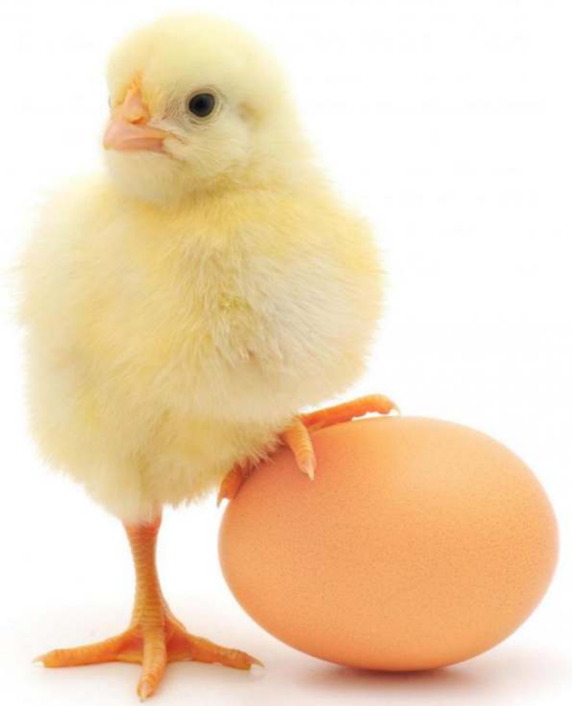
ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

OPEN ACCESS International Journal of Poultry Science

ISSN 1682-8356
DOI: 10.3923/ijps.2019.116.121

Research Article
Impact of Feeding Fermented Wet Feed on Broiler Breeder Production Performance and Some Hatchability Traits

Ziyad T.M. AL-Dhanki, W.I. AL-Jugifi and A.F.M. AL-Enzy

Department of Animal Production, College of Agriculture, University of Anbar, AL-Anbar, Ramadi, Iraq

Abstract

Background and Objective: The aim of this experiment was to investigate the effect of feeding fermented wet feed (FWF) on the live performance and some hatchability traits of broiler breeder hens. **Methodology:** The FWF was prepared by mixing 1 kg of basal broiler breeder diet with 1 L of tap water and 2 kg of manufactured locally produced probiotics (at least 10^9 *Lactobacillus* that was locally isolated from adult chickens, *Bacillus subtills* isolated from a Korean probiotic feed and 10^8 commercially available *Saccharomyces cerevisiae*). The mixture was then incubated under a plastic cover for 38 h at 37.5±2°C. After the incubation step, 216 female and 24 male broiler breeders (Ross 308) from 28-38 weeks of age (84 days) were distributed randomly among four treatment groups with three replicates per treatment (each replicate contained 18 females and two males and thus 54 females and six males were subjected to each treatment). The control, 1st, 2nd and 3rd treatment groups were administered 0% FWF:100% basal diet, 25% FWF:75% basal diet, 50% FWF:50% basal diet and 75% FWF:25% basal diet, respectively. At the end of the experiment (38 week), 500 eggs were collected and transferred to a hatchery. The incubation of the hatching eggs revealed no significant differences in the fertility percentage, total hatching percentage and hatching percentage of the fertile eggs. **Results:** No significant differences in live breeder performance were found between the treatment groups, with the exception that the 50 and 75% FWF treatments significantly increased the egg weight compared with the weights obtained with the other treatments. Similarly, these treatments resulted in a higher number of unsaleable eggs due to significant increases in the percentages of large eggs that were not suitable for hatching. Consequently, the administration of FWF increased the number of eggs transferred to the hatchery and thus increased the final breeding product, namely, the number of chicks. **Conclusion:** The current study showed that the feeding of fermented feed to broiler breeders does not significantly affect their performance parameters, with the exception of improving the egg weight and the number of unsaleable hatching eggs, which resulted in increases in the number of chicks.

Key words: Broiler breeder, fermented wet feed, hatchability traits, probiotic, production performance

Received: September 17, 2018 Accepted: December 27, 2018 Published: February 15, 2019

Citation: Ziyad T.M. AL-Dhanki, W.I. AL-Jugifi and A.F.M. AL-Enzy, 2019. Impact of feeding fermented wet feed on broiler breeder production performance and some hatchability traits. Int. J. Poult. Sci., 18: 116-121.

Corresponding Author: Ziyad T. M. Al-Dhanki, Department of Animal Production, College of Agriculture, University of Anbar

Copyright: © 2019 Ziyad T.M. AL-Dhanki *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fermented wet feed (FWF) is a microbial product that was initially proposed for improving various feed components through their pre-digestion by beneficial microorganisms (particularly cellulolytic bacteria) and the digested feed obtained from this fermentation increases the availability of some nutrients to the host. Hu *et al.*[1] suggested that the microorganisms that participate in the fermentation process will degrade proteins into small fractions (small peptides) that are soluble in water; therefore, the fermented final product contains more digestible constituents. In accordance with the previous study, Missotten *et al.*[2] reported that compared with unfermented feed, the protein and fibre of 3-day fermented feed is more easily digested in the ileal segment of the gastrointestinal (GI) tract of pigs. This increased digestibility is associated to the reduction in the pH of the GI tract obtained as a result of the acidic conditions of the fermented feed. Lyberg *et al.*[3] confirmed the results reported by Missotten *et al.* and found that the resulting acidic conditions in the GI tract would increase the number of microorganisms responsible for lytic action in the stomach, leading to a lower passage rate of digesta, consequently increasing the amount of time the feed is exposed to enzymatic action and thereby increasing digestibility. Jorgensen *et al.*[4] fermented barley and wheat for two days and fed it to pigs. They found that more than 80% of the saccharides disappeared from the final fermented product and observed a significant increase in Klason lignin, which indicates a loss of dry matter and thereby a higher protein content in the final fermented product due to carbohydrate decomposition.

FWF improves the health status of poultry by improving the environment of the GI tract, leading to better poultry performance through several modes of action. The first mode of action is the distribution of good microbiota to the whole GI tract, as revealed by the fermentation of whole poultry feed with probiotic bacteria. Briefly, Naji *et al.*[5] fermented whole feed with a locally manufactured probiotic for two days and used the fermented feed in broiler diets at proportions of 25, 50, 75 and 100%. These researchers hypothesized that this method of fermentation could spread the probiotic microorganisms to the whole GI tract, resulting in an improved health status and consequently improving broiler performance. In accordance with the results of the study conducted by Naji *et al.*[5], Jannah *et al.*[6] found that the fermentation of feed products with lactic acid bacteria will influence the microbial community of the broiler GI tract, particularly that in the ileum and caecum, leading to 0.66 and 0.48 decreases in the diversity indexes of lactic acid bacteria in these segments compared with those of the control group (0.84 and 1.05), respectively. Therefore, the microbial community in the GI tract of broilers fed fermented products becomes balanced and dominated by beneficial microorganisms (lactic acid bacteria).

Changes in the GI tract environment constitute a second mode of action observed after the feeding of fermented products. Yeh *et al.*[7] confirmed this hypothesis by fermenting feed using probiotic microorganisms and two fermentation stages: in the first stage, the feed was fermented for two days and in the second stage, the feed was fermented for five days. These researchers noted changes in the environment of the GI tract, including a significant decrease in the pH of the lumen due to a significant increase in the amount of lactic acid produced from the fermented feed, which promoted changes that occurred later in the GI tract. These results are in line with those reported by Jawad *et al.*[8], who found that feed fermented with probiotic bacteria creates an environment in the GI tract that aid the establishment of beneficial microorganisms and the exclusion of opportunistic pathogens by changing the histology of the mucus layer in the GI tract. These researchers reported significant increases in the villus height and crypt depth and these changes in intestinal histology are optimal for establishing an ideal microbial community in the GI tract of broilers because they allow the attachment of greater numbers of beneficial microbes (*Lactobacillus* spp.) to the GI tract. Additionally, Heres *et al.*[9] obtained similar results: the feeding of fermented feed suppresses the number of pathogenic bacteria (e.g., *Salmonella*) and thereby leads to improvement in the health status of the host.

Improving the health status of the host by decreasing the content of anti-nutritional substances in fermented feed is the third mode of action. Mukherjee *et al.*[10] found that the fermentation of soybean meal destroys anti-nutritional and allergenic substances in the soybean meal through their fermentation by the microorganisms dispersed in the final fermented product. Yang *et al.*[11] fermented feed with lactic acid bacteria (*e.g., Lactobacillus* spp.) and *Bacillus subtilis* and obtained results that were similar to those reported by Mukherjee *et al.*[10] found that 68% of isothiocyanates are destroyed by the 3-day fermentation of rapeseed meal for a broiler diet.

Induction of an immune response in the host is another mode of action of fermented feed. Choi *et al.*[12] found that the inclusion of fermented seaweed in broiler diets induces humeral immunity, as demonstrated by a significant increase in the concentration of serum IgM in broilers. Gao *et al.*[13] confirmed the induction of a significant immune response in broilers challenged with *Eimeria tenella* and fed a fermented diet with *Saccharomyces cerevisiae* : this enhanced cellular immune response was characterized by significant increases in CD4+ in the ileal segment of the GI tract and in sIgA secretion in the ileum in response to the coccidian challenge. Many researchers have reported on the difficulties of applying fermented feed to broiler breeder diets due to handling issues. As a result, only limited studies have investigated the use of fermented feed in broiler breeder diets[14]. Therefore, our study aimed to overcome these difficulties and investigate the effects of feeding FWF to breeders on their live performance and some hatchability traits.

MATERIALS AND METHODS

Preparation of the FWF: The FWF, which was prepared according to Jawad *et al.*[8], contained manufactured locally produced probiotics (MLPPs)[15] (each gram of MLPPs contained at least 10^9 *Lactobacillus* that was locally isolated from adult chicken, *Bacillus subtills* isolated from a Korean probiotic feed and 10^8 commercially available *Saccharomyces cerevisiae*). The feed was fermented by mixing 1 kg of basal broiler breeder diet, 1 L of tap water and 2 kg of MLPP and the resulting mixture was covered with a plastic cover for 38 h at 37.5±2°C to obtain the optimal conditions for fermentation. After 38 h of fermentation, the following treatment groups were set up: control treatment, 0% FWF:100% basal diet; 1st treatment, 25% FWF:75% basal diet; 2nd treatment, 50% FWF:50% basal diet; and 3rd treatment, 75% FWF:25% basal diet.

Experimental procedure: The treatments were experimentally tested as follows: 216 female and 24 male broiler breeders (Ross 308) from 28-38 week of age (84 days) were randomly distributed into 12 pens (220 cm length, 200 cm width and 200 cm height). Each pen contained 18 females and two males (stocking density of 4.6 birds/m^2) and represented a replicate of one of the four treatments (three replicates per treatment). According to the Ross 308 broiler breeder manual[16], the birds were exposed to 16 h of light (with an intensity of 100 Lux) from 27-38 week of age; the light was powered on at 6 am and powered off at 10 pm.

Table 1: Basal ratios for the broiler breeders (female and male) and chemical analysis of the diets

Items	Female (%)	Male (%)
Corn	50.00	40.00
Barley	0.00	30.00
Wheat bran	19.00	28.00
Soybean meal (44% protein)[1]	15.00	0.00
Protein concentrate (40% protein)	5.00	0.00
Hydrated plant fat	1.00	1.00
Dicalcium phosphate	16.00	0.00
Lime stone	7.70	0.70
Salt (NaCl)	0.70	0.30
Total	100.00	100.00
Calculated chemical analysis[2]		
Metabolizable energy (kcal kg^{-1})	2815.00	2100.00
Crude protein (%)	14.88	11.40
Lysine (%)	0.89	0.65
Methionine (%)	0.38	0.45
Meth+cys (%)	0.66	0.45
Available phosphorus (%)	3.46	0.86

[1]The chemical analysis was performed according to the NRC[18]. [2]AL-Wafi animal protein concentrate, which contained 40% crude protein, was used and the metabolizable energy was 2100 kcal kg^{-1}

Experimental diet: The quantities of the broiler breeder diet were based on the manual provided with the Ross 308 parent stock[16] and prepared according to the nutritional recommendations for the Ross 308 parent stock[17]. The basal diet composition is shown in Table 1. The male and female broiler breeders were administered a separate feeding programme, as revealed in Table 1, using different feeders in the same pen.

Production performance: The broiler breeder performance was determined according to the Ross 308 parent stock manual[16]. The performance parameters were recorded weekly until the end of the experiment (from 28-39 week) and included the egg number, egg weight and numbers of saleable eggs and unsaleable eggs (deformed, large, cracked, small, doubled, peewee and malformed eggs)[19]. The cumulative live broiler breeder traits were then calculated according to the Ross 308 parent stock manual[16] and included the cumulative egg production percentage per hen per week (HW%), the cumulative egg production (no. of eggs produced from the hen during the 84 days, eggs/hen/84 days), the egg mass (mass of the eggs produced from every hen during the 84 days, g egg/hen/84 days) and the feed conversion ratio, which represents the amount of feed consumed by a hen to produce either one egg (g feed/hen/egg/84 days) or one chick (g feed/hen/chick/84 days).

Hatching parameters and egg traits: On the final week of the experiment (38 week of age), 500 eggs were collected and transferred to a local hatchery. After 21 days of incubation in the hatchery, the hatching and fertility traits were measured. Specifically, the total hatching percentage, hatching percentage of fertile eggs, fertility percentage, percentage of live and dead pipped eggs and embryonic mortality (early, mid and late) were determined according to Buhr and Mauldin[19].

Statistical analysis: All percentage data were first subjected to an arcsine transformation and the resulting transformed data were used in the statistical analyses. Specifically, the effects of the four treatments were statistically analysed through one-way analysis of variance using the General Linear Model (GLM) procedure of the statistical package SAS version 9.1[20]. P-values less than 0.05 and 0.01 were considered to indicate significant treatment effects and the results were represented as the means/pooled SEMs.

RESULTS

Traits of hatching eggs: The traits of the hatching egg produced by the breeders fed FWF are illustrated in Table 2. As shown, significant differences in the mean egg weight were observed throughout the production period (84 days). Specifically, 50 and 75% FWF caused significant increases in the mean egg weight compared with the weights obtained with the other treatments. Similarly, these treatments significantly decreased the number of unsaleable eggs and thus increased the number of hatching eggs incubated in the hatchery. In addition, the number of large eggs was significantly increased in the control group compared with the other groups and thus, the number of hatching eggs from the control group transferred to the hatchery was lower than those obtained from the other groups (Table 2).

Hatching parameters: The hatchability traits of the broiler breeders fed FWF are shown in Table 3. As shown, significant differences were only detected in embryo mortality (early and late) and no significant differences were found in the remaining traits. The 50 and 75% FWF treatments caused significant increases in early and late embryonic mortality, respectively.

Production performance: The cumulative broiler breeder performance is shown in Table 4. The use of FWF had no effect on any of the breeder performance traits, even though some numerical differences were found among the treatments.

Table 2: Effect of feeding fermented wet feed (FWF) to broiler breeders (aged 38 weeks) on some traits of hatching eggs.

	Treatments						
[1]Traits of hatching eggs	Control	FWF (25%)	FWF (50%)	FWF (75%)	Total mean	Pooled SEM	Prob.
Egg weight (g)	48.05^{b}	47.19^{b}	59.74^{a}	59.01^{a}	53.50	1.1729	0.0001
Number of saleable eggs (eggs/hen/84 days)	35.88	36.35	46.70	46.78	41.43	2.3407	NS
Number of unsaleable eggs (eggs/hen/84 days)	10.65^{a}	8.17^{b}	6.93^{b}	6.66^{b}	8.10	0.5656	0.0159
Type of unsaleable eggs (egg/hen/84 days)							
Large eggs	6.51^{a}	4.05^{b}	3.06^{b}	3.76^{b}	4.34	0.4826	0.0276
Cracked eggs	0.24	0.20	0.18	0.11	0.18	0.0383	NS
Small eggs	0.03	0.09	0.07	0.07	0.06	0.0678	NS
Doubled eggs	2.25	2.57	2.32	1.88	2.25	0.1522	NS
Peewee eggs	1.11	0.97	0.99	0.58	0.91	0.1139	NS
Malformed eggs	0.50	0.26	0.29	0.24	0.32	0.0755	NS

All the data in the table are cumulative (84 days), with the exception of egg weight, which was calculated weekly (12 week)

Table 3: Effect of feeding fermented wet feed (FWF) to broiler breeders (aged 38 weeks) on hatchability traits

	Treatments						
[1]Hatchability traits	Control	FWF (25%)	FWF (50%)	FWF (75%)	Total mean	Pooled SEM	Prob.
Total hatching (%)	86.64	81.36	81.85	76.30	81.54	2.1982	NS
Hatching of fertile eggs (%)	87.56	86.20	88.52	82.50	86.20	1.5748	NS
Fertility (%)	98.77	94.20	92.10	91.73	94.20	1.4131	NS
Alive pipped (%)	0.00	1.23	0.00	0.00	0.31	0.3086	NS
Dead pipped (%)	1.23	0.00	0.00	1.23	0.62	0.4301	NS
Early embryonic mortality (%)	5.09^{ab}	2.35^{ab}	5.68^{a}	0.00^{b}	3.28	0.9285	0.05
Med embryonic mortality (%)	1.23	5.93	4.57	3.58	3.83	1.0655	NS
Late embryonic mortality (%)	4.57^{ab}	3.33^{ab}	0.0^{b}	9.38^{a}	4.32	1.2080	0.043

[1]All the data in the table are shown as percentages

Table 4: Effect of feeding fermented wet feed (FWF) to broiler breeders on cumulative performance (84 days)

	Treatments						
Cumulative performance (84 days)	Control	FWF (25%)	FWF (50%)	FWF (75%)	Total mean	Pooled SEM	Prob.
[1]HW (%)	61.96	61.32	61.34	62.58	61.80	0.3258	NS
[2]Egg/hen/84 days	40.00	39.59	50.18	50.10	44.97	2.3766	NS
[3]g feed/egg/hen/84 days	266.71	258.31	202.77	200.94	232.18	14.0300	NS
[4]g feed/hen/chick	323.92	305.30	240.72	255.53	281.36	16.9063	NS
[5]g egg/hen/84 days	2490.00	2407.40	3069.60	3096.30	2765.84	147.2550	NS

[1]Represents the percentage of weekly hen egg production throughout the 84 days of production. [2]Represents the cumulative egg production during the 84 days of egg production. [3]Represents the amount of feed in grams converted to the number of eggs, i.e., the feed conversion ratio. [4]Represents the amount of feed in grams converted to chicks produced from the hen during the 84 days of the experiment. [5]Represents the egg quantity in grams produced from a hen during 84 days of the experiment, i.e., the egg mass

DISCUSSION

The non-significant effects of FWF on egg production, egg mass, egg weight and the feed conversion ratio were in line with the findings of Loh *et al.*[21] These researchers used fermented feed in the diets of egg layers (Babcock B380) at the rates of 3, 6 and 9% for 14 week and found that the use of fermented feed produces acidic conditions in the GI tract due to the presence of volatile fatty acids. The resulting acidic environment of the lumen might decrease the triacylglycerols in the serum of breeders and consequently reduce the lipids needed for yolk processing and the number of yolks in the ovary, as reported by Gonzalez-Esquerra and Leeson[22] and these findings might be responsible for the significant increase in egg weight (Table 2) obtained with the 50 and 75% FWF treatments.

The non-significant effects of FWF could be due to the denaturation of some proteins and peptides in the digesta. Missotten *et al.*[2] reported that fermented feed could cause a loss of amino acids due to their transformation to biogenic amines (e.g., cadaverine due to lysine decarboxylation). In addition, Beal *et al.*[23] showed that fermented feed has the disadvantage of producing excessive amounts of acetic acid and ethanol, which causes odours in feed, consequently results in an "off-flavour" taste and thereby leads to decreased feed intake.

In spite of the non-significant differences in the fertility percentage among the experimental treatments, a significant decrease in early embryonic mortality was observed, which may be due to difficulties in distinguishing between very early embryonic mortality and unfertile eggs, so the percentage of early embryonic mortality increases. In addition, the observed significant differences in late embryonic mortality could result from elongated windows of hatching due to significant differences in egg weight (Table 2), which would consequently result in significant differences in chick weight, indicating that heavy embryos are exposed to the unfavourable hatchery conditions that cause early embryonic mortality.

CONCLUSION

The current study shows that feeding fermented feed to broiler breeders does not significantly affect their performance parameters, with the exception that the fermented feed improved the egg weight, increased the number of unsaleable hatching eggs and thereby increased the number of chicks, which constitute the final product of the breeders.

SIGNIFICANCE STATEMENT

The current experiment discovered the following positive effects of FWF: the addition of this product to feed will be useful for increasing the hatching egg weight and the number of saleable eggs and for decreasing embryonic mortality. The current experiment will help scholars improve the production performance of broiler breeders. Thus, the new discoveries will allow us to overcome the handling-related difficulties associated with the use of fermented feed in the diets of broiler breeders, which will ultimately lead to improved production performance of broiler breeders.

ACKNOWLEDGMENTS

The authors are grateful to the Department of Animal Science, College of Agriculture, University of Anbar, for the help and support provided, which contributed to the successful completion of the current study.

REFERENCES

1. Hu, J., W. Lu, C. Wang, R. Zhu and J. Qiao, 2008. Characteristics of solid-state fermented feed and its effects on performance and nutrient digestibility in growing-finishing pigs. Asian-Aust. J. Anim. Sci., 21: 1635-1641.
2. Missotten, J.A., J. Michiels, J. Degroote and S. De Smet, 2015. Fermented liquid feed for pigs: An ancient technique for the future. J. Anim. Sci. Biotechnol., Vol. 6, No. 1. 10.1186/2049-1891-6-4.

3. Lyberg, K., T. Lundh, C. Pedersen and J.E. Lindberg, 2006. Influence of soaking, fermentation and phytase supplementation on nutrient digestibility in pigs offered a grower diet based on wheat and barley. Anim. Sci., 82: 853-858.
4. Jorgensen, H., D. Sholly, A.O. Pedersen, N. Canibe and K.E.B. Knudsen, 2010. Fermentation of cereals-influence on digestibility of nutrients in growing pigs. Livest. Sci., 134: 56-58.
5. Naji, S.A.H., J.K.M. Al-Gharawi and I.F.B. Al-Zamili, 2016. The effect of fermented feed on intestinal morphology and histology of broiler. http://qu.edu.iq/agr/wp-content/uploads/The-effect-of-fermented-feed-on-intestinal-morphology-and-histology-of-broiler.pdf.
6. Jannah, S.N., H. Khotimah and R.S. Ferniah, 2018. Molecular diversity of lactic acid bacteria on ileum and coecum broiler chicken fed by *Chrysonilia crassa* fermentation. J. Phys.: Conf. Ser., Vol. 1025, No. 1. 10.1088/1742-6596/1025/1/012070
7. Yeh, R.H., C.W. Hsieh and K.L. Chen, 2017. Screening lactic acid bacteria to manufacture two-stage fermented feed and pelleting to investigate the feeding effect on broilers. Poult. Sci., 97: 236-246.
8. Jawad, H.S.A., I.H. Lokman, S.A. Naji, A.B.Z. Zuki and A.B. Kassim, 2016. Effects of dietary supplementation of wet fermented feed with probiotic on the production performance of Akar Putra chicken. Asian J. Poult. Sci., 10: 72-77.
9. Heres, L., B. Engel, F. van Knapen, M.C. de Jong, J.A. Wagenaar and H.A. Urlings, 2003. Fermented liquid feed reduces susceptibility of broilers for *Salmonella enteritidis*. Poult. Sci., 82: 603-611.
10. Mukherjee, R., R. Chakraborty and A. Dutta, 2016. Role of fermentation in improving nutritional quality of soybean meal-a review. Asian-Aust. J. Anim. Sci., 29: 1523-1529.
11. Yang, Y.X., Y.G. Kim, J.D. Lohakare, J.H. Yun and J.K. Lee *et al.*, 2007. Comparative efficacy of different soy protein sources on growth performance, nutrient digestibility and intestinal morphology in weaned pigs. Asian Aust. J. Anim. Sci., 20: 775-783.
12. Choi, Y.J., S.R. Lee and J.W. Oh, 2014. Effects of dietary fermented seaweed and seaweed fusiforme on growth performance, carcass parameters and immunoglobulin concentration in broiler chicks. Asian-Aust. J. Anim. Sci., 27: 862-870.
13. Gao, J., H.J. Zhang, S.G. Wu, S.H. Yu and I. Yoon *et al.*, 2009. Effect of *Saccharomyces cerevisiae* fermentation product on immune functions of broilers challenged with *Eimeria tenella*. Poult. Sci., 88: 2141-2151.
14. Engberg, R.M., M. Hammershoj, N.F. Johansen, M.S. Abousekken, S. Steenfeldt and B.B. Jensen, 2009. Fermented feed for laying hens: Effects on egg production, egg quality, plumage condition and composition and activity of the intestinal microflora. Br. Poult. Sci., 50: 228-239.
15. Al-Dhanki, Z.T., 2003. Locally produced probiotic and its effect on production performance of broiler, layer and broiler breeder. University in Baghdad, Iraq.
16. Aviagen, 2016. Manual guide of broiler breeder (ROSS 308). Aviagen Inc., USA. http://ap.aviagen.com/.
17. Aviagen, 2014. Ross 308 parent stock: Nutrition specifications. Aviagen Inc., USA. http://ap.aviagen.com/.
18. NRC, 1994. Nutrient Requirement of Poultry. 9th Rev. Edn., National Academy Press, Washington, DC., USA.
19. Buhr, R.J. and J.M. Mauldin, 1991. Embryonic mortality viewed at hatch time with live embryo comparison. Poster for Misset World Poultry.
20. SAS., 2004. SAS system manual. SAS Institute Inc., Cary, NC.
21. Loh, T.C., F.L. Law, H.L. Foo, Y.M. Goh and I. Zulkifli, 2007. Effects of feeding a fermented product on egg production, faecal microflora and faecal pH in laying hens. J. Anim. Feed Sci., 16: 452-462.
22. Gonzalez-Esquerra, R. and S. Leeson, 2000. Effect of feeding hens regular or deodorized menhaden oil on production parameters, yolk fatty acid profile and sensory quality of eggs. Poult. Sci., 79: 1597-1602.
23. Beal, J.D., S.J. Niven, A. Campbell and P.H. Brooks, 2002. The effect of temperature on the growth and persistence of *Salmonella* in fermented liquid pig feed. Int. J. Food Microbiol., 79: 99-104.