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PROBIOTIC EFFECT OF *LACTOBACILLUS* SP. (H-1) ISOLATED FROM SAUERKRAUT AND USED IN CHICKEN FEED

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Abstract

In this study, the *Lactobacillus* sp. (H-1) isolated from sauerkraut that was used as probiotic in chicken feed. There were 7 dietary treatments for investigation of probiotic activity on broiler chicken growth. The experiments were conducted during May 2019 to November 2019 in the Fermentation Department (Pharmaceutical Research Department, Ministry of Industry - 1, Yangon Region) and chicken farm in Ngwe Nantthar village, Hlegu Township. The chicks in the group D which were fed with 0.015% dried isolated bacteria (with nitrogen source) provide the optimal body weight gain than other group but the chicks in group E which were fed with 0.005% dried isolated bacteria (without nitrogen source) showed lowest body weight gain among 7 groups. Similarly, the chicks in the group D also provided feed consumption, reduce mortality rate compared to those of control group. It can be concluded that the best results were found in group D (0.015% dried bacteria of *Lactobacillus* sp.) fed to the chickens.

Keyword: *Lactobacillus*, nitrogen source, mortality rate.

Introduction

Antibiotics are used to fight bacterial infections. However, a selective pressure gave rise to bacteria resistant to antibiotics. This leaves scientists worried about the danger to human and animal health. Some strategies can be initiated to reduce the use of antibiotics in chicken farms. Much research has been carried out to look for natural agents with similar beneficial effects of growth promoters. The aim of these alternatives is to maintain a low mortality rate, a good level of animal yield while preserving environment and consumer health.

Among these, the most popular are probiotics, prebiotics, enzymes, phytogenic feed additives and etc. (Mehdi *et al.,* 2018). Probiotics are live microbial feed supplements that have a beneficial effect on the health and well-being of the host (Bovill *et al.,* 2001). A positive impact of probiotics supplementation in poultry has been well reported on production performance, feed intake, weight gain and feed conversion efficiency, immune responses, and body's resistance to infectious diseases and help lowering of chick mortality (Bansal *et al.,* 2011, Hatab *et al.,* 2016).

Rich medium and suitable conditions are the key environmental parameters required for good bacterial growth (Manzoor *et al.,* 2017). The nitrogen sources was necessary for the growth and product formation in microbial cultivation (Zammaretti *et al.,* 2005). Yeast extract is widely used for the cultivation of lactobacilli because it is an abundant source of nitrogen, the vitamin B group, purine, and pyrimidine (Yeo *et al.,* 2018). The aim of this study is to investigate the effects of *Lactobacillus* sp. (H-1) as probiotic in chicken feed, to study the effect of nitrogen source (yeast extract) on the biomass production and growth performance of broiler and to observe the effects of probiotic supplementation on the body weight gain, feed consumption, feed conversion rate and mortality rate.

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Materials and Methods

Microorganisms

Bacterial strain of *Lactobacillus* sp. (H-1) was isolated from sauerkraut and was incubated in the laboratory of Pharmaceutical Research Department (PRD), Ministry of Industry - 1, Yangon Region.

Culture maintenance

Strain was maintained on tomato juice agar medium slant.

Preparation of inoculum

Bacterial cells were grown in 500 ml conical flask containing 100 ml of tomato juice broth medium. Culture was incubated at 37°C in incubator.

Tomato juice agar medium (Sigma-Aldrich, 2013)

Composition per liter

Tomato juice	20.00 g
Yeast extract	10.00 g
Dextrose	10.00 g
Dipotassium phosphate	0.50 g
Monopotassium phosphate	0.50 g
Magnesium sulphate	0.20 g
Manganese sulphate	0.01 g
Ferrous sulphate	0.01 g
Sodium chloride	0.01 g
Agar	20.00 g

Fermentation (Dubey and Maheshwari, 2002)

Cells were grown in 5000 ml flasks containing 4000 ml medium. The flasks were inoculated using 10 % seed culture and then placed in the incubator for 3 days. At the end of fermentation stage, the bacteria were present as a suspension of cells in the medium. The bacteria was separated by centrifugation at 2000 rpm for 20 min. After centrifugation, the supernatant was discarded and the sediments were collected. Then, for dry weight determination these sediments were filtered by using filter paper. The filtrate was dried in oven at 60°C for 10 hours in order to get the constant weight.

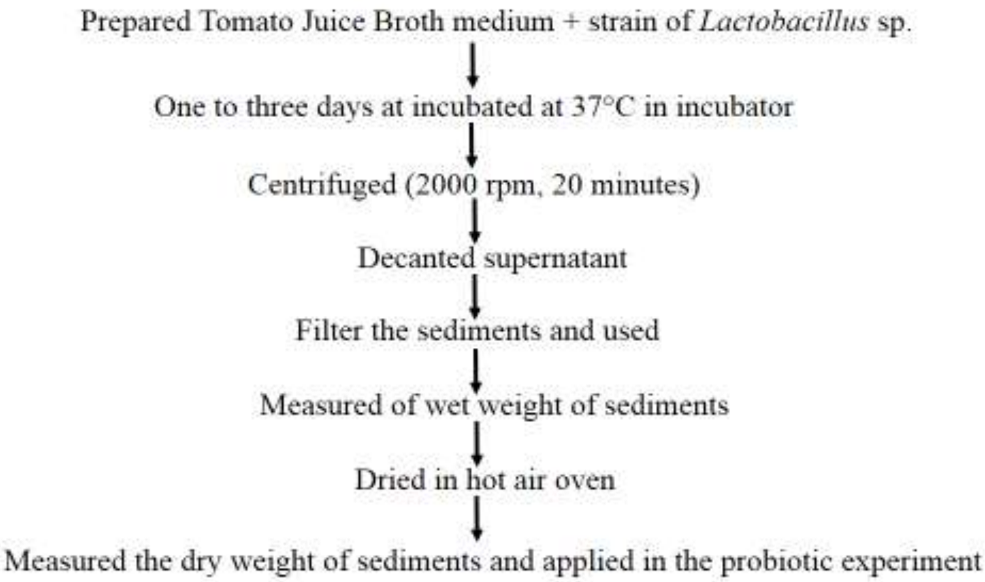

Figure 1 Flow chart for determining percentage (%) dry cell mass of *Lactobacillus* sp.

Preparation as probiotics

After drying, the dried cells was grounded to powder. The resulting powder was used as probiotics and mixed with the normal food for chicken.

Selection of chick for probiotics test

Three hundred and fifty healthy meat broiler was chosen at random. The tested birds were collected immediately after hatching from the broiler trading, Sunjin Myanmar Co., Ltd. They were transported to Ngwe Nantthar village, Hlegu Township where the research work was undertaken.

Application of *Lactobacillus* sp. (H-1) strain as probiotic in chicken feed (Pourakbari *et al.*, 2016)

The experimental broilers were divided into seven groups. Group A was fed on basal diet as control. Group B was fed with basal diet plus 0.005% of feed of *Lactobacillus* sp. (H-1) strain. Group C was fed with basal diet plus 0.010% of *Lactobacillus* sp. (H-1) strain. Group D was fed with basal diet plus 0.015% of *Lactobacillus* sp. (H-1) strain. Similarly, group E was fed with basal diet plus 0.005%, group F was fed with basal diet plus 0.010% and group G was also fed with basal diet plus 0.015% of *Lactobacillus* sp. (H-1) strain. Groups B, C, D contained nitrogen source but groups E, F, G did not contain nitrogen source in *Lactobacillus* dried cell powder.

Rearing and feeding of chick

The chicken house was divided into seven units. The units were enclosed by wire mesh netting. An experiment with 7 treatments (0%, 0.005%, 0.010% and 0.015%) including with/without nitrogen source and each included 50 chicks in the experiment. The experimental diets fed at three different breeding (starter; 0 - 10 days, grower; 11 - 28 days and finisher; 29 - 42 days). These basic constituents of the feed contained soybean meal, animal protein, broken rice, rice bran, corn, wheat bran, cassava, amino acids, minerals, vitamins, etc. The feeds were produced by a commercial mill (Green Feed) in Shwe Pyi Thar Township. Body weight and feed consumption were measured weekly. Death bird was recorded daily.

Calculation of feed utilization (Heuser, 1995)

Feed utilization (feed conversion rate or FCR) was calculated as the ratio between weight of feed consumed and body weight gained.

$$\text{Feed conversion rate} = \frac{\text{Weight of feed consumed}}{\text{Body weight gained}}$$

Vaccination program

The tested chicks were vaccinated according to the following schedule program based on Than Shwe and Fatt (2000). Environmental temperature for the first week of broiler was 32°C and gradually decreased according to the age, 25°C until the end of the experiment.

Table 1 Vaccination schedule

Vaccines	Age of chicken (day)	Method of vaccination
Infectious Bronchitis and Newcastle Disease (1st time)	3	Eye drop
Infectious Bursal Disease (1st time)	7	Mouth drop
Infectious Bronchitis and Newcastle Disease (2nd time)	14	Mouth drop
Infectious Bursal Disease (2nd time)	21	Mouth drop

Chicken feed


Weighting chick broiler chicken


One day old broiler chicken


Farm of broiler


Two week old broiler chicken


Three week old broiler chicken

Four week old broiler chicken | Five week old broiler chicken | Six week old broiler chicken

Figure 2 Development stages of broiler chicken during two to six weeks in the experiment I

Results

Determination of dry mass

Lactobacillus sp. (H-1) isolated from the sample

The optimal condition of *Lactobacillus* sp. (H-1) isolated from the sample was 3 days of culture, 10% size of inoculum and medium of pH-6 in 3 days of fermentation periods. The results showed that the maximum dried cell weight were obtained 1.38400 g per liter (with nitrogen source) and 0.57917 g per liter (without nitrogen source). The yield of dried mass were an average of 1.0 g per liter (with nitrogen source) and 0.5 g per liter (without nitrogen source). The result of bacterial dry mass were presented in Tables 2 and 3.

Table 2 Dried mass and wet weight of *Lactobacillus* sp. (H-1 with nitrogen source) at optimal condition (3 days of 10 % inoculum)

Batch number in liter	Wet weight in gram	Dry weight in gram	Dry mass%
1	3.29720	1.11490	33.81
2	3.18802	1.08125	33.92
3	4.14500	**1.38400**	33.39
4	3.00018	1.00208	33.40
5	2.95860	0.98863	33.42
6	3.15229	1.05220	33.38
7	2.69095	**0.92850**	34.50
8	2.69895	0.92952	34.44
9	3.42531	1.16625	34.05
10	3.42521	1.14525	33.44
Total	31.98171	10.79258	337.75
Average	3.198171	1.079258	33.775

Table 3 Dried mass and wet weight of *Lactobacillus* sp. (H-1 without nitrogen source) at optimal condition (3 days of 10 % inoculum)

Batch number in liter	Wet weight in gram	Dry weight in gram	Dry mass%
1	1.54237	0.50674	32.85
2	1.73745	**0.57917**	33.33
3	1.55904	0.51125	32.79
4	1.35193	0.46032	34.05
5	1.35109	**0.45990**	34.04
6	1.35278	0.46073	34.06
7	1.67201	0.56629	33.87
8	1.52990	0.50568	33.05
9	1.51603	0.50256	33.15
10	1.65702	0.55242	33.34
Total	15.26962	5.10506	334.53
Average	1.526962	0.510506	33.453

Experiment I

Application of *Lactobacillus* sp. (H-1) strain isolated from sauerkraut as probiotic in chicken feed

Data presented in Tables showed the effect of different levels of dried bacteria *Lactobacillus* sp. (H-1) on body weight gain, feed consumption, feed conversion rate and mortality rate in all treatments. At the end of experiment, mean of body weight (2996.9 g, 3037.3 g, 3074.8 g, 3108.6 g, 2991.7 g, 3005.4 g, 3029.0 g), mean of feed consumption (1050.0 g, 1089.6 g, 1096.8 g, 1123.6 g, 1049.4 g, 1085.4 g, 1087.6 g), mean of feed conversion rate (1.39 %, 1.51 %, 1.41 %, 1.45 %, 1.47 %, 1.44 %, 1.42 %), and mortality rate (4 %, 2 %, 0 %, 0 %, 4 %, 4 %, 2 %) were observed in the group A, B, C, D, E, F and G respectively.

Body weight gain was increased in all group during 0-21 days and 35-42 days. However, body weight gain was not increased during 22-34 days. In this experiment, feed consumption of groups B, C, D, F and G were increased than control (group A) during 2-6 weeks but group E was decreased than control. However, groups E, F and G were lower than groups B, C and D in the result of feed consumption at this experiment. In the 2nd week, the feed conversion ratio (FCR) is not different from that of control (1.57 g for control and groups B, C, D and, 1.66 g, 1.65 g, 1.64 g for groups E, F, G respectively). FCR is lower than that of control in (group E in 3rd week, groups E and F in 4th week, groups B, C, D, E and G in 5th week). In the 6th week, FCR of control (group A) is lower than other groups. The motility rate was much higher in control (group A) and groups E, F 4%, followed by groups B and G which were 2% and finally groups C and D was 0%. In the present study, group D (0.015 % probiotic) showed the best result in the body weight gain, feed consumption and mortality rate. The results were presented in **Tables 4-8** and **Figures 3, 4**.

Table 4 Mean body weight, weight gain, feed consumption and feed conversion and mortality rate in two week old broiler supplemented with probiotic (H-1) strain

Group	Body weight (g)	Weight gain (g)	Feed consumption (g)	Feed conversion rate	Mortality chick
A	659.6	586.6	512.4	1.57	0
B	680.4	607.2	512.4	1.57	0
C	691.0	617.4	512.4	1.57	0
D	700.8	627.0	512.4	1.57	0
E	656.4	582.8	512.4	1.66	0
F	661.6	588.8	512.4	1.65	0
G	672.0	597.2	512.4	1.64	0

A = 0% (Control); B = 0.005% (with nitrogen source); C = 0.010% (with N_2 source);
D = 0.015% (with N_2 source); E = 0.005% (without nitrogen source);
F = 0.010% (without N_2 source); G = 0.015% (with N_2 source)

Table 5 Mean body weight, weight gain, feed consumption and feed conversion and mortality rate in three week old broiler supplemented with probiotic (H-1) strain

Group	Body weight (g)	Weight gain (g)	Feed consumption (g)	Feed conversion rate	Mortality chick
A	1215.6	556.0	722.8	1.30	0
B	1242.0	561.6	750.4	1.34	0
C	1269.2	578.2	768.0	1.33	0
D	1287.4	586.6	782.2	1.33	0
E	1212.6	556.2	717.0	1.29	0
F	1219.8	558.2	747.3	1.37	1
G	1228.8	558.6	755.1	1.39	1

A = 0% (Control); B = 0.005% (with nitrogen source); C = 0.010% (with N_2 source);
D = 0.015% (with N_2 source); E = 0.005% (without nitrogen source);
F = 0.010% (without N_2 source); G = 0.015% (with N_2 source)

Table 6 Mean body weight, weight gain, feed consumption and feed conversion and mortality rate in four week old broiler supplemented with probiotic (H-1) strain

Group	Body weight (g)	Weight gain (g)	Feed consumption (g)	Feed conversion rate	Mortality chick
A	1744.9	529.3	940.8	1.86	1
B	1771.8	529.8	984.0	1.86	0
C	1802.2	533.0	1022.2	1.92	0
D	1820.6	533.2	1057.2	1.98	0
E	1731.4	518.8	925.0	1.78	0
F	1745.7	529.8	971.8	1.83	1
G	1759.6	530.8	988.6	1.86	1

A = 0% (Control); B = 0.005% (with nitrogen source); C = 0.010% (with N_2 source);
D = 0.015% (with N_2 source); E = 0.005% (without nitrogen source);
F = 0.010% (without N_2 source); G = 0.015% (with N_2 source)

Table 7 Mean body weight, weight gain, feed consumption and feed conversion and mortality rate in five week old broiler supplemented with probiotic (H-1) strain

Group	Body weight (g)	Weight gain (g)	Feed consumption (g)	Feed conversion rate	Mortality chick
A	2239.2	494.3	1060.4	2.32	2
B	2269.6	497.8	1057.0	2.12	0
C	2297.8	498.6	1110.0	2.24	0
D	2333.2	512.6	1176.0	2.29	0
E	2232.9	501.5	1039.6	2.23	1
F	2249.2	503.5	1093.3	2.34	2
G	2263.7	504.1	1095.9	2.17	1

A = 0% (Control); B = 0.005% (with nitrogen source); C = 0.010% (with N_2 source);
D = 0.015% (with N_2 source); E = 0.005% (without nitrogen source);
F = 0.010% (without N_2 source); G = 0.015% (with N_2 source)

Table 8 Mean body weight, weight gain, feed consumption and feed conversion and mortality rate in six week old broiler supplemented with probiotic (H-1) strain

Group	Body weight (g)	Weight gain (g)	Feed consumption (g)	Feed conversion rate	Mortality chick
A	2996.9	757.7	1050.0	1.39	2
B	3037.3	767.7	1089.6	1.51	1
C	3074.8	775.1	1096.8	1.41	0
D	3108.6	775.4	1123.6	1.45	0
E	2991.7	758.8	1049.4	1.47	2
F	3005.4	766.2	1085.4	1.44	2
G	3029.0	765.3	1087.6	1.42	1

A = 0% (Control); B = 0.005% (with nitrogen source); C = 0.010% (with N_2 source);
D = 0.015% (with N_2 source); E = 0.005% (without nitrogen source);
F = 0.010% (without N_2 source); G = 0.015% (with N_2 source)

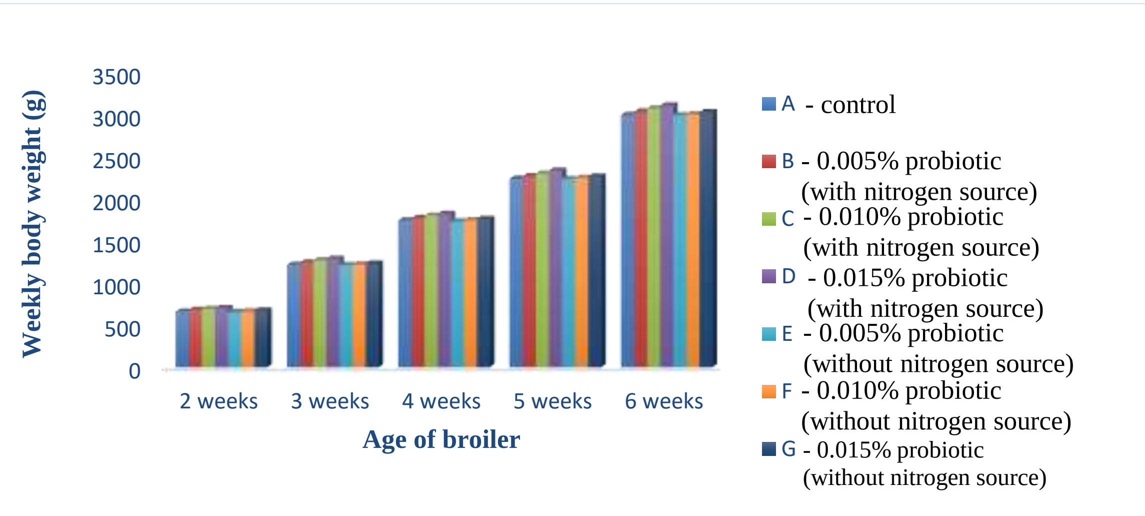

Figure 3 Comparison of weekly body weight in the experiment I

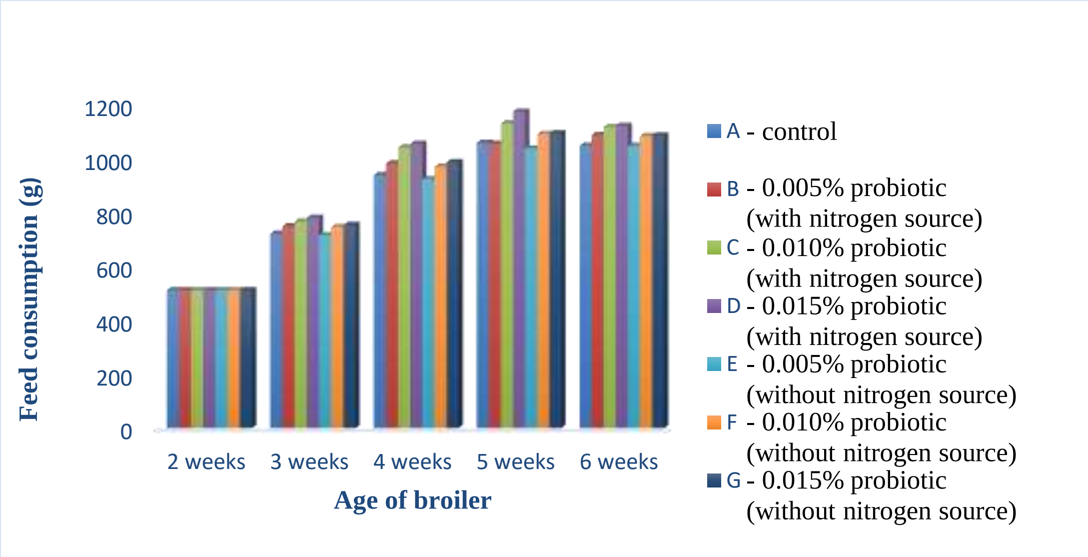

Figure 4 Comparison of weekly feed consumption in the experiment I

Discussion and Conclusion

The optimal condition of *Lactobacillus* sp. (H-1) isolated from the sample was 3 days age of culture, 10% size of inoculum and medium of pH-6 in 3 days of fermentation periods. The results showed that the maximum dried cell weight were obtained 1.38400 g per liter (with nitrogen source) and 0.57917 g per liter (without nitrogen source). The yield of dried mass were an average of 1.00 g per liter (with nitrogen source) and 0.50 g per liter (without nitrogen source).

Hwang *et al.*, 2015 observed that dried cell weight of *Lactobacillus acidophilus* was 1.06 g L^{-1} and 1.61×10^{9} CFU/ml. Rosa *et al.*, 2013 reported that maximum cell yield was achieved a pH value of 6.45 and 1.04 g L^{-1} of inoculum. The data of present research were found to be in agreement with above authors. Salma *et al.*, 2017 observed that *Lactobacillus helveticus* was found optimized at temperature of 40ºC and pH 6.25 which yielded 3.25 g L^{-1} dry cell biomass in MRS medium. According to this literatures, the results of present study were somewhat different.

Dietary probiotic significantly enhanced the feed intake and weight gain in starter phase (0-21 days) only was reported by Cengiz *et al.*, 2015. Body weight gain was increased in all group during 0-21 days and 35-42 days. However, body weight gain was not increased during 22-34 days. This results was also similar to the result of Cengiz *et al.*, 2015. In this experiment, feed consumption of groups B, C, D, F and G were more increased than control (group A) during 2-6 weeks but group E was decreased than control.

However, groups E, F and G were lower than groups B, C and D in the result of feed consumption at this experiment. In the 2nd week, the feed conversion ratio (FCR) is not different from that of control (1.57 g for control and groups B, C, D and, 1.66 g, 1.65 g, 1.64 g for groups E, F, G respectively). FCR is lower than that of control in (group E in 3rd week, groups E and F in 4th week, groups B, C, D, E and G in 5th week). In the 6th week, FCR of control (group A) is lower than other groups. The motility rate was much higher in control (group A) and groups E, F 4%, followed by groups B and G were 2% and finally groups C and D was 0%.

Khin Thu Zar Min, 2011 reported that feed conversion rate was not different between control and treatment groups. Samad *et al.,* 2011 found that feed conversion rate was lower for birds supplemented with probiotics than in control bird but no significant differences were reported between treatment groups. Afsharmanesh and Sadaghi, 2014 stated that some probiotics had no effect on feed intake and feed conversion ratio during the starter phase while feed intake increased during the grower-finisher phase. Therefore, these reported data were similar with this experiment.

Cengiz *et al.*, 2015 stated that the feed intake was reduced, whereas the feed conversion was improved significantly when birds were fed DFM at 0-7 days of age. According to Cengiz *et al*, 2015, the results of feed consumption and feed conversion ratio were somewhat different. In this experiment, body weight gain of groups B, C, D (with nitrogen source) were higher than groups E, F, G (without nitrogen source). Yeo *et al.,* 2018 reported that the yeast extract concentration (ranging between 20 and 30 g/l) enhanced the biomass production and growth rate of *L. helveticus*. Therefore, this reported data of Yeo *et al.*, 2018 was nearly the same with this experiment.

In the present study, probiotic supplementation in broiler feed was effective in improving body weight gain, feed consumption and reduce mortality rate. Results of the present study show that the treatment 0.005 %, 0.010 % and 0.015 % of dried bacteria used as probiotic had higher body weight gain and feed efficiency compared with the control group. The obtained results confirmed the previous finding of Pourakbari *et al.*, 2016. They reported that two hundred one-day-old male chickens were allocated to one of five treatments: control, and the same basal diet supplemented with 0.005%, 0.010%, 0.015% and 0.020% of probiotics. They described that probiotics in feed at 0.010% or higher levels of supplementation improved body weight gain and feed conversion rate compared with the control.

In this study, group D (0.015 % probiotic) showed the best result in the body weight gain, feed consumption and reduce mortality rate. The present result was nearly the same with those reported by Pourakbari *et al*., 2016. The present study reveals that probiotics could be successfully used as nutritional tools in poultry feeds for promotion of growth and reducing the mortality. Therefore, it may be assumed that the application of probiotic in the feeding method of poultry would be safer and effective.

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