

Dietary effect of combination of some probiotic microorganisms on productive performance of layer chickens fed up to the starter phase

M KUMARI¹, D WADHWA², V K SHARMA³, K S SHARMA⁴ and B S KATOCH⁵

CSK Himachal Pradesh Krishi Vishwavidhyalaya, Palampur, Himachal Pradesh 176062 India

Received: 20 October 2009; Accepted: 17 September 2010

ABSTRACT

Comparative biological response of combination of different strains of lactobacilli, streptococci and yeast, isolated from different indigenous sources was studied in commercial laying chickens. The treatments consisted of T₁ (control feed with culture medium only), T₂ (*Lactobacillus bulgaricus* L₄ + *Straptococcus lactis* S₁ + *Saccharomyces cerevisiae* Y₃), T₃ (*L. acidophilous* leopard excreta + *S. faecalis* leopard excreta + *Torulopsis spherica* calf dung), T₄ (*L. acidophilous* bottle gourd + *S. uberis* bitter gourd + *S. cerevisiae* bitter gourd), T₅ (*L. lactis* tomato + *S. faecium* khamir + *Pichia membranaefaciens* tomato), T₆ (T₂ + T₃) and T₇ (T₃ + T₄). In overall, the FCR was higher in probiotic fed groups as compared to that of the control. Mortality was observed in the control (T₁) and T₅ group only. Feed consumed per kg egg mass was lower and, percent hen per day egg production was higher in all the probiotic fed groups. The probiotic feeding resulted in larger egg size production. The better egg laying performance could be due to the higher total and % Gram +ve microbial counts in the intestinal tract. It could be concluded that the probiotics, fed only up to 8 weeks of age showed a carry-over effect on the laying performance throughout the laying period and there was no need to mix too many strains of microbes from different sources to increase the farm returns.

Key words: Dietary effect, Layers, Probiotics, Starter phase

Lactobacilli and *Streptococci* are the most commonly used microbes in the production of probiotics. Fuller (1986) reported that the useful strains of microbes or probiotics improve the biological performance of the layers. Sharma *et al.* (2001) reported that the microbial combinations significantly improve the productive performance of layers when the probiotics are fed up to 18 weeks of age. Discontinuous administration of *Lactobacilli* showed positive effects on the performance of layers through immune modulations (Koenen *et al.* 2004). The present study was, therefore, planned to study the carry-over effect of probiotics on the biological and productive performance of laying birds when the microbial combinations are fed only up to 8 weeks of age.

MATERIALS AND METHODS

One-week old layer chicks (147) were distributed randomly into 7 groups, each having 3 replicates of 7 chicks with almost similar average group weights. The isolation and

characterisation of *L. acidophilous* and *S. faecalis* from leopard excreta, *Torulopsis spherical* from calf dung, *S. uberis* and *S. cerevisiae* from bitter gourd, *L. lactis* and *Pichia membranaefaciens* from tomato and *S. faecium* from fermented dough of wheat flour was carried out by following standard techniques (Cowan *et al.* 1974). Lactobacilli and streptococci strains were grown on Ellikr broth while *S. cerevisiae* and *Pichia membranaefaciens* in yeast fermentation broth. Different microbial cultures as per experimental plan (Table 1) were mixed with feed quota of a particular treatment everyday and feeding of probiotics was done from 1 week to 8 weeks of age (starter phase). The standard managerial practices were followed for rearing chicks in thermostatically controlled battery brooders up to 8 weeks of age and were fed chick mash, then grower mash (Table 2) till 18th week of age or first day of laying, whichever was earlier and thereafter the feeding regimen was switched over to layer mash (Table 2). Gain in weight (GIW), feed consumption, feed conversion ratio (FCR), and mortality were recorded during starter and grower phase and the number of eggs laid, average egg weight, egg mass, percent hen day egg production, feed consumed per dozen of egg and per kg egg mass were recorded/calculated during 12 months of lay. The feed ingredients and compound feeds

Present address: ¹Scientist, Department of Biology and Environmental Sciences, College of Basic Sciences (e-mail: daisynutrition@rediffmail.com); ² Assistant Professor, ³Professor and Head, ⁴Professor, ⁵Formerly Professor and Head, Department of Animal Nutrition.

Table 1. Experimental plan

Test diet	Treatment	Sources of microbes	Microbial concentration per kg of feed
T ₁	Control (culture only)	-	-
T ₂	<i>Lactobacillus bulgaricus</i> (L ₄) ⁺ <i>Streptococcus lactis</i> (S ₁) + <i>Saccharomyces cerevisiae</i> (Y ₃)	Himachal Pradesh Krishivishav Vidyalaya, Palampur India, CFTRI, Mysore; Himachal Pradesh Krishivishav Vidyaalya, Palampur, India	2.93 × 10 ⁸ + 2.30 × 10 ⁸ +1.93 × 10 ⁷
T ₃	<i>L.acidophilus</i> (leopard excreta) + <i>S.faecalis</i> (leopard excreta) + <i>Torulopsis sphaerica</i> (calf dung)	Leopard excreta Leopard excreta Calf dung	2.93 × 10 ⁸ +2.30 × 10 ⁸ +1.93 × 10 ⁷
T ₄	<i>L.acidophilus</i> (bottle gourd) + <i>S.uberis</i> (bitter gourd) + <i>S. cerevisiae</i> (bitter gourd)	Bottle gourd Bitter gourd Bitter gourd	2.93 × 10 ⁸ +2.30 × 10 ⁸ +1.93 × 10 ⁷
T ₅	<i>L.lactis</i> (tomato) + <i>S. faecium</i> (khamir) + <i>Pichia membranaefaciens</i> (tomato)	Tomato Khamir Tomato	2.93 × 10 ⁸ +2.30 × 10 ⁸ +1.93 × 10 ⁷
T ₆	T ₂ + T ₃	(As in T ₂ and T ₃)	1.46 × 10 ⁸ +1.15 × 10 ⁸ +0.92 × 10 ⁷ +1.46 × 10 ⁸ +1.15 × 10 ⁸ +0.92 × 10 ⁷
T ₇	T ₃ + T ₄	(As in T ₃ and T ₄)	2.93 × 10 ⁸ +2.30 × 10 ⁸ +1.93 × 10 ⁷

were analysed for CP, EE, CF, total ash, NFE and Ca by AOAC (1995) and phosphorus by HMSO (1973). Metabolisable energy (ME) was calculated as per Lodhi *et al.* (1976). After the termination of experimental period, birds in each treatment were sacrificed for recording total and differential microbial counts i.e. Gram (+) and Gram (-) microbes in small intestine (Cowan *et al.* 1974). The statistical analysis of the data was done as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The chemical analysis of feeds offered to the layers showed that the formulations were as per BIS (1992) standards. The live-weight gain during the starter phase (Table 3) was the highest ($P < 0.05$) in T₃. However, there was no significant difference amongst the rest of the treatments. The FCR of T₃ treatment was also the lowest ($P < 0.05$), indicating the positive effect of probiotics on gain in weight in this group. No chick mortality was observed in any of the treatments other than control (T₁) and T₅ during the starter phase.

During the grower phase, the GIW of treatment T₄ was

the highest ($P < 0.05$). The probiotics resulted in lower ($P < 0.05$) FCR compared to the unsupplemented group (T₁) in all the treatments. However, the FCR of T₄, T₆ and T₇ were the lowest (significantly; $P < 0.05$) and did not differ amongst each other. No mortality was recorded during this phase, which could be due to the modulating effect of the probiotics on immune system (Koenen *et al.* 2004, Nayeppor *et al.* 2007 and Apata 2008) and elimination of the weaklings during the starter phase, where there was mortality of 14.28% in T₁ and T₅ treatments.

The overall response of various treatments of pullets from 1–18 weeks of age (Table 3) showed that the GIW were statistically higher in T₃, T₄ and T₇, but T₇ showed the highest (numerically) GIW. The supplementation resulted in significantly ($P < 0.05$) lower FCR in all the groups when compared to that of the control (T₁). This showed that the direct fed microbials exerted beneficial effects on layers even before the onset of laying, through saving expenditure on the feed and early maturity of birds. The effect of probiotics on growth rate and FCR of broilers is well documented (Kaistha *et al.* 1996).

The data regarding laying performance up to 1 year of

Table 2. Composition of experimental diets (Parts/100 parts)

Ingredients	Starter	Grower	Layer
Maize	54.0	37.0	25.0
Wheat	-	-	22.0
Groundnut cake	15.0	18.0	5.0
Sunflower cake	-	-	5.0
Soyflakes	18.0	3.0	2.5
Fish meal	6.0	3.0	2.5
Molasses	5.0	8.0	5.0
Deoiled rice barn	-	28.0	26.0
Dicalcium phosphate	1.0	2.0	1.5
Calcium grit	-	-	5.0
*Premix	1.0	1.0	1.0
Chemical composition (% DM)			
Crude protein	21.62	18.11	15.35
Crude fibre	3.02	6.30	54.40
Ether extract	2.39	1.98	2.40
Total ash	2.24	7.25	6.55
NFE	35.17	54.64	57.61
Ca	1.02	1.06	2.47
P	0.60	0.52	0.41
ME** (kcal/kg; calculated value)	3007	2514	2552
*Premix:			
Maize meal as carrier	675 g	765 g	765 g
(Vitamins A:82,500 IU; B ₂ : 50 mg; D ₃ : 1,300 IU and K: 10 mg/g) commercial mix	20 g	15 g	15 g
(Vitamins B ₁ : 5 mg; B ₆ : 7 mg; B ₁₂ : 50 mg; E: 45 mg; niacinamide: 60 mg and folic acid: 4 mg/g) (Vit.E: 0.02 g and Se: 0.04 mg/g) (DOT: 250 mg; and ethopabate:16 mg/g)	25 g	20 g	20 g
Choline chloride (25%)	30 g	-	-
Trace minerals (Fe ₂ O ₃ : 56 g; MnSO ₄ : 25 g; CuSO ₄ : 2 g; ZnSO ₄ : 1 g; ZnO:5 g; FeSO ₄ :10 g; KI: 250 mg and Na ₂ S ₂ O ₃ : 750 mg)	50 g	-	-
	100 g	100 g	100 g
	100 g	100 g	100 g

lay (Table 4) revealed that the average egg number was significantly ($P < 0.05$) higher and feed consumed per kg egg mass produced, was significantly ($P < 0.05$) lower in all the microbial fed groups as compared to that of the control (T_1). The average egg number was the highest in T_7 and feed consumed per kg egg mass production was the lowest in this treatment. Applegate *et al.* (2009) also reported improved feed intake –to-egg mass ratios during 8 weeks of the 12-week study on probiotic supplementation. The layers of T_7 group had numerically the highest overall GIW. It meant that there was a carry-over effect of the probiotics (because the chicks were offered probiotics up to the starter phase only). However, there was no significant difference amongst the

microbial fed groups as far as the feed consumed per dozen of egg production was concerned. Similar amounts of feed consumed per dozen egg production, but lower feed consumed per kg egg mass production in probiotic supplemented groups meant that the egg size was affected by the direct fed microbes and the higher egg mass production in the treatment group was due to comparatively larger egg size. The percent hen/day egg production was significantly ($P < 0.05$) higher in all the microbial fed groups. Upon consumption, probiotics deliver many lactic acid bacteria into the gastrointestinal tract, which have been reputed to modify the intestinal milieu and to deliver enzymes and other beneficial substances into the intestine leading to better performance by the birds (Lutful and Kabir 2009). There was an increase in the total G +ve and decrease in total G -ve microbial counts in the small intestine of the probiotic fed groups compared to that of the un-supplemented group (T_1). This suggested that the direct fed microbes exerted affect on the ratio of G +ve and G -ve counts in the GIT. It has been reported that (Ivanov 2003) a ratio of 68 to 70: 32 to 30 of G +ve and G -ve, respectively, is the most favourable ratio to have optimum growth response in the broilers. In the present study this ratio was changed from 63: 37 (control) to 72 to 78: 28 to 22 in probiotic supplemented groups. This might be due to the effective colonization of probiotics in the gastro-intestinal tract of layers (Table 5). There is a competition for the adhesion sites between microbes, to the epithelial lining of gastro-intestinal tract, thus the harmful bacteria may not find site for their adhesion and a competition for the nutrients between the direct fed microbes and the pathogenic microbes, together destroy the harmful microbes (Reddy 2001).

These results showed that on the whole, egg-laying performance (in terms of egg mass and egg size, per cent hen-day production and feed consumed per kg egg mass) was improved by supplementing the diets with probiotics up to 8 weeks of age. Sharma *et al.* (2001) reported higher laying performance to the same tune as observed in the present study, by feeding same strains, dose rates and combinations of microbes when the rations were supplemented with probiotics up to 18 weeks of age. The present study therefore, suggested that there is no need to supplement the diets with these probiotics, up to 18 weeks of age. The feed consumed per kg egg mass production was lower in the present study as compared to that reported by Sharma *et al.* (2001). Even the same isolates, with similar dose rate and strain combinations, gave better performance in the present experiment, which was difficult to explain, as there are a number of factors, which influence the feed intake. No difference in feed consumed per kg egg mass and per cent hen/day egg production within the treatment groups showed that all the treatments were equally good in enhancing the productivity. So it could be concluded from the present study that, there is no need for mixing too many strains of microbes from

Table 3. Biological performance of layer chicks under different treatments

Particulars	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇
<i>Starter phase (1–8 weeks)</i>							
Initial weight (g)	45.53±0.92	45.57±1.07	45.57±1.66	46.14±0.91	46.29±1.08	46.14±1.37	45.86±1.34
Gain in weight (g)	354 ^a ±9.03	374 ^a ±9.86	407 ^b ±17.30	359 ^a ±9.79	362 ^a ±13.60	376 ^a ±10.92	385 ^a ±15.17
Feed intake (g)	2167 ^a ±42.63	2065 ^b ±39.57	2012 ^b ±28.11	2099 ^a ±38.76	2091 ^a ±28.32	1911 ^b ±21.29	1996 ^b ±38.18
Feed conversion ratio (FCR)	6.13 ^b ±0.04	5.51 ^b ±0.09	4.95 ^a ±0.05	5.85 ^d ±0.06	5.77 ^{de} ±0.03	5.08 ^{ef} ±0.03	5.19 ^{ft} ±0.04
Mortality (%)	14.28	-	-	-	14.28	-	-
<i>Grower phase (9–18 weeks)</i>							
Gain in weight (g)	858 ^a ±44.54	886 ^a ±40.35	897 ^a ±30.66	952 ^b ±20.30	872 ^a ±26.33	918 ^a ±23.96	933 ^a ±31.27
Feed intake (g)	4102 ^a ±27.83	3990 ^a ±30.78	3911 ^b ±30.05	3918 ^b ±30.70	4010 ^a ±38.11	3948 ^b ±45.12	3921 ^b ±21.43
Feed conversion ratio (FCR)	4.78 ^d ±0.50	4.49 ^a ±0.03	4.38 ^{ab} ±0.03	4.12 ^c ±0.02	4.56 ^a ±0.06	4.31 ^{ac} ±0.03	4.20 ^{bc} ±0.05
Mortality (%)	-	-	-	-	-	-	-
<i>Over-all (1–18 weeks)</i>							
Gain in weight (g)	1212 ^a ±41.87	1260 ^a ±36.04	1304 ^b ±28.59	1310 ^b ±17.28	1235 ^a ±31.07	1294 ^a ±22.47	1318 ^b ±24.25
Feed intake (g)	6269 ^a ±66.94	6055 ^b ±75.39	5923 ^b ±29.76	6016 ^b ±46.89	6101 ^b ±59.74	5859 ^b ±46.59	5918 ^b ±49.18
Feed conversion ratio (FCR)	5.18 ^c ±0.03	4.81 ^a ±0.04	4.54 ^b ±0.04	4.59 ^b ±0.05	4.95 ^a ±0.05	4.52 ^b ±0.04	4.49 ^b ±0.03
Mortality (%)	14.28	-	-	-	14.28	-	-

Figures bearing different superscripts within a row differ significantly (P < 0.05).

Table 4. Egg laying performance up to 12 months of lay under different treatments

Particulars	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇
Average egg number	22.69 ^a ±1.01	24.05 ^b ±0.92	24.09 ^b ±0.74	23.88 ^b ±0.59	23.99 ^b ±0.53	23.92 ^b ±0.84	24.15 ^b ±0.82
Average egg weight (g)	55.08 ^a ±0.62	57.00 ^a ±0.66	57.38 ^a ±0.39	58.32 ^b ±0.87	56.91 ^b ±1.04	57.05 ^b ±0.49	58.12 ^b ±0.72
Average egg mass (g)	1250.77 ^a	1370.92 ^a	1382.28 ^b	1392.8 ^b	1308.93 ^a	1364.64 ^a	1403.60 ^b
	±102.12	±79.92	±75.56	±39.22	±57.89	±72.14	±49.95
Feed consumed per kg egg mass (g)	2641.08 ^a	2409.76 ^b	2381.89 ^b	2370.88 ^b	2417.64 ^b	2414.14 ^b	2348.92 ^b
	±59.25	±72.48	±39.49	±69.09	±59.24	±55.56	±68.25
Feed consumed per dozen of egg (g)	1744.92 ^a	1646.74 ^a	1643.84 ^a	1660.15 ^a	1650.69 ^a	1658.14 ^a	1639.77 ^a
	±42.09	±32.67	±31.19	±28.82	±47.79	±59.25	±68.44
Per cent hen day egg production	75.66 ^a ±0.92	80.17 ^b ±0.79	80.03 ^b ±0.38	79.60 ^b ±1.02	80.04 ^b ±0.67	79.87 ^b ±0.89	80.50 ^b ±0.49

Figures bearing different superscripts within a row differ significantly (P < 0.05).

Table 5. Microbial counts under different treatments

Particulars	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇
Total microbial count in small intestine (×10 ⁸)	2.00 ^a ±0.38	4.17 ^b ±0.50	5.10 ^b ±0.17	4.57 ^b ±0.38	3.60 ^b ±0.20	4.80 ^b ±0.23	5.17 ^b ±0.32
% Gram +ve microbes (in small intestine)	63.33 ^a ±3.33	76.33 ^b ±1.33	75.00 ^b ±2.89	75.00 ^b ±0.00	78.33 ^b ±4.41	72.00 ^a ±1.15	76.67 ^b ±1.01
% Gram –ve microbes (in small intestine)	36.67 ^a ±3.33	23.67 ^b ±1.33	25.00 ^b ±2.89	25.00 ^b ±0.00	21.67 ^b ±4.41	28.00 ^a ±1.15	23.33 ^b ±1.01

Figures bearing different superscripts within a row differ significantly (P < 0.05).

different sources and feeding them beyond 8 weeks of age, which also would save cost in terms of time and labour thereby, making poultry rearing more profitable. The direct fed microbes also increase the egg size. However,

future research is needed to investigate how the production and economy will be affected when the layers are fed throughout their productive life under similar managerial conditions.

REFERENCES

- AOAC. 1995. *Official Methods of Analysis*. Association of Official Analytical Chemists. 16th edn. Arlington, Virginia, USA.
- Apata D F. 2008. Growth performance, nutrient digestibility and immune response of broiler chicks fed diets supplemented with a culture of *Lactobacillus bulgaricus*. *Journal of Science of Food and Agriculture* **88**: 1253–58.
- Applegate T J, Onyango E M, Angel R and Powers W J. 2009. Effect of amino acid formulation and dietary direct-fed microbial supplementation on egg production and egg characteristics in laying hens. *Journal of Applied Poultry Research* **18**: 552–61.
- BIS. 1992. *Bureau of Indian Standards*. Poultry feeds-specifications. Manas Bhawan Bahadur Shah Zafar Marg, New Delhi, 110 012.
- Cowan S T, Holt J G, Liston J, Murray R G E, Niven C F, Ravin A W and Stoneous R Y. 1974. *Burgeys Mannual of Determinative Bacteriology*. 8th edn.
- Fuller R. 1986. Basis and efficiency of probiotics. *Worlds Poultry Scientific Journal* **44**: 69–75.
- Higgins J P, Higgins S E, Vicente J L, Wlfenden A D, Tellez G and Hargis B M. 2007. Temporal effects of lactic acid bacteria probiotic culture on *Salmonella* in neonatal broilers. *Poultry Science Journal* **86**: 1662–66.
- HMSO. 1973. *The Analysis of Agricultural Materials*. MAFF Technical Bulletin No.27, London.
- Ivanov I. 2003. A balancing act - Optimising the gut microflora. *Poultry International* **3**: 33–37.
- Kaistha M, Katoch S, Katoch B S, Kumari M, Dogra K K. and Sharma C R. 1996. Effect of dietary supplementation of useful microbes isolated from *Luffa cylindrica* and *Momordica charantia* on the performance of broilers. *Indian Journal of Poultry Science* **31**: 156–62.
- Koenen M E, Kramer J, Van der Huist R, Heres L, Jeurissen S H M and Boersma W J A. 2004. Immuneomodulation by probiotic lactobacilli in layer- and meat-type chickens. *British Poultry Science* **45**: 355–66.
- Lodhi G N, Singh D and Ichoponanai J S. 1976. Variations in nutrient content of feeding stuff rich in protein and reassessment of the chemical methods of metabolisable energy estimation. *Journal of Agricultural Sciences* **86**: 293–303.
- Lutful S M and Kabir. 2009. The role of probiotics in poultry industry. *International Journal of Molecular Science* **10**: 3535–46.
- Nayebpor M, Farhomand P and Hashemi A. 2007. Effects of different levels of direct fed microbial (Primalac) on growth performance and humoral immune response in broiler chicken. *Journal of Animal Veterinary Advances* **6**: 1308–13.
- Reddy D V. 2001. *Principles of Animal Nutrition*. Oxford & IBH Publishing Co., New Delhi.
- Sharma K S, Sharma V K, Katoch S, Kaistha M and Katoch B S. 2001. Performance of laying pullets fed microbial combinations upto eighteen weeks of age/the age of sexual maturity. *Indian Journal of Animal Sciences* **71**: 166–69.
- Snedecor G and Cochran W G. 1994. *Statistical Methods*. 6th edn. Oxford and IBH.