

**Damanhour University**

**Faculty of Veterinary Medicine**

**Department of Nutrition and Veterinary Clinical Nutrition**



**Impact of dietary supplementation of some *Bacillus subtilis* strains on performance, blood parameters, immunity and gut integrity in broilers**

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## **CONTENTS**

<b>Subject</b>	<b>Page</b>
<b>1. INTRODUCTION</b>	<b>1</b>
<b>2. REVIEW OF LITERATURE</b>	<b>3</b>
<b>3. MATERIALS AND METHODS</b>	<b>24</b>
<b>4. RESULTS</b>	<b>36</b>
<b>5. DISCUSSION</b>	<b>57</b>
<b>6. CONCLUSION</b>	<b>81</b>
<b>7. ENGLISH SUMMERY</b>	<b>82</b>
<b>8. REFERENCES</b>	<b>85</b>
<b>9. ARABIC SUMMARY</b>	

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## LIST OF TABLES

Table	Title	Page
1	The physical composition of the basal diets	25
2	Calculated and chemical analysis of basal diets	26
3	Vaccination program during the experimental period	27
4	Experimental outline throughout the whole experimental period	27
5	Volumes Recommended for Reverse Transcription of RNA Samples	32
6	The Thermal Cycler Reaction Conditions	33
7	Primers of candidate gene used in gene expression analysis	34
8	Impact of dietary <i>Bacillus subtilis</i> strains (QST713 or PB6) supplementation on body weight development (g) of broiler chickens	36
9	Impact of dietary <i>Bacillus subtilis</i> strains (QST713 or PB6) supplementation on average of starter, grower and finisher weight (g) of broiler chickens	37
10	Impact of dietary <i>Bacillus subtilis</i> strains (QST713 or PB6) supplementation on body weight gain (g) of broiler chickens	38
11	Impact of dietary <i>Bacillus subtilis</i> strains (QST713 or PB6) supplementation on average starter, grower and finisher body weight gain (g) of broiler chickens	39
12	Impact of dietary <i>Bacillus subtilis</i> strains (QST713 or PB6) supplementation on feed intake (g/bird/week) of broiler chickens	40
13	Impact of dietary <i>Bacillus subtilis</i> strains (QST713 or PB6) supplementation on average of starter, grower and finisher feed intake (g) of broiler chickens	41
14	Impact of dietary <i>Bacillus subtilis</i> strains (QST713 or PB6) supplementation on feed conversion ratio (FCR) of broiler chickens	42
15	Impact of dietary <i>Bacillus subtilis</i> strains (QST713 or PB6) supplementation on average starter, grower and finisher feed conversion ratio (FCR) of broiler chickens	43
16	Impact of dietary <i>Bacillus subtilis</i> strains (QST713 or PB6)	44

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	supplementation on average of protein efficiency ratio (PER)	
17	Impact of dietary <i>Bacillus subtilis</i> strains (QST713 or PB6) supplementation on economical efficiency (%) in different groups of broiler chickens	45
18	Impact of dietary <i>Bacillus subtilis</i> strains (QST713 or PB6) supplementation on carcass traits of broiler chickens	46
19	Impact of dietary <i>Bacillus subtilis</i> strains (QST713 or PB6) supplementation protein profile through starter, grower and finisher	47
20	Impact of dietary <i>Bacillus subtilis</i> strains (QST713 or PB6) supplementation on serum glucose through starter, grower and finisher	48
21	Impact of dietary <i>Bacillus subtilis</i> strains (QST713 or PB6) supplementation on lipid profile through starter, grower and finisher	49
22	Impact of dietary <i>Bacillus subtilis</i> strains (QST713 or PB6) supplementation on kidney function biomarkers through starter, grower and finisher	50
23	Impact of dietary <i>Bacillus subtilis</i> strains (QST713 or PB6) supplementation on liver function biomarkers through starter, grower and finisher	51
24	Morphometry of jejunum and follicular area of bursa in broilers	54

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## LIST OF FIGURES

<b>Fig.</b>	<b>Title</b>	<b>Page</b>
1	RT-PCR validation of pro inflammatory gene IL -1 $\beta$ ; Interleukin 1 beta of birds with <i>Bacillus subtilis</i> supplementation	52
2	RT-PCR validation of pro inflammatory IL-6; interleukin 6 of birds with <i>Bacillus subtilis</i> supplementation	52
3	RT-PCR validation of antioxidant gene CAT; catalase of birds with <i>Bacillus subtilis</i> supplementation	53
4	RT-PCR validation of antioxidant gene SOD1; superoxide dismutase 1 of birds with <i>Bacillus subtilis</i> supplementation	53
5	Photomicrograph of jejunal sections of four groups treated with probiotics	55
6	Photomicrograph of bursa showing the follicular (F) structure between control and probiotics treated groups	56

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## LIST OF ABBREVIATIONS

<b>Abbreviation</b>	<b>Indication</b>
A/G ratio	Albumin, globulin ratio
AGP	Antibiotic growth promotors
AI	Avian Influenza
ALT	Alanine amino transferase
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
AST	Aspartate amino transferase
AVL	Avilamycin
AWG	Average weight gain
B. coagulans	<i>Bacillus coagulans</i>
BMD	Bacitracin methylene disalicylate
BMV	Breast muscle yield
BS	<i>Bacillus subtilis</i>
BWG	Body weight gain
cm	centimeter
<i>C.perfringens</i>	<i>Colistridium perfringens</i>
CAT	Catalase
CD	Crypt depth
CE	Competitive exclusion
CFU	Colony forming unit
CON	Control
CP	Crude protein
CT cycle	Threshold cycle
DFM	Direct feed microbial
DNA	Deoxyribonucleic acid
EE	Economical efficiency
EPEC	Entero Pathogenic E, coli
EPEF	European production efficiency factor
EU	European Union
F	Follicles

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FAs	Fatty acids
FCR	Feed conversion ratio
FI	Feed intake
G	Gram
GALTs	Gut-associated lymphoid tissues
GAPDH	Glyceraldehyde-3- Phosphatedehydrogenase
GC	Germinal centre
GIT	Gastro intestinal tract
GOT	Glutamate-oxaloacetate transaminase
GPT	Glutamate- pyruvate transaminase
GSH-Px	Glutathion peroxidase
HDL	High density lipoprotein
H-E	Hematoxylin-eosin stain
HepG2	Human hepatoma cells
IB	Infectious Bronchitis
IBD	Infectious Bursal Disease
IFN $\gamma$	Interferone alpha
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IL	Interleukins
IL-1B	Interleukin- 1beta
Kg	Kilogram
LDL	Low-density lipoprotein
LL	large lymphocytes
MDA	Malondialdehyde
Mg	Milligram
mRNA	Messenger ribonucleic acid
MT	Metric ton
ND	New-Castle
NDV	Newcastle disease virus
NE	Necrotic enteritis
PER	Protein efficiency ratio
PHYTO	Phytobiotics

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PRO	Probiotics
PRRs	Pattern recognition receptors
qRT-PCR	Quantitative real-time polymerase chain reaction
RPM	Round per minute
<i>S. typhimurium</i>	<i>Salmonella typhimurium</i>
SCFA	Short chain fatty acids
sGOT	Serum glutamic oxaloacetic transaminase
sGPT	Serum glutamic pyruvic transaminase
SL	Small lymphocytes
SOD	Super oxide dismutase
SPSS	Statistical Package for Social Science
SRBCs	Sheep red blood cells
TAOC	Total antioxidant capacity
TC	Total cholesterol
TFI	Total feed intake
TG	Triglyceride
TGF	Transforming growth factor
TLR	Toll like receptors
TNF	Tumor necrotic factor
TP	Total protein
VH	Villus height
VLDL	Very low density lipoprotein
VSA	Villus surface area
VW	Villus width
WPMY-1	Human prostatic myofibroblast cell line
WWG	Whole weight gain
Xg	Xanthan gum
ZnB	Zinc bacitracin
μm	Micro meter
μg	Micro gram
2-ΔΔCt	Double delta C method



## 7. Summary

This Work was conducted at Nutrition and Clinical Nutrition Department, Faculty of Veterinary Medicine, Damanhur University to investigate the possible effect of dietary some probiotics supplementation on growth performance, some blood parameters, immune response and gut integrity in broiler chickens. A total of 160 one-day old avian chicks were used in this experiment. The broiler chickens were randomly allotted into 4 groups (40 per each) in four replicates and received out of the different diets during the experimental period (5 weeks) the first group fed on the broiler diet without supplement (control) while the second one fed on Bay mix<sup>®</sup> (0.1g/kg), the third one fed on COLSTAT<sup>®</sup> (0.5 g/kg) and the fourth one fed half dose of both Bay mix<sup>®</sup> and COLSTAT<sup>®</sup>. The obtained results can be summarized as following:

### Growth performance parameters

1-The growth performance parameters of the examined *Bacillus subtilis* strains (QST713 and PB6) showed no significant differences between the supplemented groups and the control one. However, a slight improvement ( $p \geq 0.05$ ) in final body weight (an increase of 2.8%) and weight gain (an increase of 2.9%) was observed for Group 2 (G2).

2- The feed conversion ratio was similar ( $p \geq 0.05$ ) in all supplemented groups compared with the control group.

3- There was a no difference ( $p \geq 0.05$ ) in feed intake between the control and other supplemented groups (2-4) throughout the whole experimental period.

4- The protein efficiency ratio (PER) was similar across all supplemented groups compared to the control.

5- Additionally, no mortalities were detected in any of the experimental groups throughout the study.

6- Relative economic efficiency within the groups (G1-G4) ranged from 100%, 108.9%, 96.3% and 83.6% respectively. This indicated that, the use of feed additives (QST713 supplemented group) improved the economic efficiency when compared to the control group.

7- Carcasses traits, the data of carcass weight, dressing percentage, edibles (liver, heart and gizzard), and non -edible parts (intestine) and immune organs (bursa and spleen) of birds slaughtered at 5 weeks of age revealed no significant difference found among averages of all traits for all diets except significant increase in gizzard percentage in all supplemented groups comparing with the control one.

### Serum metabolites

The average results of sera analysis at the starter, grower, and finisher phases. The results of protein profile (total protein, albumin, globulin), lipid profile (total cholesterol and triglyceride), glucose, liver function indices (GOT and GPT), and Kidney function indices

(uric acid and creatinine) showed ( $p \geq 0.05$ ) there were no significant differences in all supplemented groups when compared with the control one.

### **Immunological parameters**

- 1- Interleukin 1 beta (IL1 $\beta$ ) and interleukin 6 (IL6), two proinflammatory genes, were expressed with reduced expression ( $p < 0.001$ ) in the 0.05 gm Bay mix<sup>®</sup>(1)/Kg diet + 0.25 gm CLOSTAT<sup>®</sup>(2)/kg diet in comparison to the other diets.
- 2- The expression of antioxidant genes in the jejunum. The group that consumed 0.05 gm Bay mix<sup>®</sup>(1)/Kg and 0.25 gm CLOSTAT<sup>®</sup>(2)/kg food had higher levels of catalase and superoxide dismutase 1 (SOD1) expressions ( $p < 0.001$ ) in comparison to the other groups.

### **Histomorphological Findings**

- 1-The effects of various probiotic supplements on jejunum histomorphometric parameters in broiler chickens at (35d), including villus length, width, and crypt depth, Based on the available data, intestinal integrity was positively impacted by *B. subtilis* QST713. Our findings thus demonstrate a small increase in villus length that was not different from the control group or the group that received a combination of half dosages of the two probiotics used.
- 2- The birds that got *B. subtilis* PB6 had the longest jejunal villi ( $P < 0.0001$ ).
- 3-Additionally, there were notable differences in the villus width between the majorities of ranging profile groups. The smallest width was found in the combination group, whilst the maximum width was found in the birds who received *B. subtilis* PB6 compared to the control and *B. subtilis* QST713 receiving groups.
- 4- when comparing the group supplemented with *B. subtilis* QST713 to the control or mixture groups, no obvious difference was seen.
- 5- A larger crypt depth was seen in birds fed probiotic-containing diets than in birds fed a diet lacking probiotics.
- 6- In addition, crypt depth significantly increases in birds fed diets supplemented with mixed probiotics as opposed to groups supplied with one probiotic separately. Applying *B. subtilis* QST713 or *B. subtilis* PB6 individually to the diet resulted in nearly identical depths for the intestinal crypts.
- 7- The villus architecture of typical birds from each group. The group that received *B. subtilis* QST713 supplements exhibited a normal intestinal shape and healthy villus epithelium. The *B. subtilis* PB6-supplemented group had an undamaged villus structure with increased villi length, reflecting a functionally active and mature epithelium, which is consistent with the jejunum's morphometric study.
- 8- Comparing the combined group to the other treated groups and the control group, the bursa follicular area increased to its greatest extent. All other groups recorded varying amounts of follicular area; however, the *B. subtilis* PB6 group recorded the least. Both the

control groups and the group that received the *B. subtilis* QST713 showed no apparent increase in their bursa. Cortex and medulla are the two unique regions of these follicles. The sections presented here demonstrate how the control group's follicle is slightly elongated, containing small lymphocytes (SL) or lymphoblasts, and few large lymphocytes (LL). But the probiotic group as a whole had well-developed bursal follicles.

9- It was very clear that the germinal center of the follicles increased in the group supplemented with *B. subtilis* PB6 while the dark small lymphocytes decreased. This was in contrary to the combined group receiving both probiotics.



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