EFFECTS OF ADDING SALTOSE AS PROBIOTIC TO BROILER DIETS ON PRODUCTIVE PERFORMANCE IN THE COMMERCIAL BREEDING CIRCUMSTANCES

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ABSTRACT

Saltose is one of the biological feed additives used for controlling coccidia and clostridia in the broiler, this study was aimed to evaluate its use on productivity and profitability in commercial circumstances. A total of 60 thousand one day old Ross 308 chicks, were divided randomly into two groups, each consisting of 6 replicates distributed in 3 Commercial Closed chicken Houses that belonged to medium size company in Saudi Arabia. Each replicate contains 5,000 bird and each house contains 4 replicants, 2 replicants of them for each group. In group A, the chicks were fed on a commercial control diet, group B was fed on the same diet enriched with 0.5 gm Saltose /kg feed with two stages. Data were collected weekly during the experimental period for 35 days; broiler performance (feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR)), mortality rate, organs weight, dressing and feed cost per kilogram weight gain were estimated. The results showed that supplementation of Saltose to broiler diets caused a numerical improvement (P>0.05) in the mortality, broiler performance, organs weight and dressing through 1-21 day and 22-35 day, but these improvement effects reached to significant degree (P<0.05) only with body weight gain (BWG), feed conversion ratio (FCR), Furthermore adding "Saltose" caused Lower feed cost per kilogram weight gain than Control group by 7.25%. It was concluded that the use of "saltose" as feed additives in commercial broiler diets has productive and economic benefits in field circumstances

INTRODUCTION

In 2006 the European Union (EU) banned the use of growth-promoting antibiotics because of the increase in micro-organism resistance to antibiotics in both animals and humans (1). Thus, the importance of natural biological feed additives such as (prebiotics, probiotics, synbiotics) has increased as an alternative to growth-promoting antibiotics that work to control pathogens in the gut and thus stimulate animal productivity (2).

Probiotics are live microbial feed supplements that affect the host animal beneficially by altering its intestinal microbial balance (3). Prebiotics are non-digestible food ingredients that affect the host beneficially by selectively stimulating the growth and activity of one or a limited number of bacteria in the colon (4). Synbiotics, on the other hand, are mixtures of probiotics and prebiotics (5). Many researchers looked for alternatives to antibiotics that are safer for human health, such as prebiotics, probiotics and symbiotic (6, 7). (8)reported that probiotic improved the growth of broiler chickens and their immune responses and livability.

These days, many species of Bacillus bacteria are used as probiotic supplements in animal diets. *Bacillus* species are facultative anaerobe gram-positive bacteria that have endospores (9), also Bacillus spp. have the ability to produce different antioxidants(10), and probiotic supplementation can help to reduce oxidative stress in broilers(11), So that(12) reported that *B. subtilis* supplementation improved BW and FCR of broiler chickens during grower and finisher periods.

There are many examples of probiotics one of them "Saltose" which is new patent nonantibiotic biological product, composed of Cell Wall Lyaze (patent new enzyme) 3,700 U/g, Bacillus licheniformis, Bacillus subtilis, Bacillus pumilus 1.8 x 109 cfu/g, Enterococcus faecalis, Enterococcus faecium 2.5 x 108 cfu/g, Protease, Lipase, Cellulase, Amylase 12,000 U/g and Beta-Xylanase 350 U/g (13). So that has a positive effect on productive performance and minimized the incidence of infection with *Clostridium perfringens* (14).

This scientific paper aims to study the effect of Saltose as a supplement in commercial diets appropriate for the Ross 308 strain on the performance and economic determinants of commercial Broiler flocks under conditions of intensive commercial breeding.

MATERIALS AND METHODS

A total of 60,000 one day old Ross 308 chicks, were obtained from a commercial hatchery (Al-Khumasiyah Co., Riyadh, Saudi Arabia), were housed in three commercial closed broiler houses in parallel experimental partitions contains automatic climate control, automatic suitable

feed pans and nipples with stocking density 16/m2, so chicks were divided into Two groups, each one consists of 6 replicates, 4 replicants in a house.

Continuous lighting was provided throughout the experiment which lasted for 35 day; during brooding at one day old the ambient temperature was 35°C and gradually decreased to reach 23°C on day 26 and then kept constant.

Chicks were divided into two groups, group A as control and group B as experimental group but each group divided into six replicates each one contains 5,000 chicks while each commercial chicken house contains 4 replicates. Feed and water were provided ad libitum. Birds were fed starter (0 - 21days) and Grower (21 - 35 days) commercial mash diets based on maize and soybean meals (15), Dietary treatments were distributed randomly to each partition according to Table1, and the Dry Matter%, Crude protein% and Crude Fat% was analyzed in an accredited laboratory.

Table 1: The experimental groups treated with different feed additives.

Group A (control)	Chicks fed on basal diet without any feed additives
Group B (test)	Chicks fed on basal diet enriched with Saltose [@] powder 0.5 gm/kg.

The birds were vaccinated according to the table 2 against Newcastle disease (ND), infectious bronchitis (I.B.) and Gumboro disease (IBD).

0	Galimune 208	Oily	ND + AI	Subcutaneous.	Hatchery
0	Vaxxitek	Recombinant vaccine	IBD	Subcutaneous.	Hatchery
0	Ma5Clone30	Live vaccine	ND+IB	Spray.	Hatchery
14	Clone 30	Live vaccine	ND	D. Water.	Farm

Table2: Vaccination Program.

The tested substance was Saltose@ which is patent product produced by Poultry Industry Consultant Company(13). Gosaku Bld., 1-29-2 Nishigotanda, Shinagawa-ku, Tokyo, 141-0031, Japan and distributed in Saudi Arabia by Gozl trading Est, Dammam, Saudi Arabia.

All diets used were formulated to meet the nutrient requirement of the broilers according to recommendations of (16). The diet composition and nutrient value of basal diet used were illustrated in table 3.

	Starter 1-21 day	Grower 21-35 day
Corn	59.06	63.46
Soybean meal	35	30.5
Soyabean oil	1.5	2
Monocalcium Phosphate	1.65	1.5
Calcium carbonate	1.55	1.4
Salt	0.25	0.25
Sodium Sulfate	0.19	0.19
Vitamin premix.	0.1	0.1
Mineral premix.	0.1	0.1
DL-methionine.	0.3	0.25
L-lysine HCl	0.23	0.18
Choline Cl	0.07	0.07

Table 3: Ingredients and nutrient composition of basal diets. Ingredients:

Calculated Nutrient content:

AME (Kcal/Kg) 3100	2900	3000
Crude protein %	22.2	20.3
Available phosphorus %	0.49	0.44
Calcium %	0.96	0.87
Lys (dig) %	1.23	1.08
Met (dig) %	0.61	0.54
Met + Cys (dig) %	0.91	0.82

Analyses Nutrient content:

Dry matter (DM, %)	89.4	89.7
Crude protein %	22.32	20.41
Crude fat %	4.2	4.8

Saltose was added at the starter, grower and finisher basal diets at 0.5g/kg diet.

The body weight gain BWG (gram/bird) is calculated by subtracting the live body weight at the end of the stage minus the live body weight at the beginning of the stage.

Body weight (gram/bird) The average body weight was calculated at the beginning or the end of stage period.

Feed intake (gram/bird) was calculated at the beginning or the end of stage period, as follow the residual amount of feed was weighed and subtracted from the known weight of feed at the beginning of the stage.

Feed conversion ratio (gram feed/gram gain) was calculated at 21 day and at 35 day the end of experiment as the amount of feed consumed per unit of body gain.

Mortality rate (%), the accumulated stage or final mortality rates were calculated by subtracting the number of live birds at the end of the stage or experiment from the total number of birds at the beginning of the stage or experiment and the product is multiplied by 100 to obtain the percentage of mortality rate.

At 35 day, 20 birds from each group were selected accordiong to the average BW within the group, weighted individually, and sacrificed by cervical dislocation. Then, liver, gizzard, heart was separated from the carcass. The gallbladder was separated from the liver and the content of gizzard removed, then the liver, gizzard, heart was weighed. The organ weights were expressed as a percentage of live BW.

Also, 10 bird from each experimental group were selected according to the average BW within the group, weighted individually, and The birds were slaughtered in a halal manner, and the blood, feathers and entrails were disposed of to obtain the slaughtered bird and the weight of the slaughtered bird divided by the original weight multiplied by 100 to calculate the dressing were expressed as a percentage of live BW.

The feed cost per kilogram weight gain, as economic indicator, was calculated for the whole period of the experiment, as multiplying FCR by the average cost of the diets (17).

The statistical study aimed to reveal the possibility of an effect of adding "Saltose" to the diets on Broiler performance during the experimental stages. The significant differences were tested using the (T-Test) to study the relationship between the values of the FCR, BWG, FI and the weights of the organs of the experimental groups, as well as The statistical program used was the Statistical Package for Social Sciences (18) to compare and analyze the statistical results.

RESULTS AND DISCUSSION

Table No. (4) shows the average body weight gain BWG, consumed feed FI and the feed conversion ratio FCR for birds from 1- 21 days, from 22-35 days, and 1-35 days, and it is noticed that the increase in the average of BWG of 1-21 days and for 21-35 days for group B containing the tested material were significantly greater (P <0.05), in addition to the fact that the rest of the production data referred to group B were generally numerically better (P> 0.05) than group A as

control, and this is maybe due to the consumption of group B birds the tested material, and this is consistent with previous studies have shown that the probiotics improved broilers' growth performance and promoted greater populations of synbiotic microbiota in the intestine of broilers(19, 10, 20).

Table (4): Effect of Saltose on body weight gain, feed intake, and feed conversion ratio of broilers.

	Treatment			
	Control	Saltose	SEM^1	P- Value
(0-3) Week				
BWG (g/bird)	618	690	6.68	0.03*
FI (g/bird)	877	917	12.88	0.171
FCR (g/g)	1.42	1.33	0.218	0.423
(3-5) Week				
BWG (g/bird)	840	928	7.367	0.013*
FI (g/bird)	1527	1522	26.026	0.037*
FCR (g/g)	1.818	1.641	0.0345	0.189
Whole Period				
BWG (g/bird)	1458	1618	7.87	0.39
FI (g/bird)	2404	2439	28.22	0.197
FCR (g/g)	1.65	1.51	0.025	0.172

* Means in the row there are significantly different at $P \le 0.05.$

¹Standard error of means.

It is well known that gut health is a key point for animal performance due to its critical importance on nutrient digestion, absorption and metabolism, incidence of enteric diseases, and immune responses (21), and that was understood, because the supplementation of probiotic (*Lactobacillus* bacteria) increased intestinal villus' height and absorptive capacity in broiler chickens that led to higher final BW of the birds (22).

(23) declared that adding dietary "Saltose" improved (P<0.05) BWG, FCR of the broilers during whole period of rearing. However, the FI of the broilers in different experimental group was not different (P>0.05). (24) found that *Bacillus* bacteria improved immune function and increased the level of endogenous antimicrobial peptides in the gut, which led to higher growth performance.

In contrast to our results, another probiotics like (*B. subtilis CH201*) had no effect on birds' growth performance traits (23), and This is in agreement with the results of (25) who reported that dietary probiotics (*B. subtilis*) did not change BW and FCR of broiler chickens compared with the control group.

This may be due to the difference in the type and amount of germs studied as probiotic in these papers compared with the "Saltose". Plus, other bio-additives within "Saltose" have a synergistic effect that stimulates the action of these germs more, and leads to achieving their positive significant effect and remarkable improvement. while the studied substance, as we indicated, contains three types of *Bacillus germs*, and this gives greater potential for them synergistic action, and it can enhance the effect of using the studied substance on production efficiency.

Table No. 5 shows the mortality rate in the first week, the real mortality rate from week 2-5, as well as the mortality rate for the whole trial. In the first week the mortality rate of group A was 2.1% and slightly lower than group B which was 2.7%, while the actual mortality rate during 1-5 weeks in group A was 8.39% but higher than the group B which was 6.08%. It clearly appears that the tested material " Saltose" has a positive effect reduces the mortality rate numerically, and thus has a positive effect on the economic return from adding it.

Treatment	Total Mortality%	First week Mortality%	Actual Mortality %
Control	10.49	2.1	8.39
Saltose	8.78	2.7	6.08
SEM ¹	0.735	0.14	0.736
P-Value	0.505	0.299	0.417

Table (5): Effect of Saltose on the Mortality (1–35 d).

• There were no significant differences in the results of the T-test.

¹Standard error of means.

Group B that contains Saltose has a significant effect on the productive performance of broiler compared with control, It showed the higher final body weight, the lower feed intake, lower feed conversion ratio and minimum mortality rate at the end of trial, this related to the beneficial effect of (Saltose) as biological probiotic containing 3 type of beneficial *bacteria Bacillus licheniformis*, *Bacillus subtilis* and *Bacillus pumilus* 1.8 x 109 cfu/g(13), So that achieve positive effects on mortality by controlling gut health and this is consistent with(26, 27) who are showed that some of Bacillus strains improved intestinal microflora and inhibited pathogenic strains such as *Campylobacter* and *Clostridium* species in broiler chickens (26, 27).

Furthermore Saltose contains five enzymes Protease, Lipase, Cellulase, Amylase 12,000 U/g, Beta-Xylanase 350 U/g that increase palatability and digestibility of dietary nutrients and Cell Wall Lyaze 3,700 U/g that able to control the coccidial infection so minimizing mortality rate according to(13, 14).

Also, "Cell Wall Lyase" which is a combination of unique new patent enzymes, which can damage the cell wall of oocyst of parasite (such as Coccidiosis, Cryptosporidiosis) and bacterial agents, *Salmonellosis* and *Clostridiosis*, also Saltose reduced *C. perfringens* colonization. These were evaluated comprehensively by (29).

Table No.6 shows the effect of adding saltose on the average percentages of organ weight (Liver, Gizzard, heart and Dressing) at 35 days of age, and no significant differences (P > 0.05) were observed between studied weight percentages of organs.However, it is noted that the relative weights of organs in group B bird have a numerical difference, and this could be a reflection of the achievement of the group B birds to gain a significant weight, which can be seen clearly in Table No. 4.

Table (6): Effect of Saltose on mean percentages of organ weight (Liver, Gizzard, heart and Dressing) at 35 days of age.

Treatment	Liver%	Gizzard%	Heart%	Dressing%
Control	2.1	1.18	0.60	72.25
Saltose	2.33	2.01	0.66	73.68
SEM ¹	0.016	0.013	0.009	0.248
P-Value	0.964	0.912	0.829	0.174

• There were no significant differences in the results of the T-test. ¹Standard error of means.

Table No. 7 shows the effect of the experimental diets on feed cost per kilograms weight gain. Chicks fed the basal diet supplemented with Saltose had 92.25% lower feed cost per kg weight gain in comparison to the control group birds, despite the same level of feed energy and feed component.

Treatment	Feed cost per kg gain (RS)	%
Control	1.999	100
Saltose	1.854	92.75
SEM ¹	0.03003	
P-Value	0.192	

Table (7): Effect of Saltose on the feed c	cost per kilogram	weight gain $(1-35 \text{ d})$.
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• There were no significant differences in the results of the T-test.

¹Standard error of means.

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آثار إضافة السالتوز كبروبيوتيك إلى الخلطات العلفية المقدمة لدجاج اللحم على الأداء الإنتاجي في ظروف التربية التجارية. عبد الجبار الشيخ سليمان

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الخلاصة

سالتوز هو أحد المضافات الحيوية المستخدمة للتحكم في الكوكسيديا والمطنيات في دجاج لدجاج اللحم، وقد هدفت هذه الدراسة إلى تقييم استخدامها على الإنتاجية والربحية في الظروف التجارية. تم تقسيم ما مجموعه، ٦ ألف كتكوت روس يبلغ من العمر يوم واحد، بشكل عشوائي إلى مجموعتين، كل واحدة تتكون من ٦ مكررات موزعة على ٣ بيوت دجاج مغلقة تجارية تابعة لشركة متوسطة الحجم في المملكة العربية السعودية. كل مكرر يحتوي على ٥٠٠ طائر ويحتوي كل بيت على ٤ مكررات، ٢ مكررات، ٢ مكررات موزعة على ٣ بيوت دجاج مغلقة تجارية مكررات، ٢ مكرر لكل مجموعة. في المملكة العربية السعودية. كل مكرر يحتوي على ٥٠٠٠ طائر ويحتوي كل بيت على ٤ مكررات، ٢ مكرر لكل مجموعة. في المحموعة (أ) تم تغذية الصيصان على خلطة علفية تجارية كشاهد، بينما تم تغذية المجموعة (ب) على نفس الخلطة العلفية المضاف لها ٥٠٠ جم سالتوز/ لكل كجم علف على مرحلتين بادي ونامي. تم جمع الميانات أسبوعياً خلال فترة التجرية لمحموعة (أ) تم تغذية الصيصان على خلطة علفية تجارية كشاهد، بينما تم تغذية الميموعة (ب) على نفس الخلطة العلفية المضاف لها ٥٠٠ جم سالتوز/ لكل كجم علف على مرحلتين بادي ونامي. تم جمع الميانات أسبوعياً خلال فترة التجرية لمعنا له. وحدا إذا كل كجم علف على مرحلتين بادي ونامي. الوزني البيانات أسبوعياً خلال فترة التجرية لمدة ٣٥ يوما. تم تقدير أداء دجاج التسمين) العلف المستهلك(آ1) ، والكسب الوزني البيانات أسبوعياً خلال فترة التجرية لمدة ٣٦ يوما. تم عدا النوز إلى أعلاف دجاج التسمين) العلف المستهلك(آ2) ، والكسب الوزني البيانات أسبوعياً ذلال فترة التناتين ما ٢-11 يوم و ٢٢-٣٥ يوم، ولكن هذا التأثيرات الإيجابية وصلت إلى درجة اللحم ووزن الأعضاء والذبيحة في الفترتين من ١-11 يوم و ٢٢-٣٥ يوم، ولكن هذا التأثيرات الإيجابية وصلت إلى درجة الحم ووزن الأعضاء والذبيحة ولي النفوق وأداء دجاج اللحم ولادن اللى مردون (100 حرم) وعلى خلطة علف لكل كيلوغرام من الوزني العم ووزن الأعضاء والذبيحة في الفترتين من ١-11 يوم و ٢٢-٣٥ يوم، ولكن هذا التأثيرات الإيجابية وصلت إلى درجة معوزن الأعضاء والذبيحة ولي الغلف دجاع الحم ولاما يوزني (100 حرم) وعدان الحرم) وعدان الكرم و من وردن المورة على والدي الحرم الى ونسبة تحويل العلف (700))، وعلاوة على كلك أدن إم مان الحرم الم معنوية (2000 ح)) معلم مع الكسب الوزن من ١-11 يوم والما يحويو العلما ول

REFERENCES

- 1-Hashemi, S. & Davoodi, H., 2010. Phytogenics as new class of feed additive in poultry industry.J. Anim. Vet. Adv. 9, 2295-2304.
- 2-Garrido, M.N., M. Skjerve, H. Oppegaard and H. Sørum, 2004. Acidified litter benefits the intestinal flora balance of broiler chickens. Applied and Environmental Microbiology, 70: 5208-5213.

- **3-Hassan H.M.A., A. Samy, Amani W. Youssef and M.A. Mohamed. 2018**. Using Different Feed Additives as Alternative to Antibiotic Growth Promoter to Improve Growth Performance and Carcass Traits of Broilers. Int. J. Poult. Sci., 17 (6): 255-261.
- 4-Gibson, G.R., Scott, K.P., Rastall, R.A., Tuohy, K.M., Hotchkiss, A., Dubert-Ferrandon, A., Gareau, M., Murphy, E.F., Saulnier, D. & Loh, G., 2010. Dietary prebiotics: current status and new definition. Food Sci. Technol. Bull. Funct. Foods 7, 1-19.
- 5-Yang, Y., Iji, P. & Choct, M., 2009. Dietary modulation of gut microflora in broiler chickens: A review of the role of six kinds of alternatives to in-feed antibiotics. World's Poult. Sci. J. 65, 97-114.
- **6-Mikelsaar, M. & Zilmer, M., 2009**. Lactobacillus fermentum ME-3-an antimicrobial and antioxidative probiotic. Microb. Ecol. Health Dis. 21, 1-27.
- 7-Corcionivoschi, N., Drinceanu, D., Pop, I.M., Stack, D., Ştef, L., Julean, C. & Bourke, B.,
 2010. The effect of probiotics on animal health. Anim. Sci. Biotechnol. 43, 35-41.
- 8-Attia YA, Zeweil HS, Alsaffar AA, El-Shafy AS. 2011. Effect of non-antibiotic feed additives as an alternative to flavomycin on productivity, meat quality and blood parameters in broilers. Arch Geflügelk. 75:40–48.
- 9-Cutting, SM. 2011. Bacillus probiotics. Food Microbiol. 28:214–220.
- 10-Latorre JD, Hernandez-Velasco X, Vicente JL, Wolfenden R, Hargis BM, Tellez G. 2017. Effects of the inclusion of a Bacillus direct-fed microbial on performance parameters, bone quality, recovered gut microflora, and intestinal morphology in broilers consuming a grower diet containing corn distillers dried grains with solubles. Poult Sci. 96:2728–2735.
- 11-Shah M, Zaneb H, Masood S, Khan RU, Din S, Khan I, Tariq A, ur Rehman H. 2019a. Ameliorative effect of zinc and multistrain probiotic on muscle and bone characteristics in broiler reared under cyclic heat stress. Pak J Zool. 51:1041–1046.
- 12-Upadhaya SD, Rudeaux F, Kim IH. 2019. Effects of inclusion of Bacillus subtilis (Gallipro) to energy-and protein-reduced diet on growth performance, nutrient digestibility, and meat quality and gas emission in broilers. Poult Sci. 98:2169–2178.
- 13-Poultry Industry Consultant Biotechnology Company (PIC-BIO). 2013. Saltose. September 20. Available from www.bio-live.com/ en/ static/ pdf/saltose_A4.pdf.

- 14-El Iraqi K.G., T.M. Melegy and Shimaa A.E 2014a. NasrNon Antibiotic Improve Performance and Minimizing Shedding in Clostridium perfringens Infected Broiler. Global Veterinaria 13 (3): 342-347.
- **15-Steven, L. and D.S. John, 2008**. Feeding program for Broiler chickens. pp.229-296. In: Commercial Poultry Nutrition, 3 ed. British Library.
- 16-Ross 308 Broiler: Nutrition Specifications 2014, Aviagen, 0814-AVNR-035.
- 17-Zaghari M, Derakhshani Diba M, Moravej H, Zahroojian N. 2017. Estimation of metabolizable energy equivalency of Bacillus subtilis spore for male broiler chickens. J Livest Sci Technol. 5:9–18.
- **18-SPSS Statistical 17.0 (2009):** Statistical Package for Social Sciences-Version 17.0. Computer software 17.0, SPSS Inc., Head-quarters. Wacker Drive, Chicago, Illinois 60606, USA.
- 19-Mountzouris, K., Tsirtsikos, P., Kalamara, E., Nitsch, S., Schatzmayr, G. & Fegeros, K., 2007. Evaluation of the efficacy of a probiotic containing Lactobacillus, Bifidobacterium, Enterococcus, and Pediococcus strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. Poult. Sci. 86, 309-317.
- 20-Rhayat L, Jacquier V, Brinch KS, Nielsen P, Nelson A, Geraert PA, Devillard E. 2017. Bacillus subtilis s train specificity affects performance improvement in broilers. Poult Sci. 96:2274–2280.
- **21-Hamasalim HJ. 2016**. Synbiotic as feed additives relating to animal health and performance. Adv Microbiol. 6:288–302.
- 22-Shah M, Zaneb H, Masood S, Khan RU, Ashraf S, Sikandar A, Rehman HFU, Rehman HU. 2019b. Effect of dietary supplementation of zinc and 174 M. ZAGHARI ET AL. multi-microbe probiotic on growth traits and alteration of intestinal architecture in broiler. Probiotics Antimicrob Proteins. 11:931–937.
- 23-Zaghari M., P. Sarani & H. Hajati. 2020 Comparison of two probiotic preparations on growth performance, intestinal microbiota, nutrient digestibility and cytokine gene expression in broiler chickens. Journal of applied animal research 2020, vol. 48, no. 1, 166–175.
- 24-Hong HA, Duc LH, Cutting SM. 2005. The use of bacterial spore formers as probiotics. FEMS Microbiol Rev. 29:813–835.
- 25-Abudabos AM, Alyemni AH, Dafalla YM, Khan RU. 2017. Effect of organic acid blend and Bacillus subtilis alone or in combination on growth traits, blood biochemical and antioxidant

status in broilers exposed to Salmonella typhimurium challenge during the starter phase. J Appl Anim Res. 45:538–542.

- 26-Ashayerizadeh, A., Dabiri, N., Mirzadeh K. & Ghorbani, M., 2011. Effect of dietary supplementation of probiotic and prebiotic on growth indices and serum biochemical parameters of broiler chickens. J. Cell Anim. Biol. 5, 152-156.
- 27-Abudabos, A.M., Alyemni, A.H. & Al Marshad, M., 2013. Bacillus subtilis PB6 basedprobiotic (CloSTAT TM) improves intestinal morphological and microbiological status of broiler chickens under clostridium perfringens challenge. Int. J. Agric. Biol. 15, 978-982.
- 28-El Iraqi K.G., T.M. Melegy and A.O. Hassan, 2014b. Evaluation of New Biological Product for Control of Coccidia and Clostridia in Broiler Chickens. Global Veterinaria 12 (2): 257-263.
- 29-Hosoi, T., Ametani, A., Kiuchi, K., Kaminogawa, S. 2000. Improved growth and viability of lactobacilli in the presence of Bacillus subitlis (natto), catalase or subtilisin. Canadian J. Microbiol. 46: 892-897.