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Estimation of metabolizable energy equivalency of *Bacillus subtilis* spore in male broiler chickens

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Abstract There are many studies on the effects of probiotics on performance of broiler chickens, but none of them has evaluated the metabolizable energy (ME) equivalency values of probiotics. The aim of this study was to determine the metabolizable energy equivalency value of *Bacillus subtilis* spore and its potential for decreasing feed ME content and cost. One hundred seventy-six day-old male broilers (Ross 308) were used in a completely randomized design, with 11 treatments, of four replicates each, and 4 chickens per cage as an experimental unit. Dietary treatments contain a basal diet (2800 kcal/kg) containing graded levels of ME (2850, 2900, 2950, 3000 and 3050 kcal/kg) and *Bacillus subtilis* (4×10^9 CFU/g DSM 17299) at 0.05, 0.10, 0.15, 0.20 and 0.25 g/kg. Graded levels of feed ME and added *Bacillus subtilis were used* as independent variables to derive regression equation of performance traits on independent variables. The derived regression equations of body weight and feed conversion ratio (FCR) for ME were set to be equal with those obtained for *Bacillus subtilis* and were solved; *Bacillus subtilis* equivalence value for ME was calculated by subtracting the obtained value from ME content of the basal diet. In comparison to the basal diet, added *Bacillus subtilis* significantly improved the body weight and feed conversion ratio. Metabolizable energy equivalency of *Bacillus subtilis* for FCR and body weight at 42 days of age was 360366 and 485823 kcal/kg, respectively. Results showed that ME value of *Bacillus subtilis* was decreased by advancing chickens age.

Keywords: broiler chickens, *Bacillus subtilis*, metabolizable energy equivalency performance

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Introduction

Feed can account for up to 70% of the cost of broiler production. The role of any feed additive that potentially improves nutrient utilization should be of interest to the poultry industry. Probiotics are one of the additives that could improve nutrient utilization and use as growth promoter in poultry (Anjum et al., 2005; Opalinski et al., 2007; Apata, 2008; Zaghari et al., 2015). The use of probiotics in the fields of science, medicine and business is growing quickly (Olnood et al., 2015). An extensive variety of direct fed microbials (DFM) is utilized as probiotics, including *Bacillus*, *Lactobacillus*, *Streptococcus*, and *Enterococcus* spp. as well as yeasts (Oggioni et al., 2003; Patterson and Burkholder, 2003; Huang et al. 2004; Koenen et al., 2004; Zhang and Kim, 2014). Among the large number of probiotic products in use today are bacterial spore formers, mostly of the genus *Bacillus*. Members of the genus *Bacillus* occupy a unique position since they are delivered as spores. The long term advantages of using spores as probiotics are that they are heat-stable and can survive transit across the stomach barrier, properties that cannot be assured with other probiotic bacteria that are given in the vegetative form (Hong et al., 2005). Bacillus spore can tolerate severe environmental stress and transitions during storage and handling (Setlow, 2006; Cartman et. al., 2008). A number of mechanisms are responsible for the resistance of spores of *Bacillus* species to heat, radiation and chemicals. Study of Setlow (2006) concentrated on these mechanisms such as the water content of spore core, the spore coat proteins and other related mechanisms. Based on results obtained by Bai et al. (2016), *Bacillus subtilis* has an antioxidant capacity in broiler diets. Anjum et al. (2005) suggested that *Bacillus subtilis* could increase the secretion of protease, amylase and lipase and subsequently increase growth performance and FCR. Furthermore, some *Bacillus* species have the capacity to produce cellulase, xylanase, phytase, and keratinase (Hendricks et al., 1995; Monisha et al., 2009; Mazotto et al., 2011; Mittal et al., 2011). *Bacillus subtilis* has been shown to improve ileal nutrient digestibility and production performance in broilers. Also, the study of Wang and Gu (2010) indicated that *Bacillus Coagulans* administration in feed can increase protease and amylase activity and improve broilers growth performance. In agreement with previous research, the study of Zaghari et al. (2015) showed that *Bacillus subtilis* could improve growth in birds fed protein reduced diets. Kehlet et al. (2015) and Harrington *et al.* (2015) suggested that the addition of *B. subtilis* to broiler diets with reduced energy levels improved broiler performance. These studies evaluated the effect of *Bacillus subtilis* in reduced metabolizable energy (ME) of diets but they did not measure the precise quantity of energy that liberates by *Bacillus subtilis*. Therefore, the aim of the present study was to estimate the precise ME equivalency value of *Bacillus subtilis* spore (GalliPro®) by mathematical method and quantifying its contribution for decreasing feed ME content as well as feed cost.

Material and methods

A total of 176 1-d-old feather-sexed male broilers (Ross 308) were obtained from a local commercial hatchery and reared over a 42-d experimental period. The chicks were housed in thermostatically controlled batteries with wire floors in an environmentally controlled building. Throughout the study, the birds were kept under a 23L:1D cycle. Each cage contained a trough feeder, as well as one tube waterer. Experimental diets in mash form and fresh clean drinking water were offered *ad libitum*. Environmental temperature in the three first days of life was 32°C and afterward it was 31°C until the end of the first week. The temperature was then decreased 1°C passed every 4 days until 22°C, which was maintained until the end of the experiment.

Birds with the same average initial body weight (43±0.5 g) were randomly allotted to one of 11 treatments. Each dietary treatment consisted of 4 replications, with 4 broiler chickens per replicate. Dietary treatments contain a basal diet (2800 kcal/kg), graded levels of ME (2850, 2900, 2950, 3000 and 3050 kcal/kg; Table 1 and 2) and graded levels of *Bacillus*

Table 1. Ingredients and nutrient contents of the starter diets (0-14d)

	Diets (g/kg)					
Ingredients	Basal diet	2	3	4	5	6
Corn grain	516	504.5	493	481.5	470	458.5
Soybean meal	427.8	429.8	431.8	433.8	435.8	437.8
Sunflower oil	14.3	23.8	33.3	42.8	52.3	61.8
Di-Ca phosphate	18.9	18.9	18.9	19	19	19
Oyster shell	9.8	9.7	9.7	9.7	9.7	9.7
Common salt	3.7	3.7	3.7	3.7	3.7	3.7
Vitamin premix[1]	2.5	2.5	2.5	2.5	2.5	2.5
Mineral premix[1]	2.5	2.5	2.5	2.5	2.5	2.5
DL-Met	3.1	3.2	3.2	3.2	3.2	3.2
L-Lys HCl	0.8	0.8	0.8	0.7	0.7	0.7
L-Thr	0.6	0.6	0.6	0.6	0.6	0.6
Sum	1000	1000	1000	1000	1000	1000
Calculated nutrients (g/kg)						
ME (kcal/kg)	2800	2850	2900	2950	3000	3050
CP	230	230	230	230	230	230
Available P	4.8	4.8	4.8	4.8	4.8	4.8
Ca	9.6	9.6	9.6	9.6	9.6	9.6
Na	1.6	1.6	1.6	1.6	1.6	1.6
Dig. Lys	12.8	12.8	12.8	12.8	12.8	12.8
Dig. Met	6.3	6.3	6.3	6.3	6.3	6.3
Dig. Met + Cys	9.5	9.5	9.5	9.5	9.5	9.5
Dig. Thr	8.6	8.6	8.6	8.6	8.6	8.6

[1] Provided the following per kilogram of diet: Vitamin A, 12000, IU; Cholecalciferol, 5000 IU; Vitamin E, 80mg; Vitamin k, 3.2 mg; Vitamin B1, 3.2 mg; Vitamin B2, 8.6 mg; B6, 4.3 mg; B12, 0.017 mg; Folic acid, 2.2 mg; Niacin, 65 mg; Pantothenic acid, 20 mg; Vitamin H, 0.22 mg; Choline, 500 mg; Manganese, 120 mg; Iron, 20 mg; Selenium 0.3 mg; Cupper, 16 mg; Iodine 1.25 mg and Zinc, 110 mg.

Table 2. Ingredients and nutrient contents of the grower diets (15-42d)

	Diets (g/kg)					
Ingredients	Basal diet	2	3	4	5	6
Corn grain	571	559.5	548	536.5	525	513.5
Soybean meal	386.6	388.7	390.7	392.7	394.8	396.7
Sunflower oil	4.7	14.2	23.7	33.2	42.7	52.2
Di-Ca phosphate	16.7	16.7	16.7	16.7	16.7	16.8
Oyster shell	9.1	9.1	9	9	9	9
Common salt	3.7	3.7	3.7	3.7	3.7	3.7
Vit premix[1]	2.5	2.5	2.5	2.5	2.5	2.5
Min premix[1]	2.5	2.5	2.5	2.5	2.5	2.5
DL-Met	2.6	2.6	2.7	2.7	2.7	2.7
L-Lys HCl	0.4	0.3	0.3	0.3	0.2	0.2
L-Thr	0.2	0.2	0.2	0.2	0.2	0.2
Sum	1000	1000	1000	1000	1000	1000
Calculated nutrients (g/kg)						
ME (kcal/kg)	2800	2850	2900	2950	3000	3050
CP	215	215	215	215	215	215
Available P	4.35	4.35	4.35	4.35	4.35	4.35
Ca	8.7	8.7	8.7	8.7	8.7	8.7
Na	1.6	1.6	1.6	1.6	1.6	1.6
Dig.Lys	11.5	11.5	11.5	11.5	11.5	11.5
Dig.Met	5	5	5	5	5	5
Dig.Met + Cys	8.7	8.7	8.7	8.7	8.7	8.7
Dig.Thr	7.7	7.7	7.7	7.7	7.7	7.7

[1] Provided the following per kilogram of diet: Vitamin A, 12000, IU; Cholecalciferol, 5000 IU; Vitamin E, 80mg; Vitamin k, 3.2 mg; Vitamin B1, 3.2 mg; Vitamin B2, 8.6 mg; B6, 4.3 mg; B12, 0.017 mg; Folic acid, 2.2 mg; Niacin, 65 mg; Pantothenic acid, 20 mg; Vitamin H, 0.22 mg; Choline, 500 mg; Manganese, 120 mg; Iron, 20 mg; Selenium 0.3 mg; Cupper, 16 mg; Iodine 1.25 mg and Zinc, 110 mg.

subtilis (GalliPro®) top-dressed on the basal diet (0.05, 0.10, 0.15, 0.20 and 0.25 g/kg; Table 3).

GalliPro® is, one of the heat tolerated direct fed microbial, based on *B. subtilis* spore (*B. subtilis* 4×10^9 CFU/g DSM 17299). So that CFU per kg experimental diets (treatment number 7, 8, 9, 10 and 11) were 2×10^8, 4×10^8, 6×10^8, 8×10^8 and 1×10^9 respectively. GalliPro® was supplied by Biochem Company (Zusatzstoffe Handels-und Produktionsge sellschaft GmbH, Lohne, Germany).

Nutrient concentration was the same in the experimental diets from 1 to 6 except for ME (Table 1and 2). Energy to protein ratio of basal diets was based on the Ross 308 broiler nutrition specifications (Table 1-3). Energy and protein content of treatment 5 met the requirement recommended by Ross 308 and in treatment 6 exceeded the guidelines (Table 1and 2). A 2-phase feeding program was used, with a starter diet offered from d 1 to 14 and a grower diet from d 15 to 42.

Weekly feed intake (FI), body weight and mortality

Table 3. Ingredient contents of the starter and grower diets (treatments 7 to 11) (0-42d)

	Diets (g/kg)				
Ingredients	7	8	9	10	11
Starter period (0-14d)					
B. subtilis	0.05	0.1	0.15	0.2	0.25
Basal starter diet	1000	1000	1000	1000	1000
Sum	1000+0.05	1000+0.10	1000+0.15	1000+0.20	1000+0.25
Grower period (15-42d)					
B. subtilis	0.05	0.1	0.15	0.2	0.25
Basal grower diet	1000	1000	1000	1000	1000
Sum	1000+0.05	1000+0.10	1000+0.15	1000+0.20	1000+0.25

B. subtilis added over the top of basal starter and grower diets for making treatments 7 to 11.

Calculated nutrients content of treatment 7 trough 11 was the same as starter (2800 kcal/kg ME, 23 %CP) and grower (2800 kcal/kg ME, 21.5 %CP) basal diets.

were recorded per cage, and weight gain, FCR and survivability were calculated. At the end of the trial, one bird that was close to average of pen weight was taken from each replication and sacrificed to determine carcass, liver and abdominal fat weight.

All procedures on chicken in this research were approved by the Department of Animal Science of University of Tehran.

Economic analysis

The feed cost per kilogram weight gain of male broiler chickens was calculated, taking into consideration the cost of major feed ingredients and feed additives used at the time of the study. The feed cost per kilogram weight gain was calculated by multiplying FCR by average weighed price (AWP) of diets. The feed cost per kilogram weight gain of each diet was calculated as follows:

AWP = (% SFI×SDP) + (% GFI×GDP)

where, % SFI and SDP are starter feed intake (FI) (% of whole feed intake) and starter diet price, respectively; % GFI and GDP are grower FI (% of whole feed intake) and grower diet price.

Return on investment (ROI) was used as a rudimentary gauge of an investment's profitability. For calculation of ROI the benefit of an investment was divided by the cost of the investment, and the result is expressed as a percentage or a ratio as:
ROI = (Gains from Investment – Cost of Investment) / Cost of Investment

Statistical analysis

The cage was identified as an experimental unit. Both performance and carcass yield data were statistically analyzed. Data on body weight, feed consumption, FCR, and survivability were subjected to analysis of variance (ANOVA) in a completely randomized design (CRD) using SAS Institute (2003) statistical computer program. The Duncan's multiple range test was used for mean separation, and statistical significance was set at $P < 0.05$.

The potential energy sparing of *B. subtilis* was determined using linear regression analysis for the weekly periods across dietary ME level for BW and FCR using 4 replicates for each treatment. Linear equations were obtained for each parameter for the ME levels, and dependent variables (BW or FCR) were regressed against independent variables (ME and *Bacillus subtilis* levels). The linear regression model used was $Y = a + b \times X$ in which, Y is the parameter evaluated (BW or FCR), a is the intercept, b is the slope of the line, and X is the ME or *Bacillus subtilis* levels. The derived regression equations of body weight and FCR for ME were set to be equal with those obtained for *Bacillus subtilis* and were solved; *Bacillus subtilis* equivalence value for ME was calculated by subtracting the obtained value from ME content of the basal diet (2800 kcal/kg).

Results

Weekly body weight gains of the chicken are presented in Table 4. The results indicated that body weight at 42d was statistically different between treatments (P < 0.05), the lowest and the highest body weight were observed on the basal diet and the treatment containing 2950 kcal/kg ME, respectively. Dietary treatments had no significant effect on the average daily feed intake of broiler chickens (P > 0.05, Table 5).

Table 4. Effect of experimental diets on weekly body weight in male broiler chickens

Diets	*B. subtilis* (g/kg)	ME (kcal/kg)	Age (day)					
			7	14	21	28	35	42
Basal	0	2800	151.1	361.5	697.0	1160.0	1746.3	2394.6^{b}
2	0	2850	142.1	348.8	729.8	1252.3	1907.6	2681.3^{a}
3	0	2900	147.2	381.6	771.2	1312.6	1971.1	2661.8^{a}
4	0	2950	154.3	386.2	797.2	1361.3	2038.6	2703.3^{a}
5	0	3000	155.0	382.6	771.7	1294.5	1995.7	2639.6^{a}
6	0	3050	154.3	404.3	807.8	1328.3	1996.8	2673.1^{a}
7	0.05	2800	143.8	396.0	730.8	1247.7	1917.8	2570.6^{ab}
8	0.10	2800	146.6	367.6	730.0	1211.3	1861.6	2539.6^{ab}
9	0.15	2800	161.0	379.8	792.6	1343.5	2013.6	2657.5^{a}
10	0.20	2800	148.0	365.5	737.0	1227.0	1837.0	2516.6^{ab}
11	0.25	2800	154.5	397.5	818.2	1337.1	1984.6	2644.1^{a}
P-value			0.4245	0.1675	0.2704	0.0951	0.1184	0.0394
SEM			5.234	14.087	32.339	50.558	62.803	56.618
CV			6.961	7.491	8.489	7.912	6.491	4.338

^{abc} Means in a column with common superscript(s) do not differ ($P > 0.05$).

Table 5. Effect of experimental diets on feed intake (g/d/bird) in male broiler chickens

Diets	*B. subtilis* (g/kg)	ME (kcal/kg)	Age (day)					
			7	14	21	28	35	42
Basal t	0	2800	18.517	49.310	84.548	112.286	151.667	185.310
2	0	2850	18.544	48.536	82.250	119.536	149.018	195.880
3	0	2900	19.201	50.554	86.768	122.982	155.786	179.813
4	0	2950	19.717	50.697	88.750	126.089	161.375	185.340
5	0	3000	18.569	47.661	84.714	117.714	161.087	172.125
6	0	3050	19.785	53.191	89.952	123.024	161.810	178.450
7	0.05	2800	18.102	49.339	76.286	125.167	166.851	183.146
8	0.10	2800	17.841	50.143	82.429	113.348	154.935	177.340
9	0.15	2800	20.446	51.024	87.286	125.524	163.095	181.640
10	0.20	2800	18.774	49.857	82.964	118.179	151.911	179.340
11	0.25	2800	20.504	52.893	94.393	126.812	162.420	184.170
P-value			0.3922	0.4718	0.3519	0.2772	0.6919	0.7425
SEM			0.829	1.603	4.356	4.266	6.534	7.314
CV			8.708	6.386	10.208	7.051	8.264	8.034

Table 6 shows the effect of experimental diets on FCR. The poorest FCR was observed on the basal diet (P < 0.05). At 21 days of age, the lowest feed efficiency was observed in chicks fed the basal diet. The feed efficiency of chicks receiving the incremental levels of energy and *Bacillus subtilis*, were not different (P > 0.05, Table 6). The trend for the remaining period of the experiment was almost the same (Table 6). Effect of treatments on carcass, liver, and abdominal fat weights and survivability were not significant (P > 0.05, Table 7). Metabolizable energy equivalency value of *Bacillus subtilis* for FCR and body weight at 7, 14, 21, 28, 35 and 42 days of age for male broiler chicken is shown in Tables 8 and 9.

Discussion

Body weight at d 42 was statistically different among treatments (P < 0.05) with the lowest and the highest body weight observed in basal diet and the treatment containing 2950 kcal/kg ME, respectively. At days 21, 28 and 35 birds feeding on the non-*Bacillus* supplemented basal diet had a numerically lower body weight than their counterparts chicks (treatment 7) which received the basal diet supplemented with *Bacillus subtilis.*

Mean final body weight of chicks receiving the basal diet, diets containing incremental levels of *Bacillus subtilis* and incremental levels of ME were 2394.6, 2581.9 and 2671.8 g, respectively (P < 0.0004, Figure 1). The body weight difference between the chicks that received diets containing incremental levels of *Bacillus subtilis* and ME was not significant (P > 0.05). These results indicated that adding *Bacillus subtilis* to the basal diet, increased utilization of feed energy by the chicken. Therefore, estimation of energy equivalency value of *Bacillus*

Table 6. Effect of experimental diets on weekly feed conversion ratio in male broiler chickens

Diets	*B. subtilis* (g/kg)	ME (kcal/kg)	Age (day)					
			7	14	21	28	35	42
Basal	0	2800	0.857	1.326	1.528^{a}	1.596^{a}	1.667^{a}	1.759^{a}
2	0	2850	0.918	1.349	1.434^{b}	1.503^{bcd}	1.533^{de}	1.593^{c}
3	0	2900	0.908	1.279	1.422^{b}	1.492^{cde}	1.547^{de}	1.617^{bc}
4	0	2950	0.894	1.280	1.398^{b}	1.467^{de}	1.534^{de}	1.636^{bc}
5	0	3000	0.838	1.213	1.370^{b}	1.452^{e}	1.503^{e}	1.587^{c}
6	0	3050	0.900	1.267	1.419^{b}	1.508^{bcd}	1.569^{bcd}	1.640^{bc}
7	0.05	2800	0.822	1.330	1.450^{b}	1.549^{b}	1.616^{b}	1.703^{ab}
8	0.10	2800	0.855	1.295	1.442^{b}	1.524^{bc}	1.603^{bc}	1.663^{bc}
9	0.15	2800	0.890	1.267	1.404^{b}	1.482^{cde}	1.556^{cd}	1.657^{bc}
10	0.20	2800	0.888	1.315	1.441^{b}	1.539^{b}	1.606^{b}	1.672^{bc}
11	0.25	2800	0.929	1.294	1.436^{b}	1.542^{b}	1.612^{b}	1.699^{ab}
P-value			0.6654	0.1686	0.0405	<0.0001	<0.0001	0.0025
SEM			0.031	0.029	0.024	0.013	0.015	0.024
CV			7.082	4.574	3.386	1.801	1.925	3.014

^{abc} Means in a column with common superscript(s) do not differ (*P* > 0.05).

Table 7. Effect of experimental diets on carcass parameters in male chicken (42d)

Diets	*B. subtilis* (g/kg)	ME (kcal/kg)	Carcass (g)	AFP[1] (g)	Liver (g)	Carcass (%)	AFP (%)	Liver (%)
Basal	0	2800	1888.0	30.467	45.533	74.473	1.210	1.802
2	0	2850	2026.5	25.075	54.775	73.473	0.911	1.986
3	0	2900	1975.5	35.925	56.100	70.118	1.275	1.979
4	0	2950	2115.5	36.500	61.325	73.272	1.268	2.123
5	0	3000	2012.5	34.575	58.950	73.190	1.275	2.131
6	0	3050	2072.0	38.767	53.433	73.076	1.361	1.887
7	0.05	2800	1992.0	36.250	56.525	73.817	1.329	2.094
8	0.10	2800	1950.5	27.575	51.450	74.079	1.037	1.931
9	0.15	2800	2041.3	34.167	64.600	77.730	1.304	2.462
10	0.20	2800	1865.0	25.700	54.500	71.060	0.978	2.066
11	0.25	2800	2001.5	26.375	53.675	74.527	0.989	2.005
P-value			0.4293	0.5950	0.4116	0.0508	0.7580	0.1892
SEM			68.883	5.324	4.403	1.241	0.058	0.041
CV			6.908	33.531	15.836	3.381	0.376	0.264

[1] Abdominal Fat Pad

Table 8. Regression of feed conversion ratio (FCR) on dietary energy and *B. subtilis* levels and estimated ME equivalence value of *B. subtilis*

FCR	Regression of FCR on ME	R^2	P-value	Regression of FCR on *B. subtilis*	R^2	P-value	ME equivalency of *B. subtilis* (kcal/kg)
7d	FCR= 1.07521 -0.00006MEn	0.007	0.706	FCR=0.85355+0.24025BS	0.130	0.098	*
14d	FCR= 2.56397 -0.00043MEn	0.320	0.006	FCR=1.32161-0.1236BS	0.032	0.425	644278.8
21d	FCR= 2.70863 -0.00043MEn	0.344	0.004	FCR=1.48331-0.026245BS	0.155	0.069	259360
28d	FCR=2.53565-0.00035MEn	0.354	0.003	FCR=1.56032-0.16343BS	0.121	0.111	413571.4
35d	FCR=2.53460-0.0003MEn	0.211	0.031	FCR=1.63213-0.16891BS	0.146	0.078	366843.7
42d	FCR=2.53460-0.0003MEn	0.132	0.095	FCR=1.71631-0.19495BS	0.083	0.193	360366.6

* Regression coefficients of FCR on energy were small, therefore calculation of equivalence value was not possible.

Table 9. Regression of body weight on dietary energy and *B. subtilis* levels and estimated ME equivalence value of *B. subtilis*

BW	Regression of BW on ME	R^2	P-value	Regression of BW on *B. subtilis*	R^2	P-value	ME equivalence of *B. subtilis* (kcal/kg)
7d	BW= 35.766 +0.039MEn	0.084	0.189	BW=146.970+26.367BS	0.058	0.278	881536
14d	BW= -191.113+0.194MEn	0.235	0.022	BW=354.441+144.486BS	0.215	0.029	793296
21d	BW= -390.821+0.394MEn	0.180	0.048	BW=701.659+384.630BS	0.313	0.006	867744
28d	BW=-352.2229+0.560MEn	0.181	0.048	BW=1187.994+514.895BS	0.230	0.023	721040
35d	BW=-637.283+0.884MEn	0.242	0.020	BW=1822.265+559.529BS	0.134	0.093	562124
42d	BW=679.092+0.668MEn	0.162	0.063	BW=2476.608+616.066BS	0.168	0.057	485823

subtilis seems more logic in economical point of view. Study of Harrington et al. (2015) indicated that birds in *B.Subtilis* groups were significantly heavier than birds in non-*B. Subtilis* supplemented groups.

Dietary treatments had no significant effect on average daily feed intake of broiler chickens ($P > 0.05$). Our result was in agreement with results of Zaghari et al. (2015). But, Sen et al. (2012) demonstrated that probiotic supplementation in the diet, significantly increased feed intake. Although the study of Opalinski et al. (2007) did not show any effects of *B. subtilis* (DSM17299) supplementation on broiler weight gain, they reported that *B. subtilis* could decrease feed intake and improve FCR in broiler chickens. Table 6 shows the effect of the experimental diets on FCR. The poorest FCR was observed in the birds fed with the basal diet ($P < 0.05$).

Figure 2 compares the FCR for birds consuming the basal diet (2800 kcal/kg ME), average of five incremental levels of *Bacillus subtilis* (0.05, 0.1, 0.15, 0.2 and 0.25 g/kg) added to the basal diet, and five incremental levels of ME (2850, 2900, 2950, 3000 and 3050 kcal/kg).

In comparison to basal diet, added *Bacillus subtilis* to the basal diet improved FCR ($P < 0.05$). These results are in agreement with those of Zhang and Kim (2014),

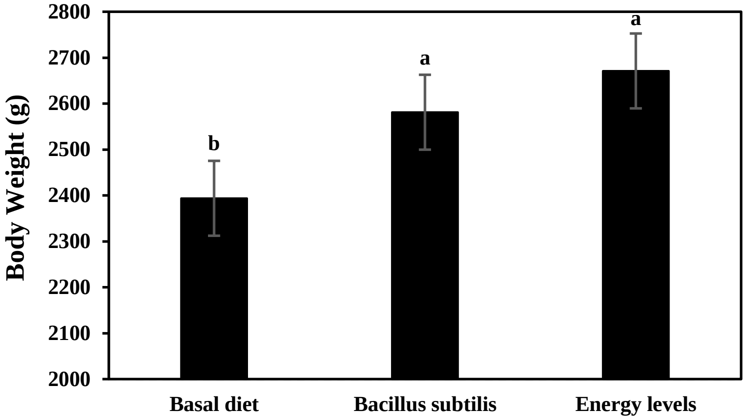

Figure 1. Comparison of mean body weight of chicken (at 42 d) receiving the basal diet, and basal diet containing five levels of *Bacillus subtilis* or metabolizable energy.

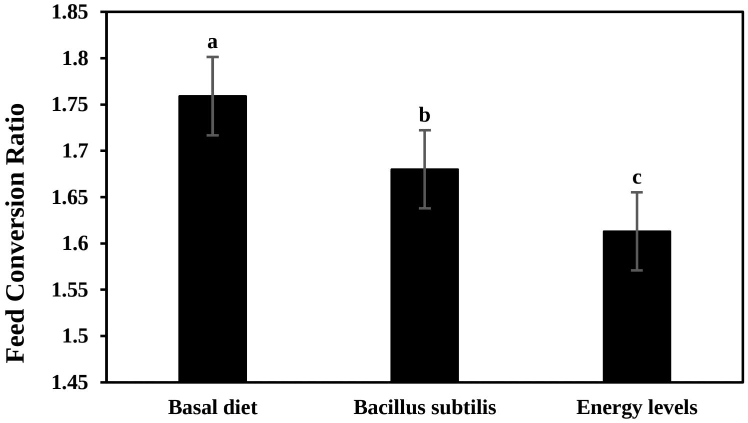

Figure 2. Comparison of feed conversion ratio of chicken (at 42 d) receiving the basal diet, and basal diet containing five levels of *Bacillus subtilis* or metabolizable energy

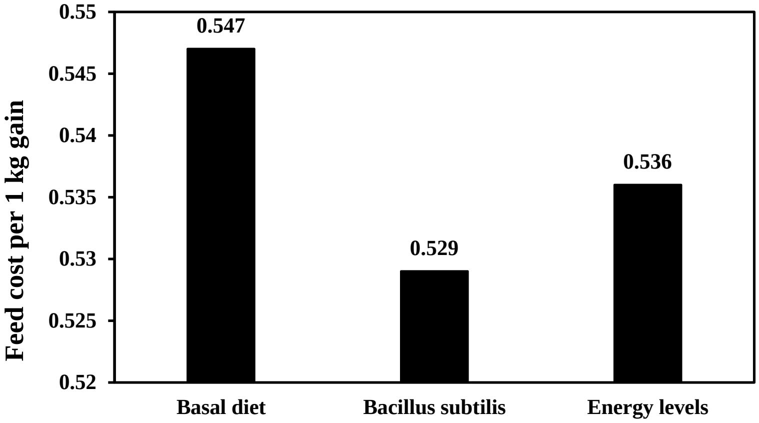

Figure 3. Comparison of average feed cost (US $) per kg gain of broiler chickens receiving the basal diet, and the basal diet containing five levels of *Bacillus subtilis* or metabolizable energy

Wang and Gu (2010) and Opalinski et al. (2007). The study of Zhang and Kim (2014) indicated that probiotic treatments increased apparent ileal digestibility of most essential amino acids compared with control. Similarly, Zaghari et al. (2015) reported that added *Bacillus subtilis* (GalliPro$^{®}$) in the diet could reduce chickens' protein and amino acid requirements. According to the study of Sen et al. (2012), *B. subtilis* LS 1-2 supplementation increased villus height and villus height to crypt depth ratio in both duodenum and ileum and improved intestinal microbial balance and gut health. These findings may explain the improvement of broilers' growth performance through added probiotics in diet.

The effect of treatments on carcass, liver and abdominal fat weights and survivability was not significant (P > 0.05) which is in harmony with Zaghari et al. (2015), Cengiz et al. (2015) and Chen et al. (2013) who reported that dietary probiotic did not affect the relative weight of breast, liver and abdominal fat. In contrast to our study, the study of Molnar et al. (2011) indicated that there was no effect of *B. subtilis* supplementation on the carcass, breast and thigh yields, or the abdominal fat content. However, the absolute and relative weights of the liver were significantly smaller in the group which was supplemented with the highest amount of *B. subtilis* (45.3g, 1.83%), compared with the control group (54.4g, 2.20%).

Average calculated ME equivalency value of *Bacillus subtilis* of the entire experiment period for FCR was about 408884 kcal/kg (Table 8). But the same value for body weight as a dependent variable in regression analysis was 718594 kcal/kg (Table 9).

Assuming the use of 200 g GalliPro per ton of feed, its contribution in to male broiler diets energy would be 82-144 kcal/kg. Data presented in Tables 8 and 9 indicated that from the first to seventh weeks of age, the contribution of *B. subtilis* to the diet ME decreased by 80%. Probably due to development of gastrointestinal functionality. Harrington et al. (2015) showed that *B. subtilis* had a ME contribution of 62 kcal/kg feed. The difference between the present study and that of Harrington et al. (2015), may be due to the mathematical method used for estimating the equivalency of energy. Furthermore, they measured the contribution of *B. subtilis* at two stages (0-21 and 22 to 42) while it was estimated at weekly intervals in the current study.

Figure 3 shows the effect of the experimental diets on feed cost per kilograms weight gain. Chicks fed the basal diet supplemented with *B. subtilis* had 3.3% lower feed cost per kg weight gain in comparison to the control birds, despite the same level of feed energy. The results indicated that ROI of GalliPro diet was 1.25:1, indicating that the investment earning power (net income) was 25%.

In conclusion, *B. subtilis* had a minimum 408000 kcal/kg feed metabolizable energy equivalency value for broiler chickens. Therefore, using energy equivalency of *B. subtilis* in feed formulation may have the potential to reduce the feed cost.

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برآورد معادل انرژی قابل سوخت و ساز باسیلوس سوبتیلیس (*Bacillus subtilis*) در جوجه‌های گوشتی نر

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چکیده پژوهش‌های فراوانی در باره تاثیر پروبیوتیک‌ها بر عملکرد جوجه‌های گوشتی انجام شده است، اما هیچیک مقدار انرژی قابل سوخت و ساز معادل پروبیوتیک‌ها را ارزیابی نکرده است. هدف از این پژوهش تعیین معادل انرژی قابل سوخت و ساز اسپور باسیلوس سوبتیلیس و امکان کاهش انرژی و هزینه خوراک بود. این پژوهش، با ۱۷۶ جوجه یک روزه نر سویه تجاری راس ۳۰۸ در یک طرح کاملا تصادفی با یازده تیمار، چهار تکرار و چهار جوجه در هر قفس انجام شد. تیمارهای آزمایشی شامل جیره پایه (۲۸۰۰ کیلوکالری در کیلوگرم)، جیره‌های دارای سطوح مختلف انرژی قابل سوخت ساز (۲۸۵۰، ۲۹۰۰، ۲۹۵۰، ۳۰۰۰ و ۳۰۵۰ کیلوکالری در کیلوگرم) و باسیلوس سوبتیلیس (DSM ۱۷۲۹۹ ۴×۱۰^{۹}CFU/g) به میزان ۰/۰۵، ۰/۱۰، ۰/۱۵، ۰/۲۰ و ۰/۲۵ گرم در کیلوگرم بود. سطوح مختلف انرژی قابل سوخت و ساز و باسیلوس سوبتیلیس به عنوان متغیر مستقل برای برآورد معادلات تابعیت صفات عملکردی به عنوان متغیرهای وابسته مورد استفاده قرار گرفت. معادلات تابعیت وزن بدن و ضریب تبدیل خوراک از میزان انرژی قابل سوخت و ساز و سطوح باسیلوس سوبتیلیس مساوی هم قرار گرفت، پس از حل معادله و کسر مقدار انرژی جیره پایه، مقدار معادل انرژی قابل سوخت و ساز پروبیوتیک به دست آمد. در مقایسه با جیره پایه افزودن باسیلوس سوبتیلیس به خوراک موجب بهبود وزن بدن و ضریب تبدیل خوراک شد. انرژی قابل سوخت و ساز معادل باسیلوس سوبتیلیس برای ضریب تبدیل خوراک و افزایش وزن در سن ۴۲ روزگی به ترتیب ۳۶۰۳۶۶ و ۴۸۵۸۲۳ کیلوکالری بود. نتایج نشان داد که با افزایش سن جوجه، معادل انرژی باسیلوس سوبتیلیس کاهش یافت.