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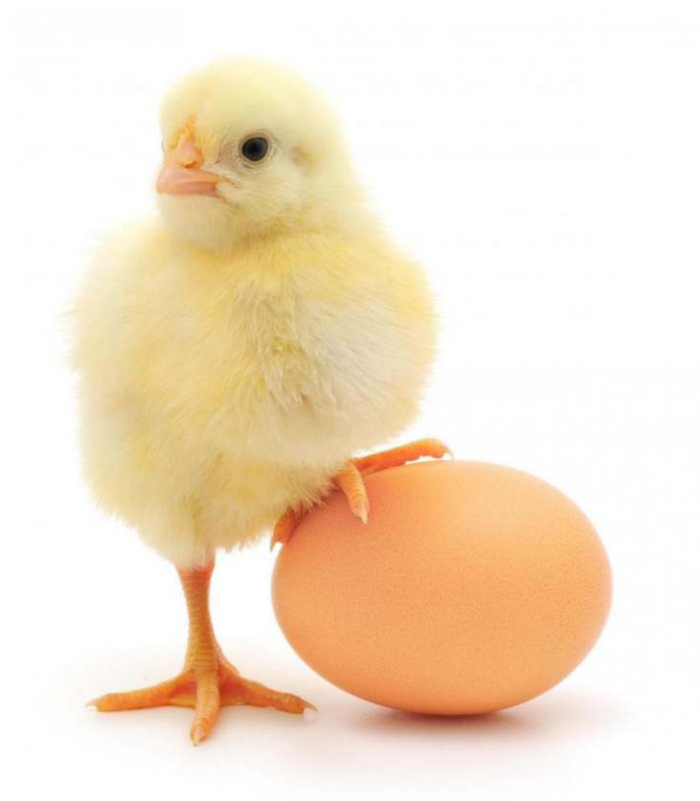

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Effect of Dry Probiotic Supplemented Fermented Feed on Production Performance of Akar Putra Chicken

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Abstract: During the recent years, solid state fermented feed (SSFF) has been introduced with great success in poultry nutrition. Thus, the present experiment was conducted to evaluate the effect of dry fermented feed with prepared probiotic (PP) on the live body weight, weight gain, feed intake and feed conversion ratio of a local Malaysian chicken (Akar Putra). The experiment comprised of 3 treatments (24 chicken/treatment), with 3 replicates of each (8 chicken/replicate). The treatments consisted of a control group (T1), the mixture of SSFF and PP at the rate 1:1:1 (1 kg of commercial broiler feed+1 liter tap water+1 g PP) in T2 and 1:1:2 (1 kg of commercial broiler feed+1 liter tap water+2 g PP) in T3. The results revealed remarkably significant ($p<0.01$) improvement in both male and female chicken in terms of final body weight gain, feed intake and feed conversion ratio in treatment groups (T2 and T3) when compared to the control group. The variation ratio of production performance parameters was calculated and best results were indicated in T2 group wherein, 1 g prepared probiotic was used.

Key words: Fermented feed, probiotic, Akar Putra chicken

INTRODUCTION

Akar Putra is a local Malaysian chicken, developed in the University of Putra Malaysia by Professor A.B. Kassim. It has a robust growing process than their parents because the maturation period is shorter (less than 13 weeks). It can lay 120-200 eggs per year and it has more resistance to diseases (Jawad *et al*., 2015).

Fermentation has been practiced for quite a long time as a means to improve the quality of food. Fermentation is the chemical transformation of organic substances into simpler compounds by the active enzymes or, complex organic catalysts, produced by microorganisms such as bacteria, yeasts, or molds. Enzymes act by hydrolysis, a process of breaking down or predigesting complex organic molecules to form smaller (more easily digestible) compounds and nutrients (Shurtleff and Aoyagi, 2007). Fermentation process has been used to improve the nutritive value of various feed ingredients like soybean (Mathivanan *et al*., 2006), copra meal (Hatta and Sundu, 2009) and tofu waste (Rasud, 2009). It creates conducive conditions for the growth of microorganisms that break down fiber and anti-nutrients, influences the bacterial ecology of gastrointestinal tract and reduce the level of Enterobacteriaceae in different parts of gastrointestinal tract as has been reported in pigs (Winsen *et al*., 2001) and broiler chicks (Heres *et al*., 2003). Although most microbial fermentations are accomplished in liquid phase, solid-state fermentations (SSFF): has many advantages viz. low medium cost and capital investment, less water output and, is more practical when carried out in the fields (Adams *et al*., 2002).

Since 2006, antibiotics were banned for use as feed additives by the European Union., because their continued use resulted in common problems such as development of drug resistant bacteria, imbalance of normal microflora and drug residues in animal products (Chen *et al*., 2009). This necessitated the need for other alternatives like probiotics. Probiotics have become important as replacement feed additives (Steiner, 2006). A probiotics is a live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance. Probiotics have been classified as GRAS (Generally Recognized as Safe) by Food and Drug Administration (FDA). The concept of their use relates to maintaining the equilibrium of intestinal microflora by the addition of beneficial microorganisms (Goldin, 1998). Many studies have reported the benefits of probiotic utilization on productive indices (Cavazzoni *et al*., 1998; Jin *et al*., 1998; Sogaard

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and Suhr-Jessen, 1999; Besnard *et al*., 2000; Campos *et al*., 2002). Al-Gharawi (2012) also reported that use of probiotics, improved the growth performance, feed efficiency, immunity parameters and disease resistance. The major probiotic strains include *Lactobacillus*, Saccharomyces, Streptococcus and Aspergillus (Tannock, 2001). Presently, Bacillus, *Lactobacillus* and Saccharomyces are the major strains used in broilers (Zhang *et al*., 2005; Chen *et al*., 2009).

Since very few investigations on the fermentation of feed with probiotics in chicken have been done, therefore, the present study was undertaken to evaluate the effect of dry fermented feed on production performance of local Malaysian chicken (Akar Putra).

MATERIALS AND METHODS

Preparation of fermented feed: The commercial broiler starter and finisher diets (Table 1) were purchased from local markets. The Akar Putra chicks were fed starter diet for the first 3 weeks of age, thereafter they were shifted to finisher diet till the completion of experimental period (12 weeks).

The fermented feed was prepared by mixing commercial broiler feed, tap water and probiotic (PP). These mixtures were placed in a plastic tray and incubated for 38 hours. at 37±2°C for complete fermentation and used after drying.

The probiotic was prepared in the University of Putra Malaysia (UPM). Each one gram of PP contained at least 10^9 cfu of *Lactobacillus acidophilus*, *Bacillus subtilis*, *Bifidobacterium* and at least 10^8 cfu of *Saccharomyces cerevisia*. Fermented feed has been characterized by having high lactic acid concentration (up to 260 mmol/kg feed) and moderate amounts of acetic acid (20-30 mmol/kg feed), besides high number of lactic acid bacteria (Log 9-10 cfu/g. feed) and pH of approximately 4.5-5.0 as described by Cutlure *et al*. (2005).

Chicken husbandry and experimental design: The experiment was carried out at the poultry farm of Faculty of Veterinary Medicine, University of Putra Malaysia (UPM), Malaysia, during the period from 15th December 2014 to 15th March 2015. A total of 72 one-day old Akar Putra chicks were assigned in Complete Randomized Design (CRD) into three experimental groups as follows:

T1: Control group fed basal feed
T2: Fed dry fermented feed mixture at the rate of 1:1:1 (1 kg of commercial broiler feed+1 liter tap water+1 g PP)
T3: Fed dry fermented feed mixture at the rate of 1:1:2 (1 kg of commercial broiler feed+1 liter tap water+2 g PP)

Each treatment group was replicated three times with 8 (4 males and 4 females) chicks per replicate. The chicks were reared in battery cages (5" x 4") and reared in temperature and humidity controlled room with 24-h. constant light schedule. They were given *ad libitum* access to water and feed throughout the experiment.

Table 1: Composition of basal diet

	------------ Basal diet ------------	
Items	1 to 22 d (Start diet)	23 to 84 d (Finisher diet)
Corn	44.9	53.10
Wheat	18.0	15
Soybean meal (45%)	33	27
Mineral and vitamin premix	1	1
Oil	2	3
Limestone	0.8	0.6
Dicalcium phosphate	0.3	0.3
Total (%)	100	100
Calculated analysis		
Crude protein (%)	21.92	19.70
Metabolism energy (kilo calorie/kg/diet)	2990	3100
Calcium (%)	0.93	0.85
Phosphorus (%)	0.48	0.45
Methionine (%)	0.55	0.50
Lysine (%)	1.35	1.25
Methionine+Cysteine (%)	0.85	0.91
Folic acid	1.1	1.2

Calculated analysis according to NRC (1984)

Sampling procedure and analytic methods:

1: Body weight, weight gain, feed intake and feed conversion ratio for males and females were recorded separately from week 1 until week 12
2: Growth rate was calculated at the marketing age based on the formula of Brody (1945):

GR : Growth rate
ASLW : Average starting live weight (weight of chick at first-day of age)
AFLW : Average finishing live weight (weight of bird at marketing age)

3: Variation ratio of production performance parameters were recorded based on the formula of Jawad *et al*. (2015):

$$\frac{A-B}{B}\times 100$$

where, A: Treatment data (Here, T2 and T3), B: Control group data (Here, T1).

Statistical analysis: Data generated from the present experiment was subjected to statistical analysis using the GLM procedure of SAS (2001) statistical software package. When significant differences were noted, means were compared by using Duncan's multiple range tests (1955).

RESULTS AND DISCUSSION

Table 2 presents the effect of dry probiotic supplemented fermented feed on mean weekly body weight of male Akar Putra Chicken. Birds in T2 treatment

Table 2: Effect of dry probiotic supplemented fermented feed on mean weekly body weight (gm) of Akar Putra male chicken

	Treatments		
Week	T1	T2	T3
1	62.667±3.48	62.333±2.404	62.667±2.333
2	104±2.887^{b}	113.667±2.603^{ab}	117.333±3.18^{a}
3	150±4.041^{b}	199.667±0.882^{a}	196±1.732^{a}
4	277±6.928	302±4.933	292.333±5.239
5	345±11.547^{b}	443±10.116^{a}	417.667±10.99^{a}
6	499±14.434^{b}	612.667±12.129^{a}	567.667±12.441^{a}
7	610±9.815^{c}	789.333±9.244^{a}	715.667±8.667^{b}
8	869±11.547^{b}	931.333±9.262^{a}	854.333±10.99^{b}
9	1041±17.898^{a}	1056±16.462^{a}	969.333±17.324^{b}
10	1165±19.053^{b}	1240.333±17.629^{a}	1147±18.193^{b}
11	1290±20.207^{b}	1523±18.502^{a}	1274±19.348^{b}
12	1390±20.785^{b}	1813±19.079^{a}	1401.667±17.975^{b}
Growth rate	190.277±0.342^{b}	192.779±0.153^{a}	190.619±0.108^{b}

Mean values with common superscript in row differ significantly (p<0.01)
Mean values at week 2, 9 and 10 differ significantly (p<0.05)

Table 3: Effect of dry probiotic supplemented fermented feed on mean weekly body weight (gm) of female Akar Putra chicken

	Treatments		
Week	T1	T2	T3
1	61.667±3.756	59.667±2.963	61.333±3.48
2	104.2±3.062^{b}	113.667±2.603^{a}	116±2.082^{a}
3	178.3±4.304^{b}	199.667±3.756^{a}	194.333±2.728^{a}
4	277.133±7.044	300.333±6.36	289.333±5.548
5	344.667±11.26^{b}	442.333±10.138^{a}	417.667±10.414^{a}
6	468.333±13.86	495.333±13.017	474±12.741
7	516.667±9.528^{b}	618.333±8.413^{a}	605.333±9.244^{a}
8	624.267±11.779^{c}	689.667±10.414^{b}	729.333±10.975^{a}
9	714.667±17.61^{b}	794±16.197^{a}	814.667±16.756^{a}
10	815.333±18.478^{b}	893.667±17.072^{a}	916.333±17.629^{a}
11	876.667±19.919^{b}	1023.667±19.064^{a}	999.333±19.633^{a}
12	937.333±20.21^{b}	1158±19.079^{a}	1104.667±20.497^{a}
Growth rate	186.155±0.523^{b}	189.688±0.304^{a}	188.64±0.408^{a}

Mean values with common superscript in row differ significantly (p<0.01)
Mean values at week 2, 3, 9 and 10 differ significantly (p<0.05)

Table 4: Effect of dry probiotic supplemented fermented feed on weekly feed consumption (gm) of male Akar Putra chicken

	Treatments		
Week	T1	T2	T3
1	44±4.041^{b}	63.333±3.48^{a}	47±3.215^{b}
2	82±2.887^{b}	101.667±2.603^{a}	96.333±2.333^{a}
3	126±6.928	151.333±6.36	138.667±6.642
4	196±5.196	207.667±4.096	201.333±4.631
5	270±6.928^{a}	272.667±6.642^{a}	242.333±6.36^{b}
6	269±9.815^{b}	379±8.963^{a}	274.333±9.244^{b}
7	407±11.547^{a}	428.333±10.138^{a}	360.333±10.975^{b}
8	410±13.279^{ab}	438.667±12.143^{a}	376.667±11.319^{b}
9	500±12.124^{b}	508.667±10.99^{a}	410.333±10.713^{b}
10	440±14.434^{c}	489±13.577^{b}	712.667±14.146^{a}
11	534±16.166^{a}	569±15.308^{a}	455±14.468^{b}
12	507±15.588^{b}	579±14.731^{a}	414.667±14.449^{c}
Total	3785±118.934	4188.333±108.987	3729.667±108.429

Mean values with common superscript in row differ significantly (p<0.01)
Mean values at week 1, 5, 7 and 8 differ significantly (p<0.05)

showed highly significant (p<0.01) effect compared with T3 and control group in terms of live body weight trait at the marketing age, while as, in female chicken of both T2 and T3 group, a highly significant (p<0.01) difference was observed when compared to the control group (Table 3). With regard to the growth rate criteria (Table 2 and 3), males of T2 treatment outperformed T3 and

Table 5: Effect of dry probiotic supplemented fermented feed on weekly feed consumption (gm) of female Akar Putra chicken

	Treatments		
Week	T1	T2	T3
1	44.1±4.128^{b}	63.333±3.48^{a}	47.667±3.756^{b}
2	82.3±3.15^{b}	101±2.082^{a}	96.667±2.603^{a}
3	125.333±6.36	151±6.083	137.333±5.548
4	195.667±4.91	208±4.359	201.333±4.631
5	230.667±6.642^{b}	272.333±6.36^{a}	242.733±6.699^{b}
6	276.333±9.244^{a}	176±8.145^{b}	255.333±8.413^{a}
7	248.333±10.975^{b}	205.333±10.138^{c}	312.667±9.597^{a}
8	289.667±12.991^{b}	157.667±12.143^{c}	338±11.59^{a}
9	266.667±11.837^{ab}	242±10.44^{b}	301±11.269^{a}
10	357.667±14.146^{a}	221±12.741^{c}	293.667±10.899^{b}
11	260±15.308^{a}	178.333±14.746^{b}	292.333±13.92^{a}
12	307.333±14.17^{a}	179.667±13.618^{b}	334.667±14.449^{a}
Total	2684.067±113.834^{a}	2155.667±104.284^{b}	2853.4±103.246^{a}

Mean values with common superscript in row differ significantly (p<0.01)
Mean values at week 1, 5 and 9 differ significantly (p<0.05)

Table 6: Effect of dry probiotic supplemented fermented feed on weekly weight gain of male Akar Putra chicken

	Treatments		
Week	T1	T2	T3
1	28±1.732	29±1.732	29±1.732
2	41.333±0.667^{c}	51.333±0.882^{b}	54.667±0.882^{a}
3	46±1.155^{c}	86±1.732^{a}	78.667±1.453^{b}
4	127±2.887^{a}	102.333±4.096^{b}	96.333±3.528^{b}
5	68±4.619^{b}	141±5.292^{a}	125.333±5.925^{a}
6	154±2.887^{b}	169.667±2.186^{a}	150±2.082^{b}
7	111±4.619^{c}	176.667±2.963^{a}	148±3.786^{b}
8	259±1.732^{a}	142±1^{b}	138.667±2.333^{b}
9	172±6.351^{a}	124.667±7.311^{b}	115±6.429^{b}
10	124±1.155^{c}	184.333±1.202^{a}	177.667±0.882^{b}
11	125±1.155^{b}	282.667±0.882^{a}	127±1.155^{b}
12	100±0.577^{c}	290±0.577^{a}	127.667±1.856^{b}
Total	1355.333±19.055^{b}	1779.667±18.55^{a}	1368±17.436^{b}

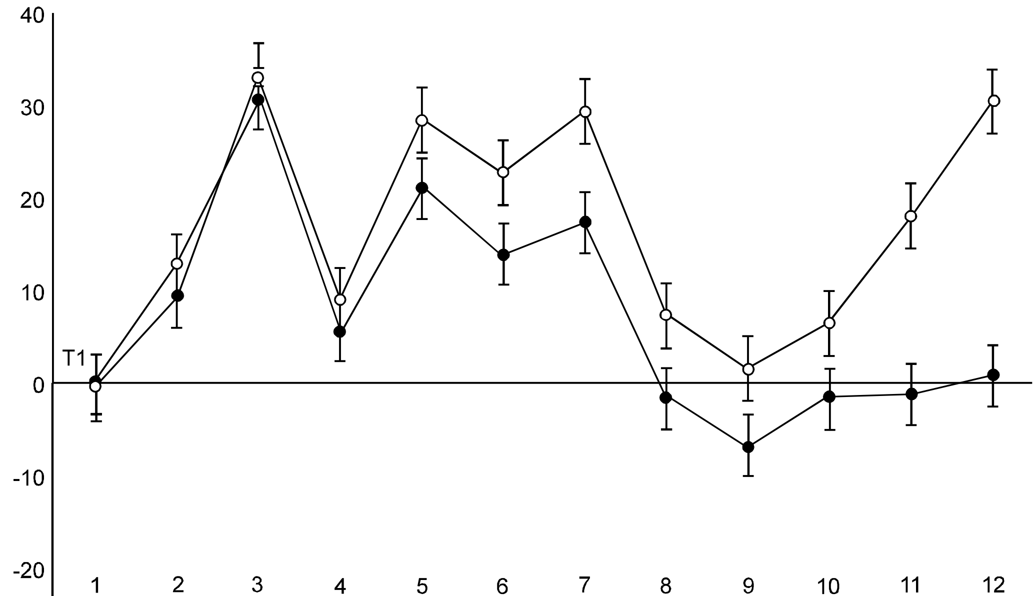
Mean values with common superscript in row differ significantly (p<0.01)

Table 7: Effect of dry probiotic supplemented fermented feed on weekly weight gain of female Akar Putra chicken

	Treatments		
Week	T1	T2	T3
1	28±1.732	29±1.732	29±1.732
2	42.533±0.742^{b}	54±0.577^{a}	54.667±1.453^{a}
3	74.1±1.242^{c}	86±1.155^{a}	78.333±0.667^{b}
4	98.833±2.744	100.667±2.603	95±2.887
5	67.533±4.221^{b}	142±3.786^{a}	128.333±4.91^{a}
6	123.667±2.603^{a}	53±2.887^{b}	56.333±2.333^{b}
7	48.333±4.333^{b}	123±4.619^{a}	131.333±3.528^{a}
8	107.6±2.272^{b}	71.333±2.028^{c}	124±1.732^{a}
9	90.4±5.839	104.333±5.783	85.333±5.783
10	100.667±0.882	99.667±0.882	101.667±0.882
11	61.333±1.453^{c}	130±2.082^{a}	83±2.082^{b}
12	60.667±0.333^{c}	134.333±0.333^{a}	105.333±0.882^{b}
Total	903.667±18.187^{b}	1127.333±17.901^{a}	1072.333±18.765^{a}

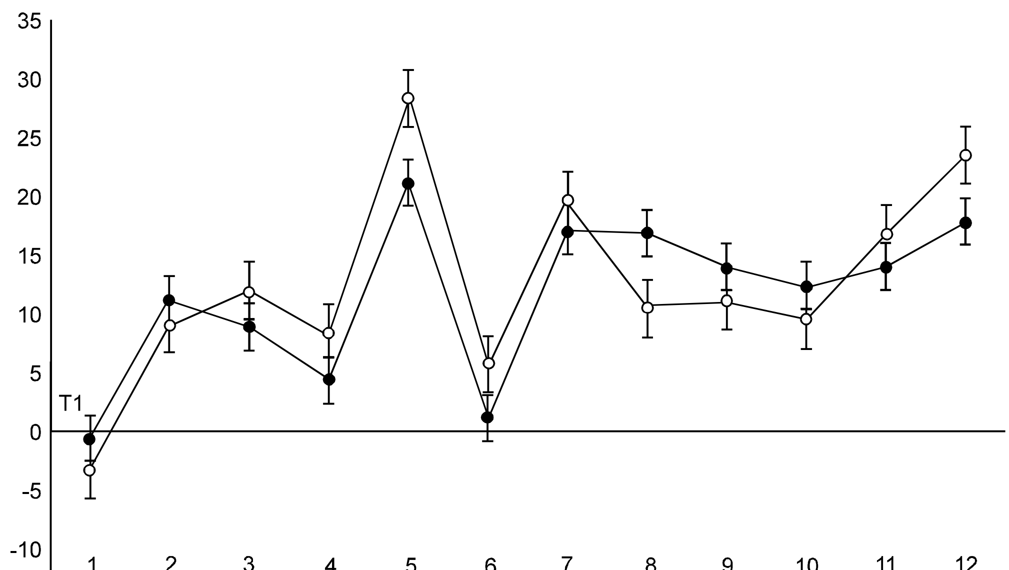
Mean values with common superscript in row differ significantly (p<0.01)

control group male chicken with variation ratios as 1.315 in T2 and 0.179 in T3. However, in case of female chicken, no such effect was observed between the two treatment groups which had the variation ratios as 1.898 and 1.335 in T2 and T3, respectively. The genetic and non-genetic factors control the growth trait in animals. Growth in domestic chicken is commonly measured by body weight and body conformation, which are the most important parameters for growth estimation. The factors



	1	2	3	4	5	6	7	8	9	10	11	12
—○— T2	-0.532	9.295	33.111	9.025	28.406	22.779	29.399	7.173	1.441	6.466	18.062	30.432
—●— T3	0.000	12.821	30.667	5.535	21.063	13.761	17.322	-1.688	-6.884	-1.545	-1.240	0.839

Fig. 1: Effect of dry probiotic supplemented fermented feed on variation ratio curve of body weight of male' Akar Putra chicken from 1 to 12 weeks of age



	1	2	3	4	5	6	7	8	9	10	11	12
—○— T2	-3.243	9.085	11.984	8.371	28.337	5.765	19.677	10.476	11.101	9.608	16.768	23.542
—●— T3	-0.541	11.324	8.992	4.402	21.180	1.210	17.161	16.830	13.993	12.388	13.992	17.852

Fig. 2: Effect of dry probiotic supplemented fermented feed on variation ratio curve of body weight of female Akar Putra chicken from 1 to 12 weeks of age

involved in the growth of chickens are too complex to be explained only under univariate analysis because all related traits are biologically correlated due to pleiotropic effect of genes and linkage of loci (Rosario *et al*., 2008; Udeh and Ogbu, 2011).

Table 4 shows the effect of dry probiotic supplemented fermented feed on mean weekly feed intake of Akar Putra Chicken. No significant effect ($p > 0.01$) was observed among all the 3 groups in terms of feed intake, however in case of females, a highly significant

Table 8: Effect of dry probiotic supplemented fermented feed on weekly feed conversion ratio (g/feed/g/gain) of male Akar Putra chicken

	Treatments		
Week	T1	T2	T3
1	1.566±0.048[b]	2.185±0.015[a]	1.619±0.023[b]
2	1.987±0.1	1.981±0.049	1.762±0.014
3	2.735±0.082[a]	1.764±0.109[b]	1.767±0.117[b]
4	1.543±0.006[b]	2.033±0.044[a]	2.092±0.032[a]
5	3.994±0.171[a]	1.936±0.028[b]	1.938±0.044[b]
6	1.746±0.031[b]	2.233±0.03[a]	1.828±0.049[b]
7	3.688±0.258[a]	2.428±0.098[b]	2.442±0.137[b]
8	1.582±0.041[c]	3.09±0.089[a]	2.715±0.039[b]
9	2.91±0.037[c]	4.099±0.159[a]	3.58±0.111[b]
10	3.547±0.083[b]	2.652±0.057[c]	4.011±0.06[a]
11	4.27±0.09[a]	2.013±0.048[c]	3.581±0.082[b]
12	5.069±0.127[a]	1.996±0.047[c]	3.252±0.154[b]
Total	2.791±0.049[a]	2.353±0.037[b]	2.725±0.045[a]

Mean values with common superscript in row differ significantly (p<0.01)

Table 9: Effect of dry probiotic supplemented fermented feed on weekly feed conversion ratio (g/feed/g/gain) of female Akar Putra chicken

	Treatments		
Week	T1	T2	T3
1	1.569±0.05[b]	2.185±0.015[a]	1.64±0.033[b]
2	1.938±0.105	1.871±0.054	1.773±0.095
3	1.689±0.058	1.755±0.047	1.752±0.057
4	1.98±0.005[c]	2.067±0.01[b]	2.12±0.016[a]
5	3.43±0.118[a]	1.918±0.008[b]	1.893±0.02[b]
6	2.233±0.028[c]	3.324±0.033[b]	4.536±0.04[a]
7	5.263±0.7[a]	1.68±0.145[b]	2.388±0.136[b]
8	2.689±0.065[a]	2.204±0.109[b]	2.724±0.056[a]
9	2.958±0.062[b]	2.323±0.029[c]	3.542±0.11[a]
10	3.551±0.11[a]	2.215±0.109[c]	2.887±0.083[b]
11	4.232±0.152[a]	1.369±0.093[c]	3.519±0.091[b]
12	5.064±0.21[a]	1.337±0.101[c]	3.175±0.112[b]
Total	2.968±0.066[a]	1.91±0.063[c]	2.659±0.051[b]

Mean values with common superscript in row differ significantly (p<0.01)

reduction in feed consumption was recorded in T2 when compared to T3 and control (Table 5). Further, the highest body weight gain was observed in T2 both in case of male and female chicken compared to T3 and control (Table 6 and 7).

The effect of dry probiotic supplemented fermented feed on mean weekly feed conversion ratio of male Akar Putra Chicken has been shown in Table 8. The results revealed that using combination of SSFF+1 gm probiotic (T2) showed significant improvement in chicken compared to T3 and T1, whereas, in female chicken, incorporation of probiotic at both the levels (T2 and T3) showed improvement in comparison to control (Table 9). These results are in concordance with Ayanwale *et al.* (2006), Silva *et al.* (2000), Day, (1997) but in disagreement with those of earlier workers (Panda *et al.*, 1999; Ergun *et al.*, 2000; Mutus *et al.*, 2006) who reported that the supplement of probiotic did not have any effect on feed conversion ratio of broiler chicken. Yousefi and Karkoodi (2007) also reported that feed consumption and feed conversion ratio of layer chicken were not affected by the dietary probiotic and yeast supplementation. In the same regard, Ahmad (2004) did not detect any difference in the feed conversion ratio of broilers as compared with the control following probiotic supplementation. The reason for the variable effect of biological additives may be confounded by variations in gut flora and environmental condition (Mahdavi *et al.*, 2005). Several researchers reported that when chicks were housed in a clean environment, a probiotic had no effect on their performance (Gunal *et al.*, 2006; Aderson *et al.*, 1999).

Figure 1 shows the variation ratio curves of average body weight of male Akar Putra chicken. Noticeable significantl increase in the body weights were observed in T2 and T3 during first 7 weeks. In subsequent 2-3 weeks, the weights decreased gradually but maintained superiority in T2 than T3 and control group.

Thereafter, body weight again increased from week 10 until the completion of study (week 12) especially in T2. Figure 2 also shows remarkable increasing in the variation ratio curves of body weight of female chicken in T2 and T3 treatments. On week 6, decrease in the body weight rate was observed, followed by very noticeable increase thereafter till week 12, especially in T2.

Conclusion and suggestion: Based on the results, it could be concluded that using dry fermented feed with probiotic, especially at the rate of 1:1:1 (1 kg of commercial broiler feed+ 1 liter tap water+1 g PP) results in significant improvement in the production performance of Akar Putra chicken. Further, it is assumed that fermented feed generally improves the bacterial ecology of the gastrointestinal tract and immunity response, so could prove an important tool in controlling the chicken diseases in future.

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