), the birds received competitive exclusion supplementation at hatchery and farm level (groups B and C, respectively) and same groups challenged with *E. coli* (groups E and F) were recorded significantly (P<0.01) higher live body weight and weight gain compared to control groups A and D. The birds challenged with *E. coli* infection (group D) showed significantly (P<0.01) lowest live body weight and weight gain compared to all other treatment groups. In context to the overall performance (0-42 days) the birds received competitive exclusion supplementation at hatchery (group B) was recorded significantly (P<0.01) higher live body weight and weight gain as compared to all other treatment groups except group E. The results of the present study are in agreements with Samli *et al.* [11] who reported that supplementation of *Enterococcus faecium* enhanced weight gain in broilers. However, Teo and Tan [12] also reported that broilers supplemented with *Bacillus subtilis* and challenged with *E. coli* showed significantly (P<0.05) higher weight gain, feed conversion ratio than those fed the basal diet without any probiotic. Khose *et al.* [13] reported that the broiler birds supplemented with probiotic recorded significantly (P<0.01) higher average live weights and weight gains as compared to control group at 6th week of age. Amer *et al.* [14] founded that the probiotic treated chicks appeared healthy with higher and excellent body performance including body weights and body gains in comparison with the non probiotic and *E. coli* challenged groups. In contrasts with Abudabos [15] recorded that the birds challenged with *Clostridium perfringens* on 16th day of post hatch and supplemented with CE did not influence (P>0.05) the body weight gain in finisher stage of performance, whereas in this study it was recorded that the body weight gain was significantly (P<0.05) resulted in higher side due to CE treatment. Hajati *et al.* [16] administered Competitive Exclusion culture through drinking water and found that Competitive Exclusion culture improved body weight gain numerically in broilers.

3.2 Feed intake

During the initial stage (0-14 days), there was non-significant differences (P>0.01) for feed intake in all treatment groups (Table 2). However, at starter phase (15-28 days) feed intake was significantly (P<0.01) increased in birds challenged with *E. coli* without CE supplementation (group D) compared to all other treatment groups. In finisher stage (29-42 days), the birds received competitive exclusion (CE) supplementation at hatchery and on farm arrival (group B and C, respectively) was recorded significantly (P<0.01) higher feed intake as compared to groups A and D. The birds challenged with pathogenic *E. coli* after 72 hrs of hatch with supplemented competitive exclusion at hatchery (group E) was recorded significantly (P<0.01) higher feed intake than birds challenged with *E. coli* after 72 hr of hatch (group D) and control group A. Overall performance (0-42 days) of the birds received competitive exclusion culture supplementation at hatchery (group B) was recorded significantly (P<0.01) higher feed intake as compared to the birds reared in control group (Table 3). In accordance to our study Remus *et al.* [17] explained when broilers were infected with *E. coli*, their feed intake was reduced by 7% and their growth rate was reduced by 10%. Hassan *et al.* [18] observed that *E. coli* colonized in intestinal wall and affect intestinal integrity that reflected on feed intake while in groups receiving probiotics and acidifier were the highest feed consumption which was based on explanation elaborated by Jin *et al.* [19] who summarized probiotic effect in points as it maintain normal intestinal micro-flora by competitive exclusion and antagonism second by alternating metabolism by increasing digestive enzyme.

Table 2: Live body weight, weight gain, Feed Intake and FCR of broilers in different treatment groups

Parameters	Treatment groups						SEM	P Value
	A	B	C	D	E	F		
	0-14 days							
Live body weight (g/b)	392	399	400	390	400	399	3.24	0.927
Weight gain (g/b)	346	355	354	345	362	354	3.60	0.809
Feed Intake (g/b)	532	491	494	568	524	532	8.66	0.090
FCR	1.36	1.23	1.24	1.46	1.31	1.34	00.02	0.075
	15-28 days							
Live body weight (g/b)	1116	1144	1144	1116	1134	1123	6.15	0.636
Weight gain (g/b)	723	745	743	726	734	724	6.48	0.901
Feed Intake (g/b)	1195^{b}	1143^{b}	1161^{b}	1295^{a}	1150^{b}	1151^{b}	13.77	0.001
FCR	1.66^{ab}	1.54^{b}	1.57^{b}	1.79^{a}	1.56^{b}	1.59^{b}	00.02	0.005
	29-42 days							
Live body weight (g/b)	1942^{c}	2220^{a}	2126^{b}	1832^{d}	2151^{ab}	2077^{b}	29.56	0.000
Weight gain (g/b)	826^{c}	1075^{a}	982^{ab}	715^{d}	1017^{ab}	953^{b}	28.09	0.000
Feed Intake (g/b)	1421^{b}	1804^{a}	1689^{a}	1449^{b}	1704^{a}	1620^{ab}	38.87	0.009
FCR	1.72^{b}	1.68^{b}	1.71^{b}	2.03^{a}	1.67^{b}	1.70^{b}	00.03	0.000
	0-42 days							
Live body weight (g/b)	1942^{c}	2220^{a}	2126^{b}	1832^{d}	2151^{ab}	2077^{b}	29.56	0.000
Weight gain (g/b)	1895^{b}	2175^{a}	2080^{b}	1787^{d}	2106^{ab}	2032^{b}	29.58	0.000
Feed Intake (g/b)	3148^{b}	3439^{a}	3345^{ab}	3312^{ab}	3374^{ab}	3303^{ab}	32.97	0.186
FCR	1.62^{b}	1.55^{c}	1.57^{bc}	1.81^{a}	1.57^{bc}	1.59^{bc}	00.02	0.000
Mortality (%)	4.54	2.27	0	11.36	2.27	4.54		

^{abcd} Means with different superscripts within rows differs significantly (P<0.01), SEM-Standard error of difference between mean values, P-value is probably significance value.

3.3 Feed conversion ratio
During initial stage (0-14 days), the FCR was not significantly influenced in all treatment groups (Table 2). At 15-28 days phase, FCR was significantly (P<0.01) better in treatment groups B, C, E and F supplemented competitive exclusion at hatchery and farm with or without *E. coli* as compared to group D challenged with pathogenic *E. coli* strain after 72 hrs of hatch. During the finisher stage of production (29-42 days), the birds in treatment groups A, B, C, E and F recorded significantly (P<0.01) better FCR as compared to their counterpart control group A birds reared without CE supplementation. Overall performance (0-42 days) of the birds received competitive exclusion culture supplementation at hatchery (group B) was recorded significantly (P<0.01) better FCR as compared to control group and treatment group D. Whereas, significantly (P<0.01) poor FCR was recorded in treatment group D as compared to all other treatment groups (Table 2). Similarly, Amer *et al.* [14] who reported that the probiotic treated groups showed no clinical signs or lesions and the chicks appeared healthy with higher body performance including FCR and body gains in comparison with the non-probiotics treated groups, whereas, the birds challenged with *E. coli* showed reduced feed consumption and these results were matched with the results with present study. However, Amerah *et al.* [20] recorded that the feed conversion ratio (FCR) exceeded over the 42 days trial period, *Bacillus subtilis* addition to the basal diets tended to improve the FCR by ~4 points with a numerical improvement in body weight gain of 60g. Khose *et al.* [13] reported that the significantly (P<0.01) better weekly FCR was recorded in probiotic supplemented groups as compared to control group at sixth week in boilers.

3.4 Mortality
The birds exposed with *E. coli* challenge (group D) recorded maximum mortality (11%) compared to other treatment groups (Table 2). The birds were sent for post mortem examination, which revelled that the cause of the death was *E. coli* infection. In accordance to present findings Hassan *et al.* [18] summarized mortality was highest in groups infected with *E. coli* followed by those received diet with *Lactiflor plus* and oxytetracyclin. Similarly, Nakamura *et al.* [21] who recorded that the chicks pre-treated with Competitive Exclusion culture and inoculated with either *Salmonalla Typhimurium* or *Salmonella Entritidis* were protected from overwhelming tissue colonization and death as evidenced from their low mortality and rate of colonization. Similarly, in this study it was revealed that the mortality was due to *E. coli* infection, but the birds exposed for *E. coli* challenge and treated with CE at hatchery and on farm arrival showed less number of mortality.

3.5 Economics of broiler production
The economics of broiler production (0-42 days) of the experimental birds were calculated and depicted in Table 3. The cost of production expressed rupees per kg on live weight basis in treatment groups A to F was Rs. 62.38, 58.69, 60.05, 68.63, 59.73 and 60.90, respectively. The net profit in treatment groups A to F (Rs./kg) on live weight was Rs. 7.62, 11.31, 9.95, 1.37, 10.27 and 9.10, respectively. The highest net profit was observed in treatment group B followed by E, C, F, A and D. The net profit (Rs./kg live weight) was reduced at Rs. 6.25 due to *E. coli* infection compared to control group. The findings are accordance with Khose *et al.* [13] revealed that the economics of broiler production considerable increased in net profit rupees per kg for supplementation of probiotics in broiler diets.

Table 3: Overall growth performance and economics of the broiler production

Particulars	Treatment groups					
	A	B	C	D	E	F
Chick cost (Rs./chick)	30	30	30	30	30	30
Feed cost (Rs./kg)	28	28	28	28	28	28
Cost of CE culture (Rs./bird)	0	1	1	0	1	1
Miscellaneous cost.(Rs.)	3	3	3	3	3	3
Total feed consumed (g/bird)	3148	3439	3345	3312	3374	3303
FCR	1.62	1.55	1.57	1.81	1.57	1.59
Live body weight (g/bird)	1942	2220	2126	1832	2151	2077
Mortality (%)	4.54	0	0	11.36	2.7	4.54
Feed cost (Rs./bird)	88.14	96.29	93.66	92.74	94.47	92.48
Production cost (Rs./bird)	121.14	130.29	127.66	125.74	128.47	126.48
Production cost (Rs/kg)	62.38	58.69	60.05	68.63	59.73	60.90
Sale rate (Rs./kg) live weight)	70	70	70	70	70	70
Gross profit (Rs./bird)	135.94	155.40	148.82	128.24	150.57	145.39
Net profit (Rs./bird)	14.80	25.11	21.16	2.50	22.10	18.91
Net profit (Rs./kg live weight)	7.62	11.31	9.95	1.37	10.27	9.10

4. Conclusion
Based on the results of the present experiment, the feeding of competitive exclusion culture at hatchery and farm level with or without *E. coli* challenged birds showed better growth performance and economic returns in broilers. Thus, it is concluded that the feeding of competitive exclusion culture @ 12.5 g/1000 chicks by beak dipping method at hatchery level were benefited in term of achieving highest live body weights, better feed conversion ratio, maximum liveability and a good amount of net profit returns in broilers.

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