

Effect of supplementing combination of direct fed microbial isolated from vegetable sources on biological performance of different strains of broiler chicken

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ABSTRACT

The dietary performance of the combination of direct fed microbial (DFM) namely *Lactobacillus acidophilus*, *Streptococcus uberis* and *Saccharomyces cerevisiae* (T2), which were isolated from over ripe *Luffa cylindrica* and *Momordica charantia* was studied vis-à-vis the combination of their respective standard direct fed microbial (DFM) *Lactobacillus acidophilus* (L4), *Streptococcus lactis* (S1) and *Saccharomyces cerevisiae* (Y3) (T1) and control diet (T0) in strains of commercial poultry broilers up to 6 weeks of age. Strain specific growth response in poultry broilers was exhibited owing to supplementation of isolated DFM. Significantly higher gain in live weight was exhibited by Vancobb strain of broiler birds supplemented with standard combination of DFM whereas nonsignificant difference for gain in live weight was exhibited by both Starbro and Kegbro strain. Overall results revealed poor feed conversion in all the broiler strains supplemented with both isolated and standard DFM. Difference in mean for mortality and dressing percentage exhibited was nonsignificant in all the treatments. Significantly higher growth response in standard DFM supplemented treatment comprising Vancobb strain of broilers appeared to be due to effective colonization of standard DFM in gastro- intestinal tract as evidenced by significantly higher total and differential microbial counts. It is thus concluded that direct fed microbials not only are strain specific but may have specific compatibilities for particular strain of broilers to exhibit desirable beneficial effects.

Key words: Broiler, Dietary supplementation, Microbial isolates

The nutritionally well balanced and efficient feeding is vital for economic poultry production. Evidence has been accumulated to show that there is a link between risk of zoonotic disease and growth promoting antibiotic usage in livestock and poultry (Edens 2003). The possible alternative to these tools are probiotics or direct fed microbials. Although the mechanism of action of probiotics is still unclear but these were successful in increasing both growth rate and feed utilization in trials with fattening cattle, pigs, broilers, laying hens and turkeys (Hertrampf 1979). The probiotics appear to colonize the GIT of the chicks of a particular strain and also show proper compatibility only with a combination of suitable microbes, thus the useful strains of microbes showed beneficial effects in poultry depending upon their source of

isolation, type of feed given to birds and the strain of chicks used (Fuller 1986). Thus there appear several factors and considerations for DFM to exert beneficial effect and thus varied findings have been published. Several studies have reported higher growth performance and feed utilization in poultry with the use of direct fed microbials (Nahashon *et al.* 1994, England *et al.* 1996, Mohan *et al.* 1996, Jin *et al.* 1998, Schneitz *et al.* 1998, Zulkifli *et al.* 2000, Bozkurt 2005, Chafai *et al.* 2007, Knarreborg *et al.* 2008) whereas many other studies have not reported any beneficial effect (Goodling *et al.* 1987, Maiolino *et al.* 1992, Owings *et al.* 1992, Chumpawadee *et al.* 2008).

The objective of the experiment was to determine if the addition of a isolated DFM could enhance the growth performance in broiler chicken of different strains. Present investigation was thus undertaken by supplementation of a combination of isolated DFM namely *Lactobacillus acidophilus*, *Streptococcus uberis* and *Saccharomyces cerevisiae* isolated from over ripe *Luffa cylindrica* and *Momordica charantia* vis-à-vis the combination of their standard counterparts i.e standard direct fed microbial (DFM) namely, *Lactobacillus acidophilus* (L4), *Streptococcus lactis*

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(S1) and *Saccharomyces cerevisiae* (Y3) in strains of commercial poultry broiler birds strain 1, 2 and 3.

MATERIALS AND METHODS

One-week old, 180 broiler chicks each of strain 1, 2, 3 were divided at random in 3 treatments in 18 compartments of battery brooders having 20 chicks in each treatment with 2 replicates of 10 chicks in each case. Experimental plan comprised 3 treatments i.e. T0-control (culture medium); T1-standard combination of DFM namely *Lactobacillus bulgaricus* L4 (2.26×10^8 cfu/kg feed, CSK HPKV, Palampur) + *Streptococcus lactis* S1 (2.27×10^8 cfu/kg feed, CFTRI, Mysore) + *Saccharomyces cerevisiae* Y3 (1.97×10^7 cfu/kg feed, CSK HPKV, Palampur); T2- combination of isolated DFM namely *Lactobacillus acidophilus* (2.26×10^8 cfu/kg feed, over ripe *Luffs cylendrica*) + *Streptococcus uberis* (2.27×10^8 cfu/kg feed, over ripe *Momordica charantia*) + *Saccharomyces cerevisiae* (1.97×10^7 cfu/kg feed, over ripe *Momordica charantia*).

Lactobacillus acidophilus, *Streptococcus uberis* and *Saccharomyces cerevisiae* were isolated from over ripe *Luffs cylendrica* and over ripe *Momordica charantia* and

characterized by using morphological and biochemical characteristics (Cowan *et al.* 1974). *Lactobacilli* and *Streptococci* were grown in Elliker broth while *Sacchromyces* in yeast fermentation broth. Different microbial cultures (1 ml diluted with 100 ml glass distilled water) as per experimental plan were mixed with feed quota of a particular treatment before feeding everyday and it was continued during entire period of the feeding trial. The starter and finisher diet was prepared as given in (Table 1). Standard management practices were followed for rearing and parameters like gain in weight (GIW), feed consumption, feed conversion ratio (FCR), dressing % and mortality % were recorded on regular basis during starter and finisher phases. At the end of starter phase, a digestibility trial was conducted for 5 days. All the experimental broilers were sacrificed after completion of trial to record dressing percentage, total and differential microbial counts in different organs of GIT Baba *et al.* (1991). Standard methods were used for determining the proximate composition, calcium AOAC (1985) and phosphorus (Parks and Dunn 1963). Data were analyzed statistically (Snedecor and Cochran 1968).

RESULTS AND DISCUSSION

Chemical composition of starter and finisher diets (Table 1) revealed that nutrient principles were as per BIS (1992) specifications. Results exhibited for gain in live weight during starter phase (Table 2) exhibited strain specific results. Whereas gain in live weight in strain 3 was significantly higher in standard DFM supplemented treatment T2, the results exhibited nonsignificant difference in strains 1 and 2. Further, mean differences for FCR exhibited nonsignificant difference in all the 3 strains during starter phase.

During finisher phase (Table 2) similar growth trend was exhibited by standard DFM supplemented treatment (T1) with significantly higher gain in live weight in strain 3 of broilers coupled with nonsignificant but numerically 9.39% higher gain in live weight compared to control treatment (T0) in strain 2. Further, supplementation of isolated DFM in treatment T2 revealed nonsignificant difference for gain in live weight in all the 3 strains, compared to control treatment (T0), and exhibited significantly lower depressed gain in live weight compared to standard DFM supplemented treatment (T1) in strain 3. Perusal of results revealed significantly higher and poor FCR both in standard as well as isolated DFM supplemented treatment T1 and T2 compared to control treatment (T0).

Overall results (Table 3) revealed significantly higher gain in live weight in standard DFM supplemented treatment (T1) in strain 3, whereas in strains 1 and 2, the mean difference for gain in live weight was nonsignificant but exhibited 2.78% higher live body weight in strain 2 compared to control treatment (T0). Further, supplementation of isolated DFM in treatment T2 did not exhibit any significant difference for gain in live weight compared to control treatment (T0) in all

Table 1. Composition of test diets

Ingredients (%)	Broiler starter	Broiler finisher
Maize	23.5	31
Wheat	22.5	26
Groundnut extraction	10	5
Sunflower extraction	5	5
Soya flakes	25	19
Fish meal	5	5
Molasses	6	5
DCP	2	3
Lime stone	1	1
Premix*	+	+
<i>Chemical composition of diets (% d.m.b.)</i>		
Crude protein	23.01	20.02
Crude fat	3.48	3.08
Ether extract	2.5	2.67
Total ash	5.8	5.3
Nitrogen free extract	65.21	68.93
Acid insoluble ash	2.5	2.12
Calcium	1.46	2.35
Phosphorus	0.72	1.16
Metabolisable energy (kcal/kg)	2826	2897

*Premix was prepared by mixing the following in 200 g maize flour: Ventri-mix DS: 10 g (vit.A, 82,500 I.U, B₂ -50 mg, D₃ 12,000 I.U. vit K 10 mg/g); Ventri bee plus: 20 g (vit B₁ 25 mg, B₆ 35 mg, B₁₂ 250ug, vit E 225 mg, pantothenate 225 mg, naicinamide 300 mg, folic acid 20 mg/5 g); E-care-Se - forte: 25 g (vit E 0.2 g and Se 0.04 mg/g); trace minerals 100 g; ferrous oxide 2 g, dicalcium phosphate 54 g, copper sulphate 2 g, manganese sulphate 3 g, zinc oxide 1 g, zinc sulphate 0.6 g, ferrous sulphate 10 g, pottasium iodide 0.5 g, magnesium sulphate 25 g; Super Dot 50 g (dinitro-otoulamide 250 mg and ethopabate, 16 mg/g).

Table 2 Effect of supplementing isolated and standard direct-fed microbial (DFM) on biological performance of broiler chicken of different strains in starter (1 to 28 d of age) and finisher phase (28 to 42 d of age)

	1–28 days of age			28–42 days of age		
	28 days wt	Gain in weight	FCR	42 days wt	Gain in weight	FCR
	g/bird			g/bird		
Strain 1						
T0	580.33	507.67	2.14	1328.00	742.67	2.17 ^a
T1	572	500.40	2.32	1310.00	738.00	2.44 ^c
T2	595.99	523.33	2.14	1286.66	690.67	2.78 ^b
SEM	12.80	16.69	0.08	22.60	35.26	10.14
Strain 2						
T0	558.25	491.50 ^c	2.24	1216.75	658.50	1.69 ^a
T1	523.75	456.25 ^a	2.43	1250.50	726.75	2.37 ^b
T2	573.5	507.25 ^b	2.34	1253.75	680.25	1.63 ^a
SEM	9.51	15.09	0.11	20.76	24.32	0.16
Strain 3						
T0	635.5	569.25 ^b	1.95 ^a	1240.50	605.00 ^a	2.51 ^a
T1	699	632.5 ^c	2.07 ^a	1387.90	688.90 ^b	3.12 ^b
T2	562.5	496 ^a	2.54 ^b	1189.05	626.55 ^a	2.84 ^b
SEM	16.16	15.79	0.09	23.85	20.1	0.18

Figures bearing different subscripts are significantly ($P \leq 0.05$) different from each other.

the 3 strains of broilers but on the contrary exhibited significantly lower weight compared to standard DFM supplemented treatment T1 in strain 3. Broiler strain related, variation in results exhibited, for gain in live weight are in consonance with the findings of Jin *et al.* (1997), that addition of pure lactobacilli culture or mixed cultures with other bacteria produced variable results and there appears to be specific conditions for probiotics use and specific effects of the probiotics under different conditions. Beneficial influence of probiotics on production parameters in pigs, cattle and poultry depending on the age, physiological status, type of production and environmental conditions (Grela *et al.* 1999). So even though higher gain in live weight was exhibited in strain 3 but similar results were not exhibited in strains 1 and 2 (Table 3).

Overall results revealed significantly higher and poor FCR both in standard as well as isolated DFM supplemented treatment, T1 and T2 compared to control treatment (T0) in strains 1 and 3. It seems that the poor food conversion efficiency of standard DFM supplemented treatment (T1) is attributable to a higher food consumption because those birds gained the most weight, during the period. It is possible that the increased food consumption results from a self-regulatory mechanism or appetite. In view of this, if DFM supplement is beneficial under stressful conditions, it is likely that, due to post-ingestion feedback, the birds consumed more DFM supplemented diet to match their physiological requirements. Zulkifli *et al.* (2000) also reported that extra intake of feed in broiler birds did not produce a corresponding increase in body tissue and thus, resulted in a poorer food efficiency.

The difference for mortality and dressing % (Table 3) were

nonsignificant amongst all the treatment groups and strain of broilers though numerically 1.11 and 3.82% higher dressing percentage was exhibited in standard DFM supplemented treatment (T1) compared to control (T0) in strains 2 and 3 of broilers.

Nonsignificant difference for digestibility of nutrients (Table 3), viz. DM, CF, EE, NFE, ADF, NDF, cellulose and hemicelluloses was exhibited in all the 3 strains of broilers. Similar results were exhibited for the balance of nutrients, viz. nitrogen, calcium and phosphorus. Our results revealed non-specific pattern in digestibility studies. Though numerically standard supplemented DFM treatment (T1) exhibited numerically 5.98 and 11.27% higher crude fibre digestibility in strains 1 and 2 but was on the lower side in strain 3 when compared to control (T0).

Pattern of microbial colonization in different parts of gastro-intestinal tract was studied and compared amongst treatments T1 and T2 and their comparison to control T0. Perusal of the results obtained for total microbial count (Table 4) exhibited varied results in different strain of broilers under study, whereas nonsignificant difference for TMC amongst all the treatments was exhibited in strain 1, significantly higher TMC was exhibited in small and large intestine in both standard and isolated DFM supplemented treatment T1 and T2 in strain 2 and similar trend was continued in treatment T1 in strain 3. Similarly Mostafa *et al.* (2007) reported that the total count of micro-organisms increased by increasing probiotic in dietary treatment in colon of hens compared with the control group. Colonization pattern of Lactobacillus and Streptococcus (Table 4) exhibited significantly higher count in strains 2 and 3 in lower part of

Table 3. Digestibility %, balance of nutrients and overall (1–6 weeks) growth performance

	Digestibility%								Balance of nutrients			Overall gain in weight			
	*DM	CF	EE	NFE	ADF	NDF	Cellulose	Hemi cellulose	N	Ca	P	g/bird	FCR	Mortality %	Dressing %
<i>Strain 1</i>															
T0	64.11	36.11	74.14	73.8	65.5	80.23	59.14	75.14	48.18	39.4	58.15	1255.34	2.16 ^a	3.2	62.89
T1	62.36	38.41	72.21	73.62	64.80	80.14	57.16	73.23	38.16	55.12	1238.40	2.39 ^b	3.2	62.52	
T2	63.12	34.71	75.63	74.2	64.06	81.47	58.52	75.89	47.21	38.22	58.28	1214.00	2.50 ^b	Nil	63.84
SEM	2.48	1.61	2.88	3.33	3.92	4.44	2.95	4.37	3.54	1.43	2.19	34.24	0.17		
<i>Strain 2</i>															
T0	61.8	21.19	69.5	76.9	55.4	75	62.6	76.12	55.2	60.7	61.5	1150.00	1.92 ^a	5.2	60.44
T1	63.29	23.58	75.78	74.2	52.3	76.12	59.12	78.61	56.5	60.5	63.1	1183.00	2.39 ^b	6.6	61.12
T2	61.28	25.18	73.95	72.8	54.51	75.28	63.28	78.98	58.21	61.21	62.8	1187.50	1.94 ^a	5.2	58.94
SEM	3.76	4.95	2.82	2.83	2.58	2.04	3.21	2.65	2.27	2.28	1.48	26.43	0.12		
<i>Strain 3</i>															
T0	68.11	40.51	71.37	75.28	68.14	77.12	61.1	84.41	55.41	44.14	65.5	1174.25 ^a	2.24 ^a	3.6	62.66
T1	70.09	38.16	72.82	75.7	72.21	78.14	62.31	79.19	55.41	42.23	64.29	1321.40 ^b	2.61 ^b	3.6	65.15
T2	69.12	42.2	73.38	76.70	88.75	82.60	75.81	81.05	42.81	65.28	1122.55 ^a	2.70 ^b	3.6	64.56	
SEM	4.09	1.41	3.37	2.37	2.75	4.96	3.13	3.1	1.70	1.83	2.24	24.27	0.14		

Figures bearing different subscripts are significantly ($P < 0.05$) different from each other. *DM, dry matter; CF, crude fibre; EE, ether extract; NFE, nitrogen free extract; ADF, acid detergent fibre; NDF, neutral detergent fibre; N, nitrogen; Ca, calcium; P, phosphorus.

Table 4. Effect of dietary supplementation of combinations of microbes on lactobacillus, streptococcus and yeast counts ($\times 10^6$ cfu) in different compartments of GIT in different strains of broilers

	Lactobacillus count $\times 10^6$ cfu					Streptococcus count $\times 10^6$ cfu					Yeast count $\times 10^6$ cfu					Total microbial counts ($\times 10^6$ cfu)				
	1 C	2 P	3 G	4 SI	5 LI	1 C	2 P	3 G	4 SI	5 LI	1 C	2 P	3 G	4 SI	5 LI	1 C	2 P	3 G	4 SI	5 LI
<i>Strain 1</i>																				
T ₀	0.2	0.24	0.19	1.18	0.14	0.38 ^b	0.33	0.27 ^b	1.58 ^c	0.2	0.15	0.15	0.18	1.58	0.08	0.76	0.82	0.74 ^b	4.1	0.51
T ₁	0.2	0.22	0.21	1.03	0.13	0.30 ^c	0.35	0.26 ^c	1.23 ^a	0.21	0.14	0.15	0.15	1.3	0.08	0.73	0.82	0.72 ^b	4.05	0.48
T ₂	0.19	0.19	0.15	0.83	0.11	0.24 ^a	0.3	0.20 ^a	1.13 ^a	0.18	0.1	0.09	0.14	0.83	0.08	0.69	0.73	0.60 ^a	3.3	0.46
SEM	0.04	0.07	0.06	0.38	0.09	0.09	0.08	0.09	0.11	0.05	0.09	0.08	0.07	0.65	0.01	0.12	0.15	0.08	0.25	0.1
<i>Strain 2</i>																				
T ₀	0.14 ^a	0.13 ^a	0.1 ^a	0.17 ^a	0.0 ^a	0.24 ^a	0.20 ^a	0.16 ^a	0.32 ^a	0.09 ^a	0.12 ^a	0.15 ^a	0.12 ^a	0.19 ^a	0.06 ^a	0.63 ^a	0.85 ^a	0.46 ^a	0.94 ^a	0.28 ^a
T ₁	0.23 ^b	0.19 ^b	0.2 ^b	1.28 ^b	0.1 ^b	0.42 ^b	0.33 ^b	0.39 ^b	1.83 ^b	0.22 ^b	0.26 ^b	0.23 ^b	0.23 ^b	1.48 ^c	0.13 ^b	1.04 ^c	0.87 ^b	0.93 ^b	5.15 ^b	0.57 ^b
T ₂	0.16 ^c	0.10 ^a	0.1 ^a	1.60 ^b	0.1 ^c	0.27 ^a	0.29 ^c	0.22 ^a	1.70 ^b	0.13 ^a	0.23 ^c	0.17 ^c	0.21 ^b	0.95 ^b	0.14 ^b	0.85 ^b	0.69 ^a	0.75 ^b	4.90 ^b	0.50 ^b
SEM	0.07	0.06	0.07	0.46	0.05	0.09	0.1	0.09	0.09	0.06	0.11	0.08	0.06	0.39	0.06	0.19	0.16	0.12	1.33	0.13
<i>Strain 3</i>																				
T ₀	0.12	0.15	0.13	0.19 ^a	0.0 ^a	0.22	0.24 ^a	0.23	0.25 ^a	0.11 ^a	0.13 ^c	0.08	0.11 ^c	0.11 ^a	0.03	0.54 ^a	0.55	0.51 ^a	0.63 ^a	0.26 ^a
T ₁	0.18	0.19	0.16	0.75 ^b	0.1 ^b	0.29	0.33 ^b	0.28	0.88 ^b	0.17 ^b	0.16 ^b	0.09	0.15 ^b	1.00 ^b	0.15	0.71 ^b	0.7	0.69 ^b	3.10 ^b	0.57 ^b
T ₂	0.15	0.16	0.14	0.19 ^a	0.0 ^a	0.25	0.25 ^a	0.26	0.24 ^a	0.10 ^a	0.07 ^a	0.08	0.08 ^a	0.11 ^a	0.04	0.56 ^a	0.56	0.54 ^c	0.61 ^a	0.28 ^a
SEM	0.1	0.05	0.05	0.09	0.08	0.09	0.09	0.05	0.37	0.04	0.08	0.05	0.07	0.95	0.15	0.13	0.2	0.17	1.07	0.11

Figures bearing different sub-scripts are significantly ($P \leq 0.05$) different from each other. 1–C, Crop; 2–P, proventriculus; 3–G, gizzard; 4–SI, small intestine; 5–LI, large intestine; Cfu: colony forming unit.

the gastro intestinal tract in standard DFM supplemented treatment (T1). The results are not without precedent. Yu *et al.* (2008) reported that broiler birds supplemented with lactobacillus had higher total anaerobic and lactobacillus count at 37 days of age in all segments of gastrointestinal tract of chicks offered TLB as compared to control chicks.

Similarly lactobacillus, streptococcus and yeast count was significantly higher in small intestine only in strain 2 supplemented with isolated DFM treatment T2. Results

(Table 4) revealed that the supplemented DFM, viz. standard and isolated namely, lactobacillus, streptococcus and yeast were not able to colonize especially in lower part of gastro-intestinal tract of strain 1. Thus broiler strain specific establishment and colonization pattern of supplemented direct fed microbials was exhibited. The results obtained are not without precedent. McLean *et al.* (2005) while studying the effect of supplementation of *Bacillus subtilis* on chicken performance concluded that the microflora in the gut

appeared to have been beneficially modified (as indicated by spore counts recovered from the caecae) and thus had an effect on performance of the birds. Knarreborg *et al.* (2008) reported that feeding chicken with gallipro-a commercial DFM enhanced chick performance as well as enriched the birds with a more diverse and complex bacterial composition in the gut, which expectedly provides a more robust microbiota less susceptible towards diseases and infections.

Higher gain in live weight exhibited only by strain 3 of broilers may be attributable to highly effective colonization in lower part of gastro intestinal tract by supplemented standard direct fed microbial (DFM) namely lactobacilli, streptococci and yeast. These results have also confirmed our earlier report, Katoch *et al.* (2000) exhibiting significantly higher gain in live weight in strain 3 broilers offered the combination of standard microbes (*L.bulgaricus* L4 + *S.lactis* S1 + *Saccharomyces cerevisiae* Y3). It is thus concluded that direct fed microbial isolates not only are strain specific but may have specific compatibilities for particular strain of broilers to produce desirable effects.

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