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EFFECT OF DIETARY SUPPLEMENTATION OF BACTERIA AS GROWTH PROMOTERS ON PERFORMANCE OF BROILER CHICKENS

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ABSTRACT: The experimental study was carried out to determine the effects of bacteria (B. subtilis or P. acidilactici) alone or combined supplementation in broiler diets on productive performance, bacteria enumeration and some physiological responses. A total number of birds (360 Cobb broiler chickens at seven d of age) were distributed into nine treatments (40 chicks each), each treatment contained four replicates of ten birds as follows: -

1- Chicks were fed the control diet (T1).
2- (T1) + 0.5 g Bacillus subtilis /kg diet.
3- (T1) + 1 g Bacillus subtilis /kg diet.
4- (T1) + 0.5 g Pediococcus acidilactici /kg diet.
5- (T1) + 1 g Pediococcus acidilactici /kg diet.
6- (T1) + 0.5 g Bacillus subtilis+0.5 g Pediococcus acidilactici /kg diet.
7- (T1) + 0.5 g Bacillus subtilis+1 g Pediococcus acidilactici /kg diet.
8- (T1) + 1 g Bacillus subtilis+0.5 g Pediococcus acidilactici /kg diet.
9- (T1) + 1 g Bacillus subtilis+1 g Pediococcus acidilactici /kg diet.

The obtained results of the experiment showed that chicks fed combined supplementation of B.subtilis 1g+ P. acidilactici 0.5g had significantly higher Live body weight, body weight, growth rate, performance index, Immune organs (bursa and thymus) and improved feed conversion ratio, blood parameters, and microbial load.

Generally, it could be concluded that the combination of B.subtilis 1g+ P. acidilactici 0.5g can be used in broiler diets as a growth promoter because this combination improved growth performance, gut health and intestinal microbial balance of broilers.

Keywords:feed additives - Bacillus subtilis-Pediococcus acidilactici- probiotic.

INTRODUCTION

A world trend in the recent time to decrease the antibiotics use in animal feed due to its residues in the meat products (Menten, 2001). Probiotics have been increasingly adopted in poultry diets as an alternative to antibiotic growth promoters (Mountzouris et al., 2010 and Zhang and Kim, 2014). Numerous microorganisms have been considered as probiotics including yeast, fungi, bacteria and mixed cultures comprising of several microbes. In poultry nutrition, probiotic species such as Streptococcus, Lactobacillus, Bifidobacterium, Bacillus, Candida, Saccharomyces and Aspergillus are widely used to avoid poultry diseases and pathogens and improve growth performance of broilers (Awad et al., 2009). Generally, two genera of bacteria are testified including lactic acid bacteria of the genus Bifodobacteria (Willis and Reid, 2008) and Lactobacllus (Lee et al., 2010a). Other bacteria that have been used, though to a lesser degree in animal and poultry probiotics include Enterococcus, Pediococcus, Bacillus, Lactococcus, Streptococcus, etc (Lee et al., 2010b and 2015).

Gong et al. (2002) reported B. subtilis as a member of the mucosa correlated bacterial population in the chicken caceum. Pedicoccus acidilactici is gram positive cocci homofermentative that can produce in a wide variety of temperature, pH and exert antagonism against other microorganisms, including enteric pathogens, mainly through the secretion of bacteriocins identified as pediocins and production of lactic acid (Klaenhammer, 1993). Pedicoccus acidilactici has not been indicated in any early paper to have toxic effects. But, health benefits include better of the normal microflora, prohibition of infectious diseases (Ooi and Liong, 2010). In particular, Bacillus sp. have been revealed to inhibit pathogen colonization, improve performance, improve nutrient digestibility, enhance immune activities and positively modulate intestinal microflora in the gut of broiler chickens (Park and Kim, 2014).

Chen et al. (2017) found improvement performance when fed broiler on a probiotic mixture (B. subtilis, B. licheniformis and S. cerevisiae) compared with a control and an antibiotic (avilamycin) treated group. In addition, Zhang and Kim (2014) reported an overall increase in body weight gain (BWG) in chicken fed with multi strain probiotics compared to that of control group. Song et al. (2014) reported significant increase in BWG in broilers fed with probiotics Bifidobacterium, Lactobacillus, Clostridium, and coliforms species. Chen et al. (2009) point out that applied the B. subtilis as a probiotics enhanced the growth performance of broilers. Salim et al. (2013) described that, microbials that contained a mixture of B. subtilis, B. licheniformis and Saccharomyces cerevisiae improved FCR. Dalloul et al. (2005) recommended a positive effect of the probiotic in stimulating some of the immune responses against E. acervulina, resulting in improving local immune resistances against coccidiosis. The earlier finding of Fuller (1977) who established that host-specific Lactobacillus strains were able to decline E.coli in the small intestine and crop.

Pedicoccus acidilactici is a probiotic bacterium that exhibits positive impacts on the role and the balance of the intestinal flora; also it reinforces the immune defense and improves the animal performance (Stella et al, 2005).

The aim of this study was investigated the impact of bacteria (B. subtilis or P. acidilactici) alone or combined supplementation at different levels in broiler diets on performance, bacteria enumeration, intestinal pH and blood parameters.

MATERIALS AND METHODS

The experimental work was carried out at El-Azab Poultry Research Station, Fayoum, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Dokki, Egypt, during the period from February to April 2016. Chemical analyses were performed in the laboratories of the Animal Production Research Institute, Agricultural Research Center. A total number of birds (360 Cobb broiler chickens at 7 d of age) were distributed randomly into nine treatments (40 chicks each), each treatment contained four replicates of ten birds as follows:

1- Chicks were fed the control diet (T1).
2- (T1) + 0.5 g Bacillus subtilis /kg diet.
3- (T1) + 1 g Bacillus subtilis /kg diet.
4- (T1) + 0.5 g Pediococcus acidilactici /kg diet.
5- (T1) + 1 g Pediococcus acidilactici /kg diet.
6- (T1) + 0.5 g Bacillus subtilis+0.5 g Pediococcus acidilactici /kg diet.
7- (T1) + 0.5 g Bacillus subtilis+1 g Pediococcus acidilactici /kg diet.
8- (T1) + 1 g Bacillus subtilis+0.5 g Pediococcus acidilactici /kg diet.
9- (T1) + 1 g Bacillus subtilis+1 g Pediococcus acidilactici /kg diet.

Birds were raised in batteries with wire mesh floors and had a free access to the fresh water from nipple drinkers (2nipples/cage) and feed throughout the experiment. Batteries were sited into a room provided with fans for ventilation and light 23 hours daily. The chicks were fed starter diet from 7 to 14-d, grower diet from 15 to 21-d and finisher diet from 22 to 38 d of age (end of the experiment).

The experimental diets were supplemented with vitamins and minerals mixture and L-lysine HCl and DL-methionine to cover the recommended requirements according to the strain catalog recommendations and were formulated to be iso-caloric and iso-nitrogenous. The composition and calculated analysis of the control diets are presented in (Table 1).

Bacillus subtilis bacteria content was $3*10^{8}$ CFU /g and Pediococcus acidilactici content was $1*10^{10}$ CFU /g. The birds were weighed and feed intake was recorded to calculate feed conversion ratio using the following formula FC = <u>FI (g)/bird during a certain period</u>

Weight gain (g)/bird during the same period and body weight gain using the following formula: $LBWG_{7-38}=LBW_{38}–LBW_{7}$.

Growth rate and performance index were calculated as follows:

GR7-38= (LBW38–LBW7)/0.5(LBW38+LBW7), PI = (LBW, Kg/FC) x100.

At end of the experiment, slaughter test were performed using 20 chicks around the average LBW. Total giblet, abdominal fat, half breast, half rear % were recorded.

At time of slaughter test, 4 samples of ileum content for each treatment were taken. Total microflora of ileum content was enumerated. The pH of intestinal contents was directly determined by pH-meter. At 38 d, individual blood samples from 4 birds of each treatment were taken. The blood samples for hematological analysis were expelled gradually into tubes containing EDTA;

the sample for biochemical analysis was collected in tubes without anticoagulant. The blood samples were centrifuged at 3000 rpm for 20 minutes to obtain serum, and stored at–20°Ċ until the time of chemical determinations by colorimetrically methods using commercial kits. Statistical analysis of results was submitted using the GLM procedure of the SPSS software (SPSS, 2008), according to the follow general model:

$Y_{ij} = \mu + T_i + e_{ij}$

Where:

Y_{ij}: observed value μ: overall mean

T_i: treatment effect (i: 1 to 9). e_{ij}: random error

Means indicating significant differences ($P \leq 0.05$) were tested using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Growth Performance:

Impact supplementation of Bacillus subtilis, Pediococcus acidilactici or combined on growth performance of Cobb strain broiler chicks during the period from 7 to 38 d of age are listed in Table 2.

Data presented in Table 2 indicate live body weight (LBW), body weight gain (BWG), feed conversion (FC), performance index (PI) and growth rate (GR) were significant ($P \leq 0.01$) for LBW at 38 days and BWG, FC, PI and GR during the period from 7 to 38 d. Birds fed experimental diets containing B.subtilis 1g+ P. acidilactici 0.5g had higher LBW (2107.25 g), BWG (1961.35), GR (1.74) and PI (40.65) and better FC (1.65) during the previous period, while, those feed control diet had lower values. No significant differences in FI were found between chicks fed bacteria (B.subtilis, P. acidilactici) alone or combined and the control group. Chicks fed B.subtilis 1g+ P. acidilactici 0.5g experimental diet had best significantly FC(1.65) at (7-38) days copmpard with the control group. These results agree with the findings of many authors being, Reis et al. (2017) and Shokryazdan et al. (2017). Since the effect observed due to supplementation of probiotic (bacteria) to broiler may be attributed to increase the number of lactic acid bacteria, which have several beneficial effects on growth performance, especially in terms of improving digestion and minerals absorption. Similarly, Shokryazdan et al. (2017) observed an improvement of chicken weight with other probiotics. The reports of Hegazy et al. (2014) recommended that probiotic addition improved broilers performance. Probiotic have various advantages as nutritional influence by metabolic reactions regulation that acts stimulator for endogenous enzymes, produces toxic substances, also by production of antimicrobial substances and vitamins. Probiotics are useful microorganisms that can be suitably used to improve growth performance and health of broiler chickens. Also, These results are in agreement with those found by Bai et al. (2013), who have found nonsignificant variation in FI between the control and probiotic bacteria group during the finisher and overall periods. They added that probiotic bacteria (B.subtilis, P. acidilactici) supplementation to broiler diets improved FCR compared with those fed un-supplemented diet and supplementation of single bacteria (B.subtilis or P. acidilactici). Fritts et al. (2000) illustrated that, dietary B. subtilis addition resulted in improving of growth performance of broiler chickens. The helpful impact of probiotics on the FC reflected a greater efficiency of nutrient utilization (Awad et al., 2006; Mountzouris et al., 2007 and Awad et al., 2009). Also, dietary supplementation with multi strain probiotic bacteria significantly improved FC in broiler chickens compared with the control Hossain et al., 2015). In another studies, improvement in GR and PI of broilers supplemented with various strains of probiotics were found (Lee et al., 2010a and Park and Kim, 2014).

Slaughter parameters:

All slaughter parameters did not affect except, half rear% which significantly affected by addition of bacteria (Table 3). Chicks fed the control diet were significantly higher half rear% (16.4) than the other treated groups. Numerically, chicks fed diet containing supplementation of bacteria B. subtilis 0.5g and the control group had highest abdominal fat% (1.11 and 1.10% respectively) while, chicks fed diet containing bacteria P. acidilactici 1 g and B.subtilis 1g+ P. acidilactici 1g (0.84 and 0.90%) showed the lowest figures, but differences were not significant.

Some studies indicate a positive effect of probiotics on slaughter parameters whereas other ones indicate no role of probiotics in this regard. Novak et al. (2011) found that supplementation of Bacillus licheniformis and Bacillus subtilis had lower abdominal fat weight compared to the control and Bacillus cereus supplemented. However, Rekiel et al. (2005) found no significant difference in carcass yield by probiotic addition compared to the control.

Immune organs:

Results of relative weight of immune organs listed in (Table 3). No significant differences were noticed in spleen% as affected by the treated birds in comparison with the control, while chicks fed combined supplementation of bacteria (B. subtilis and P. acetolactic) had significantly higher bursa and thymus% compared to the control. Chicks fed addition of bacteria B.subtilis 1g+ P. acidilactici 0.5g had higher bursa% and thymus% (0.18 and 0.57, respectively) compared with the control (0.11 and 0.41 respectively). It can be concluded that bacteria supplementation to broiler diets improved significantly bursa and thymus% compared to the control group, also improved spleen%, but differences were not significant (Table 3). Many enteric diseases could be prohibited if an ideal gut microflora was maintained, it was suggested probiotics have antimicrobial properties as potential protective agents in the gut against several pathogens (Alvarez-Olmos and Oberhelman, 2001). At that time, it seemed that all of these protective effects could be attributed to the competitive exclusion effects of probiotics. However, probiotics were found to enhance systemic antibody production (Macpherson and Uhr, 2004) as well as activate innate immunity (Matsuzaki and Chin, 2000).

Sikandar et al. (2017) shown augmented cortex/medulla ratio and thymus cortical width in 0.1 Bacillus Subtillis group compared to the control, also bursal follicles improved. Results encountered in this study is in harmony with that of Molnár et al. (2011).

The favorable action of probiotic on the immune response indicates the boost of the formulating bacteria on an acquired immune response exerted by B and T lymphocytes. The forthright effect might be associated to excite the lymphatic tissue (Kabir et al., 2004).

Blood parameters: Results listed in Tables 4 and 5 showed the effects of bacteria (B. subtilis, P. acidilactici) alone or combined supplementation to broiler diets on some blood hematological parameters. The results indicated no significant differences due to supplementation of B. subtilis and P. acidilactici, except, WBCs, heterophils, lymphocyte and H/L ratio which were significantly affected. Chicks fed diets supplemented with 1g B. subtilis +1g P, acidilactici had higher value of WBCs, also birds fed dietary 0.5g P. acidilactici had higher values of WBCs, but those fed the control diet had lower values of WBCs and lymphocyte, and higher value of heterophils (Table 4). Chicks fed diets supplemented with 1g B. subtilis +0.5g P.acidilactici had higher value of lymphocyte and lower values of H/L ratio and heterophils (Table 4).

As shown in (Table 5), serum triglycerides, total cholesterol, albumin, globulin, total protein, Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were estimated at 38 d of age. Concerning treatments effect, no significant difference ($P>0.05$) among treatments in serum parameters between the chicks treated with bacteria and control groups except AST, chicks fed diets supplemented with 1g B. subtilis +1g P, acidilactici and 1g B. subtilis +0.5g P, acidilactici had significantly ($P\leq0.05$) lower AST (135 and 138 respectivaly), while, chicks fed the control diet had higher values (158.66). There was numerical decrease in levels of triglyceride and cholesterol in the treated group compared to control group, but differences were not significant. Schiffrin et al. (1995) suggested an enhance innate immune response with supplementation of probiotics, also probiotics have been reported to enhance production of antibody and promote antibody isotype switching (Haghighi et al., 2006). These conclusions agree with those established by Matsuzaki and Chin (2000) and Siadati et al. (2017) who found that, increase of number WBCs with probiotic bacteria suplemention compare with the control groups. Chen et al. (2017) illustrated a reduced AST, ALT, cholesterol and triglycerides in broilers diets containing probiotics . Same result reported by Hedayati et al. (2015) who found that triglyceride and cholesterol was decreased with probiotics.

Intestinal microflora:

The effect of bacteria (B. subtilis or P. acidilactici) alone or combined supplementation in broiler diets on intestinal pH, some pathogenic (E. coli), beneficial gut microorganisms (lactobassullus bacteria) and immune response to Influeza (AI) and Newcasle (ND) was presented in (Table 6).

Concerning intestinal PH, obtained results showed that significant higher ($P\leq0.01$) in the control group (6.54) than the other treated groups (6.2-5.88). The lowest ($P\leq0.01$) values were obtained for birds fed diets supplemented with combined bacteria groups (1gB.subtilis +1g P. acidilactici, 1gB. subtilis+ 0.5g P. acidilactici, 0.5g B.subtilis+0.5 P. acidilactici and 0.5g B. subtilis+1g P. acidilactici (5.88, 5.94, 6 and 6.03 respectively). Obtained results exhibited that significant ($P\leq0.01$) increase in count of total bacteria and lactobassullus bacreia in groups fed diets supplemented with bacteria compared with the control, while there is significant ($P\leq0.01$) reduction in E. coli. However, the use of combine bacteria (Bacillus subtilis, Pediococcus acidilactici) leads to significant increase ($P\leq0.01$) in total bacteria and Lactobassullus bacteria and significant (P≤0.01) decrease in E. coli bacteria (Table 6). Data revealed that there were no significant differences of Newcastle and Influenza values among the treated groups compared to the control groups Table 6. B. subtilis is not a lactic acid bacteria, but Bacillus spp. can increase the acid lactic bacteria population (Knarreborg et al., 2008), which is related to a pH alteration (Wu et al., 2011 and Alloui et al., 2014). Useful impacts of probiotics are by repress enteric pathogenic bacteria growth and boost beneficial bacterial species growth, resulting in betterment of intestine health. Subsequently, probiotics effect such as enhanced intestinal environment and enteric immune responses modulation might influence the E.coli proliferation. These results are harmony with those obtained by Awad et al. (2009 and 2010), Mountzouris et al. (2010) and Chen et al. (2013) who concluded that there are suppress for pathogenic bacteria and improvement in the beneficial bacteria in the intestine. Our results denoted that probiotic supplementation had beneficial impact on growth performance and immune response reflecting better digestion and absorption. In other words, probiotic administration modulated the activities and the composition of cecal micoflora, resulting significant effect and a metabolic stimulation of these microflora .

Therefore, Pascual et al. (1999) stated that probiotic consumption should result in the making of gut micro-ecology conditions that repress unsafe microorganisms and support useful microorganisms and finally improve gut health. Also, earlier studies showed that the lactobacilli intake in the intestine of chickens might reach their highest figures so that the addition strains might contribute to their presence in intestinal microbiota, but supplementation of probiotic had the advantage of lower E. coli populations and increasing the abundance and prevalence of Lactobacillus spp, in the intestine (Mountzouris et al., 2015). Contrary, using probiotics (multi-strain of bacteria) have revealed alterations in profile of gut microflora of broilers (Chen et al., 2017 and Siadati et al., 2017).

IN CONCLUSION,

the present results clearly indicates that the simultaneous inclusion of B.subtilis 1g+ P. acidilactici 0.5g in broiler chicken diets having synergistic effect on the overall growth performance and improvement of intestinal PH and immune system when compared to control.

Table (1): Composition and calculated analyses of the control starter, grower and finisher diets.

Items%	Starter (7-14 days)	Grower (15-21days)	Finisher (22-38 days)
Yellow corn, ground(8.5%CP)	64.37	70.40	74.22
Soybean meal (44%CP)	23.08	16.78	12.30
Corn gluten meal (60%CP)	8.56	9.00	10.00
Dicalcium phosphate	1.80	1.70	1.50
Calcium carbonate	0.90	0.85	0.80
Vit. and Min. premix*	0.30	0.30	0.30
Salts (Nacl)	0.30	0.30	0.30
DL–Methionine	0.24	0.20	0.15
L-Lysine Hcl	0.45	0.47	0.43
Total	100.0	100.0	100.0
Calculated analysis%**:			
Crude protein (CP)	21.50	19.50	18.5
Crude fat	2.84	3.03	3.17
Crude fiber	3.00	3.00	3.00
Calcium	0.90	0.84	0.76
Available phosphorus	0.45	0.42	0.38
Methionine	0.50	0.48	0.50
Methionine+Cystine	0.98	0.89	0.82
Lysine	1.32	1.19	1.05
ME, kCal./Kg	3008.00	3086.00	3167.00

* Each 3.0 kg of premix supplies one ton of the diet with: Vit. A, 12000000 I.U; Vit. D3, 2000000 I.U.; Vit. E, 40g; Vit. K3, 4g; Vit. B1, 3g; Vit. B2, 6g; Vit.B6, 4g; Vit.B12, 30mg; Niacin, 30gm; Biotin, 80mg; Folic acid, 1.5g; Pantothinic acid, 12g; Zn, 70g; Mn, 70g; Fe, 40g; Cu, 10g; I, 1.5g; Co, 250mg; Se, 200mg; Choline, 350g and complete to 3.0 Kg by calcium carbonate. **According to NRC, 1994.

Table (2): Effects of single or combined supplementation of probiotic bacteria (Bacillus subtilis, Pediococcus acidilactici) to broiler diets on growth performance.

Item	**Body weight (g)**		**Body weight gain (g)**	**Feed intake (g)**	**Feed conversion**	**Growth rate**	**Performance index**
	7 days	38 days	7-38 days	7-38 days	7-38 days	7-38 days	7-38 days
Control	146.40	1911.72^{d}	1765.33^{d}	3302.45	1.87^{a}	1.72^{d}	33.58^{d}
0.5 g B.subtilis	146.07	1976.25^{bcd}	1830.17^{bcd}	3292.73	1.80^{ab}	1.72^{bcd}	36.13^{cd}
1 g B.subtilis	146.60	1960.25^{cd}	1813.65^{cd}	3233.50	1.78^{abc}	1.72^{cd}	35.96^{cd}
0.5g P. acidilactici	146.25	1983.5^{bcd}	1837.25^{bcd}	3281.23	1.78^{abc}	1.73^{bcd}	36.37^{cd}
1g P. acidilactici	146.35	1985.25^{bcd}	1838.9^{bcd}	3259.60	1.78^{bc}	1.72^{bcd}	36.88^{bc}
0.5 g B.subtilis +0.5g P. acidilactici	145.90	2025.50^{abc}	1879.6^{abc}	3271.30	1.78^{bcd}	1.73^{abc}	37.56^{bc}
0.5 g B.subtilis +1g P. acidilactici	145.85	2060.25^{ab}	1914.4^{ab}	3280.87	1.74^{bcd}	1.74^{ab}	38.83^{abc}
1g B.subtilis + 0.5g P. acidilactici	145.90	2107.25^{a}	1961.35^{a}	3236.53	1.65^{d}	1.74^{a}	40.65^{a}
1 g B.subtilis + 1g P. acidilactici	145.75	2074.95^{a}	1929.20^{a}	3262.10	1.69^{cd}	1.74^{a}	39.64^{ab}
Overall Mean±SEM[1]	146.12±0.207	2009.43±9.06	1863.3±9.1	3268.9±12.5	1.76±0.01	1.73±0.001	37.29±0.29
P value	0.984	0.001	0.001	0.915	0.001	0.001	0.0001

a,b and c means in the same column within different letters, different significantly (at P≤0.05) [1] Pooled SEM

Table (3): Effects of single or combined supplementation of probiotic bacteria (Bacillus subtilis, Pediococcus acidilactici) to broiler diets on some slaughter parameters %. and immune organs.

Item	Live body weight (g)	Total giblet%	Abdominal fat%	Half breast%	Half rear%	Immune organs%		
						Bursa	Thymus	Spleen
Control	2093.00^{d}	4.13	1.11	19.11	16.40^{a}	0.11^{c}	0.41^{b}	0.14
0.5 g B.subtilis	2111.00^{d}	4.10	1.10	17.20	14.60^{b}	0.15^{ab}	0.48^{ab}	0.15
1 g B.subtilis	2262.00^{abc}	3.75	0.97	17.40	14.90^{b}	0.15^{ab}	0.48^{ab}	0.17
0.5g P. acidilactici	2165.00^{cd}	3.86	1.06	18.10	14.20^{b}	0.14^{bc}	0.47^{ab}	0.17
1g P. acidilactici	2209.00^{bcd}	3.65	0.84	17.30	14.80^{b}	0.13^{bc}	0.49^{ab}	0.17
0.5 g B.subtilis +0.5g P. acidilactici	2322.00^{ab}	3.94	1.03	18.50	15.00^{b}	0.16^{ab}	0.57^{a}	0.12
0.5 g B.subtilis +1g P. acidilactici	2290.00^{abc}	3.82	1.00	18.10	14.90^{b}	0.14^{bc}	0.52^{a}	0.16
1g B.subtilis + 0.5g P. acidilactici	2384.00^{a}	4.03	0.98	17.80	15.00^{b}	0.18^{a}	0.57^{a}	0.17
1 g B.subtilis + 1g P. acidilactici	2361.00^{a}	3.87	0.90	18.5	14.10^{b}	0.15^{ab}	0.54^{a}	0.15
Overall Mean±SEM[1]	2244±14.9	3.93±0.04	1±0.037	18.04±0.33	14.9±0.12	0.145±0.004	0.5±0.01	0.15±0.005
P value	0.001	0.23	0.73	0.9	0.01	0.04	0.044	0.174

a,b and c means in the same column within different letters, different significantly (at P≤0.05) [1] Pooled SEM

Table (4): Effects of single or combined supplementation of probiotic bacteria (Bacillus subtilis, Pediococcus acidilactici) to broiler diets on some blood parameters.

Item	RBC (10^6/ mm^3)	Blood index				WBC (103/mm 3)	Differential count%				H/L ratio
		Hematocrit %	(MCV) μ^2	(MCH) μμg	(MCHC) %		Heterophils (H)	Lymphocyte (L)	Monocytes %	Eosinophiles %	
Control	3.02	27.82	92.34	39.67	42.91	28.00^{c}	18.67^{a}	70.00^{d}	5.00	6.33	0.26^{a}
0.5 g B.subtilis	3.15	28.56	90.64	38.36	42.36	28.41^{c}	18.00^{ab}	70.33^{cd}	5.00	6.67	0.25^{ab}
1 g B.subtilis	3.13	27.35	87.33	36.82	42.51	29.18^{c}	18.00^{ab}	70.33^{cd}	4.00	7.67	0.25^{ab}
0.5g P. acidilactici	3.16	27.40	86.73	37.92	43.71	33.35^{a}	16.33^{bc}	73.33^{b}	4.67	5.67	0.23^{abc}
1g P. acidilactici	3.09	26.54	85.69	39.8	46.94	32.14^{ab}	16.33^{bc}	72.33^{bcd}	4.33	7.00	0.22^{abcd}
0.5 g B.subtilis +0.5g P. acidilactici	3.02	26.13	86.32	37.32	43.29	30.16^{bc}	16.00^{bc}	71.67^{bcd}	5.00	7.33	0.21^{bcd}
0.5 g B.subtilis +1g P. acidilactici	3.00	27.93	93.09	38.97	41.87	33.11^{a}	16.00^{bc}	73.00^{bc}	4.33	6.67	0.21^{bcd}
1g B.subtilis + 0.5g P. acidilactici	3.04	26.61	87.35	38.53	44.08	33.62^{a}	14.33^{c}	76.33^{a}	4.67	4.67	0.18^{d}
1 g B.subtilis + 1g P. acidilactici	3.18	27.33	87.21	39.01	45.00	34.21^{a}	15.67^{c}	76.00^{a}	4.33	4.00	0.20^{cd}
Overall Mean±SEM[1]	3.09± 0.20	27.33±0.30	88.52 ±0.82	38.49 ±0.59	43.58±0 .78	31.36±0. 27	16.59±0 .23	72.59±0 .29	4.59±0. 08	6.22±0.29	0.23±0. 005
P value	0.71	0.66	0.34	0.94	0.91	0.001	0.007	0.001	0.085	0.120	0.013

a,b and c means in the same column within different letters, different significantly (at $P \leq 0.05$) [1] Pooled SEM

H. B= Hemoglobin; RBC= Red blood cells; HCT=Hematocrit; MCV= Mean corpuscular volume; MCH= Mean corpuscular hemoglobin; MCHC= Mean corpuscular hemoglobin concentration; WBC= White blood cells count.

Table (5): Effects of single or combined supplementation of probiotic bacteria (Bacillus subtilis, Pediococcus acidilactici) to broiler diets on serum blood parameters.

Item	Total protein g/dL	Albumin(A) g/dL	Globulin (G)g/dl	A/G ratio	ALT(U/L)	AST(U/L)	Triglycerides mg/dl	Total cholesterol, mg/dl
Control	2.93	1.30	1.63	0.79	27.33	158.66^{a}	91.00	179.00
0.5 g B.subtilis	3.43	1.43	2.00	0.69	27.00	158.56^{a}	84.00	173.33
1 g B.subtilis	3.26	1.36	1.90	0.71	26.33	146.00^{abc}	81.66	163.33
0.5g P. acidilactici	3.30	1.33	2.10	0.52	25.00	153.00^{bc}	86.33	165.66
1g P. acidilactici	3.20	1.13	2.06	0.56	25.00	154.00^{bc}	87.33	167.33
0.5 g B.subtilis +0.5g P.acidilactici	3.40	1.30	2.13	0.62	24.00	138.00^{bc}	87.66	157.66
0.5 g B.subtilis +1g P. acidilactici	3.36	1.16	2.20	0.52	24.66	150.00^{abc}	76.00	161.66
1g B.subtilis + 0.5g P.acidilactici	3.50	1.26	2.23	0.56	23.00	138.00^{bc}	75.66	157.66
1 g B.subtilis + 1g P. acidilactici	3.56	1.26	2.30	0.54	22.33	135.00^{c}	75.00	156.33
Overall Mean±SEM[1]	3.33±0.056	1.26±0.035	2.07±0.043	0.61±0.02	24.96±0.38	148.14±1.7	82.74±1.53	164.66±1.78
P value	0.31	0.50	0.054	0.07	0.08	0.024	0.17	0.10

a,b and c means in the same column within different letters, different significantly (at $P \leq 0.05$) [1] Pooled SEM

ALT= Alanine aminotransferase AST= Aspartate aminotransferase

Table (6): Effects of single or combined supplementation of probiotic bacteria (Bacillus subtilis, Pediococcus acidilactici) to broiler diets on bacterial count and immune response to Influenza (AI) and Newcastle (ND)

Item	Intestinal pH	Bacterial count			Titration against	
		Total Count	E. coli 10^{*4}	lactobacillus	Newcastle	Influenza
Control	6.54^{a}	10.66^{f}	4.65^{a}	5.03^{f}	7.33	7.00
0.5 g B.subtilis	6.24^{b}	10.67^{ef}	4.10^{bc}	5.39^{e}	7.67	7.33
1 g B.subtilis	6.11^{bcd}	10.80^{de}	4.17^{bc}	5.49^{de}	7.67	8.00
0.5g P. acidilactici	6.133^{bc}	10.74^{ef}	4.21^{b}	5.40^{e}	7.67	7.33
1g P. acidilactici	6.04^{bcd}	10.91^{cd}	4.05^{bcd}	5.39^{e}	8.00	8.00
0.5 g B.subtilis +0.5g P. acidilactici	6.00^{cde}	10.94^{c}	3.99^{cde}	5.65^{c}	7.67	7.33
0.5 g B.subtilis +1g P. acidilactici	6.03^{cde}	11.13^{b}	3.88^{def}	5.54^{cd}	8.33	8.33
1g B.subtilis + 0.5g P. acidilactici	5.94^{de}	11.21^{ab}	3.84^{ef}	5.76^{b}	9.00	8.67
1 g B.subtilis + 1g P. acidilactici	5.88^{e}	11.30^{a}	3.77^{f}	5.91^{a}	9.00	8.67
Overall Mean±SEM[1]	6.11±0.19	10.93±0.013	4.07±0.02	5.5±0.012	8.04±0.11	7.85±0.14
P value	0.00	0.00	0.00	0.00	0.16	0.09

a,b and c means in the same column within different letters, different significantly (at P≤0.05) [1] Pooled SEM

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الملخص العربي

تأثير إضافة البكتيريا كمحفز نمو علي أداء بداري التسمين

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تم إجراء هذه الدراسة بهدف دراسة تاثيرات البكتيريا (باسيلس سابتلس وبكتيريا بيدوكوكس اسيدو لاكتيسي) منفردا او خليط منهما في علائق كتاكيت التسمين علي الأداء الأنتاجي وميكوفلورا الأمعاء وبعض الصفات الفسيولوجية. استخدم فى هذه الدراسة 360 كتكوت غير مجنس من سلالة كب عمر سبعة ايام ، وزعت الى تسعه معاملات كل معاملة مقسمة الى اربعة مكررات .

وتم تلخيص النتائج المتحصل عليها كما يلي: اظهرت الكتاكيت المغذاه علي علائق تحتوي علي خليط من البكتيريا (اجرام باسيلس سابتلس +0.5 جرام بيديكوكس اسيدولاكتيسي) تحسنا معنويا في وزن الجسم الحي ومعدل الزيادة في وزن الجسم ومعدل النمو والاداء الانتاجي وبعض صفات مقاييس الدم واعضاء المناعة (البيرسا والثيموثية) وأفضلية في معدل التحويل الغذائي، وميكروفلورا.

ويستخلص من نتائج الدراسة أنه يمكن اضافة خليط من البكتيريا (1 جرام باسيلس سابتلس +0.5 جرام بيديكوكس اسيدولاكتيسي) الى علائق كتاكيت التسمين كمحفز نمو لانه أدت الى تحسن الأداء الأنتاجي والتوازن الميكروبى بالأمعاء للكتاكيت مع تحسن وظائف الامعاء.

الكلمات الداله: الاضافات الغذائية، باسيلس سابتلس، بيديكوكس اسيدولاكتيسي، بروبايوتك