CLOSTAT™ 365

Scientific Publications of *Bacillis subtilis* PB6 Book of Abstracts

KEMIN INTESTINAL HEALTH

100+ Scientists
30+ Publications
7 Species
Strain *Bacillus subtilis* PB6

Dear Reader,

Continuous development in genetics, improved nutritional practices helps to enhance the efficiency in animal protein production. However, the higher productivity throws challenge on gut health and immunity of the animal. Necrotic enteritis is one such chronic challenge that need critical attention for better gut health and economic animal production.

Specific acting active microbials (second-generation probiotics) are the promising alternates after the era of traditional antibiotic growth promoters (AGPs). These active microbials not only help to control the pathogens but also benefits intestinal microbiota stability, non-resistance, compatibility, etc. by producing secondary metabolites with antimicrobial properties.

Kemin Industries had identified and studied the properties of one such specific acting active microbials, *Bacillus subtilis* PB6. The whole genome study of *Bacillus subtilis* PB6 indicates its performance in livestock like broiler, layer, swine, aqua, equine, ruminants, etc. with maximum output and compatibility with other additives like anticoccidials, antibiotics, organic acids, etc.

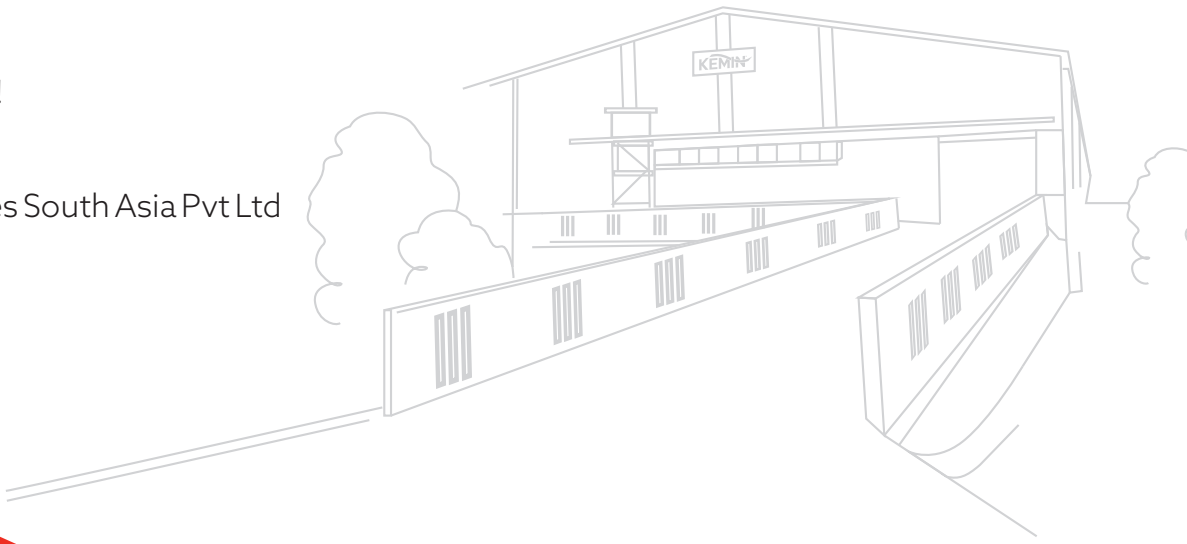
Bacillus subtilis PB6 secretes many secondary metabolites that promote the growth of commensals and inhibit the pathogens. Its spores are also stable at feed processing conditions. The special property of *Bacillus subtilis* PB6 is that it does not contain plasmids, due to which it cannot transfer the antimicrobial gene resistance to other microorganisms. These properties of *Bacillus subtilis* PB6 make it suitable for use in the feed as antibiotic-free chicken production for a healthy and safe future.

This manuscript is the compiled abstracts of scientifically published peer-reviewed papers by the active academia and specialists in the nutritional and health field. These abstracts aim to give an understanding of *Bacillus subtilis* PB6 performance as well as suitability for the current global need.

Kemin wishes to be your partner to produce a healthier, efficient, and safe animal protein.

Happy Reading!

Kemin Industries South Asia Pvt Ltd



KEMIN INTESTINAL HEALTH

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Kemin Internal Documents

Image References

Kemin Internal document Shutterstock_1042543318, 1457018426, 18100840, 367715540, 120477529, 683030524, 769091569, 117645061, 285560903, 742595047, 1364899397, 1126689623, 1126689635.

Inhibition of *Clostridium perfringens* by a novel strain of *Bacillus subtilis* PB6 isolated from the gastrointestinal tracts of healthy chickens

Alex Yeow-Lim Teo and Hai-Meng Tan

Republic of Singapore
APPLIED AND ENVIRONMENTAL MICROBIOLOGY,
Aug. 2005, Vol. 71, No. 8

KEMIN INTESTINAL HEALTH

Inhibition of *Clostridium perfringens* by a novel strain of *Bacillus subtilis* PB6 isolated from the gastrointestinal tracts of healthy chickens

Necrotic enteritis, caused by *Clostridium perfringens*, is an intestinal disease in modern broiler birds. Necrotic lesions developed on the wall of the gut in poultry leads to mortality. Traditionally, antibiotic growth promoters treat *Clostridium perfringens* infection. However, the discovery of antibiotic-resistant strains of *Clostridium perfringens* and other pathogens in livestock changed the trend. The market has also pursued non-antibiotic-based treatment courses which resulted in finding a probiotic solution.

A bacterium isolated from the intestine of healthy chicken gut, *Bacillus subtilis* PB6 (ATCC PTA 6737) found effective against pathogenic *Clostridium perfringens*. It is a spore-forming bacterial strain isolated by Kemin Scientists- Hai-Meng Tan and Alex Yeow-Lim Teo.

The efficacy of the antimicrobial activity of the anti-clostridial factor of bacterial strains checked by various tests showed that it was not affected by treatment at 100°C or 121°C or by treatment with any of the organic solvents used. In terms of the antimicrobial spectrum, the anti-clostridial factor was inhibitory toward various strains of *C. perfringens* implicated in necrotic enteritis in poultry. *Clostridium difficile, Streptococcus pneumoniae, Campylobacter jejuni, Campylobacter coli. Bacillus subtilis* PB6 is the primary ingredient in CLOSTAT™ product.

The proteinaceous nature of the antimicrobial factor and the retention of the inhibitory activity after heat treatment are the desirable characteristics of *Bacillus subtilis* PB6. Thus, *Bacillus subtilis* PB6 is an attractive probiotic for the prevention of necrotic enteritis in poultry.

Summary

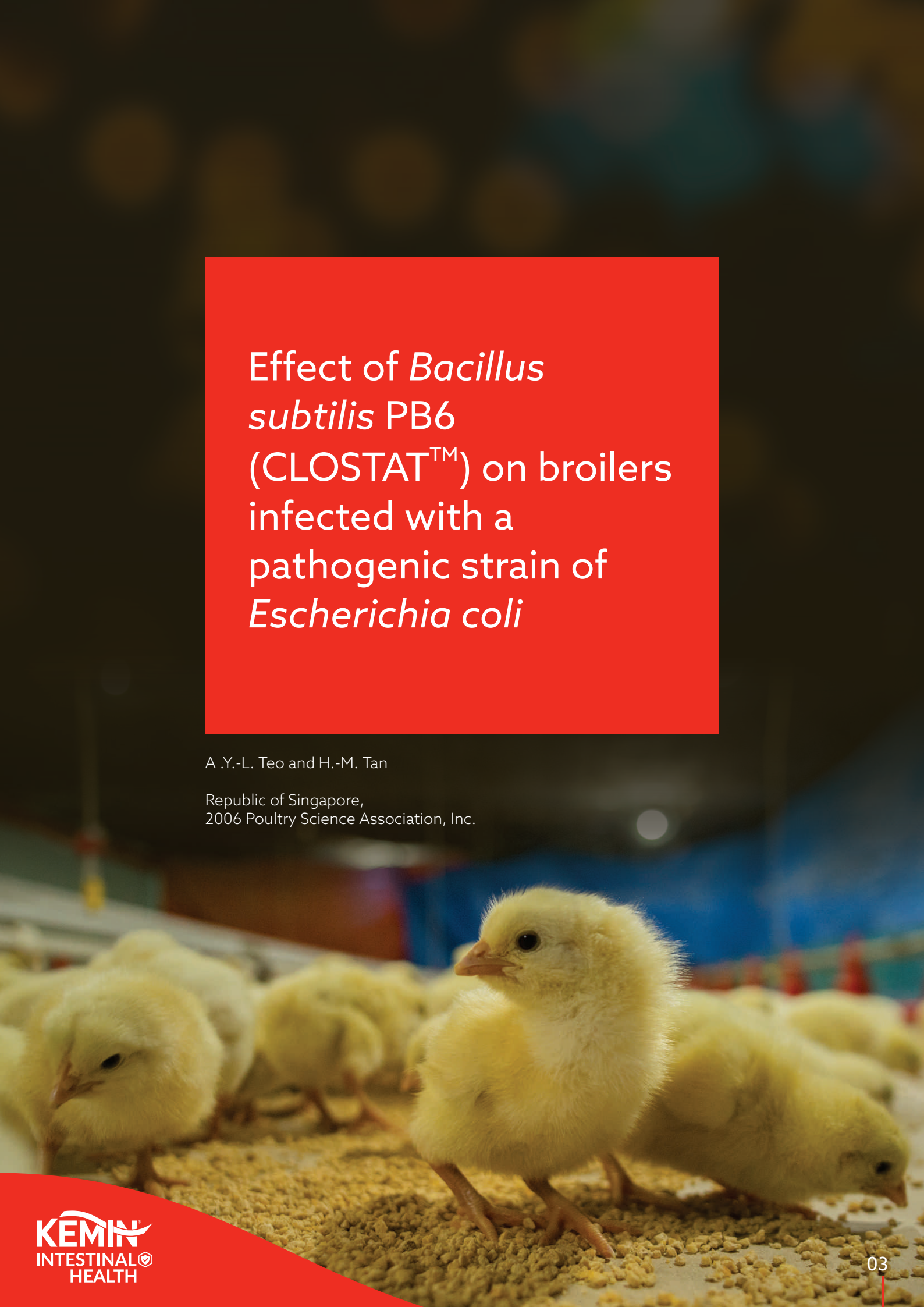
Antimicrobial activity against *Clostridium perfringens* was observed by cells of *Bacillus subtilis* PB6 due to antimicrobial compounds produced.

The thermostable nature of *Bacillus subtilis* PB6 showed no significant decreases in the anti-clostridial activities when heated at 70, 80, 90, 100, and 121°C for 15 min.

Effect of *Bacillus subtilis* PB6 (CLOSTAT™) on broilers infected with a pathogenic strain of *Escherichia coli*

A .Y.-L. Teo and H.-M. Tan

Republic of Singapore,



KEMIN INTESTINAL HEALTH

Effect of *Bacillus subtilis* PB6 (CLOSTAT™) on broilers infected with a pathogenic strain of *Escherichia coli*

The fact that *Bacillus subtilis* PB6 exists as spore cells or vegetative cells is advantageous, as they are heat resistant and tolerant to bile salts. On the other side, there are non-sporulated strains available that are not thermostable to heat as well as not compatible with bile acids produced by poultry birds. Therefore, in the present study, the primary objective was to evaluate the effect of *Bacillus subtilis* PB6 on the performance of broilers. In addition, the protective effect of *Bacillus subtilis* PB6 was evaluated on broilers challenged with a pathogenic strain of *Escherichia coli*.

Six hundred 1-day old broiler chicks were divided randomly into three groups.

- Group 1- Negative Control
- Group 2-An antibiotic control (Zinc bacitracin + Colistin sulfate)
- Group 3- CLOSTAT™

The main groups are divided into two subgroups, A and B. Of the two subgroups, the broilers in subgroup B infected with a pathogenic strain of *E. coli*. Each subgroup had five replicates with twenty birds in each replicate.

Throughout the 42-days, broilers supplemented with *Bacillus subtilis* PB6 saw an increase in the trend of weight gain and improved feed conversion ratio, in both uninfected and infected subgroups, compared with those in the antibiotic and the negative control groups.

Additionally, there was an increased body weight gain associated with the PB6 treatment observed during the first twenty-one days. Uninfected broilers treated with *B. subtilis* resulted in an increased weight gain of 2.1% and 3.9% when compared with antibiotic and negative controls, respectively.

CLOSTAT™ significantly improved both body weight gain and FCR of broiler chickens when compared with those infected with E. coli. CLOSTAT™ also increased the counts of Lactobacillus within the intestinal tracts of the uninfected broilers when compared to the birds in the negative and antibiotic controls. The cell counts of Lactobacillus were similar in infected and uninfected birds fed diets containing CLOSTAT™ and antibiotics.

Table : The Effect of antibiotic, *Bacillus subtilis* PB6, and *Escherichia coli* challenge on broiler performance.

Treatment	BW of 21 d old birds (g)	FCR of 21 d old birds	BW gain from 21 to 42 d old birds (g)	FCR from 21 d to 42 d old birds	BW of 42 d old birds (g)	FCR of 42 d old birds
Negative control						
Unchallenged	912 ± 50^{ab}	1.73 ± 0.11^{ab}	1,199 ± 63^{ab}	2.05 ± 0.21^{a}	2,111 ± 86^{bc}	1.91 ± 0.15^{a}
Challenged	878 ± 26^{b}	1.77 ± 0.05^{a}	1,172 ± 57^{ab}	2.11 ± 0.09^{a}	2,050 ± 54^{c}	1.96 ± 0.04^{a}
Zn Bacitracin + Colistin sulphate – Antibiotic control						
Unchallenged	929 ± 33^{ab}	1.68 ± 0.06^{ab}	1,171 ± 76^{b}	2.06 ± 0.14^{a}	2,100 ± 48^{bc}	1.9 ± 0.05^{ab}
Challenged	913 ± 33^{ab}	1.69 ± 0.08^{ab}	1,206 ± 44^{a}	2.04 ± 0.05^{a}	2,119 ± 52^{ab}	1.9 ± 0.04^{ab}
CLOSTAT™						
Unchallenged	949 ± 38^{a}	1.62 ± 0.08^{b}	1,259 ± 51^{a}	1.95 ± 0.10^{a}	2,208 ± 68^{a}	1.81 ± 0.07^{b}
Challenged	933 ± 24^{a}	1.64 ± 0.08^{b}	1,269 ± 53^{a}	1.93 ± 0.08^{a}	2,202 ± 68^{a}	1.81 ± 0.07^{b}

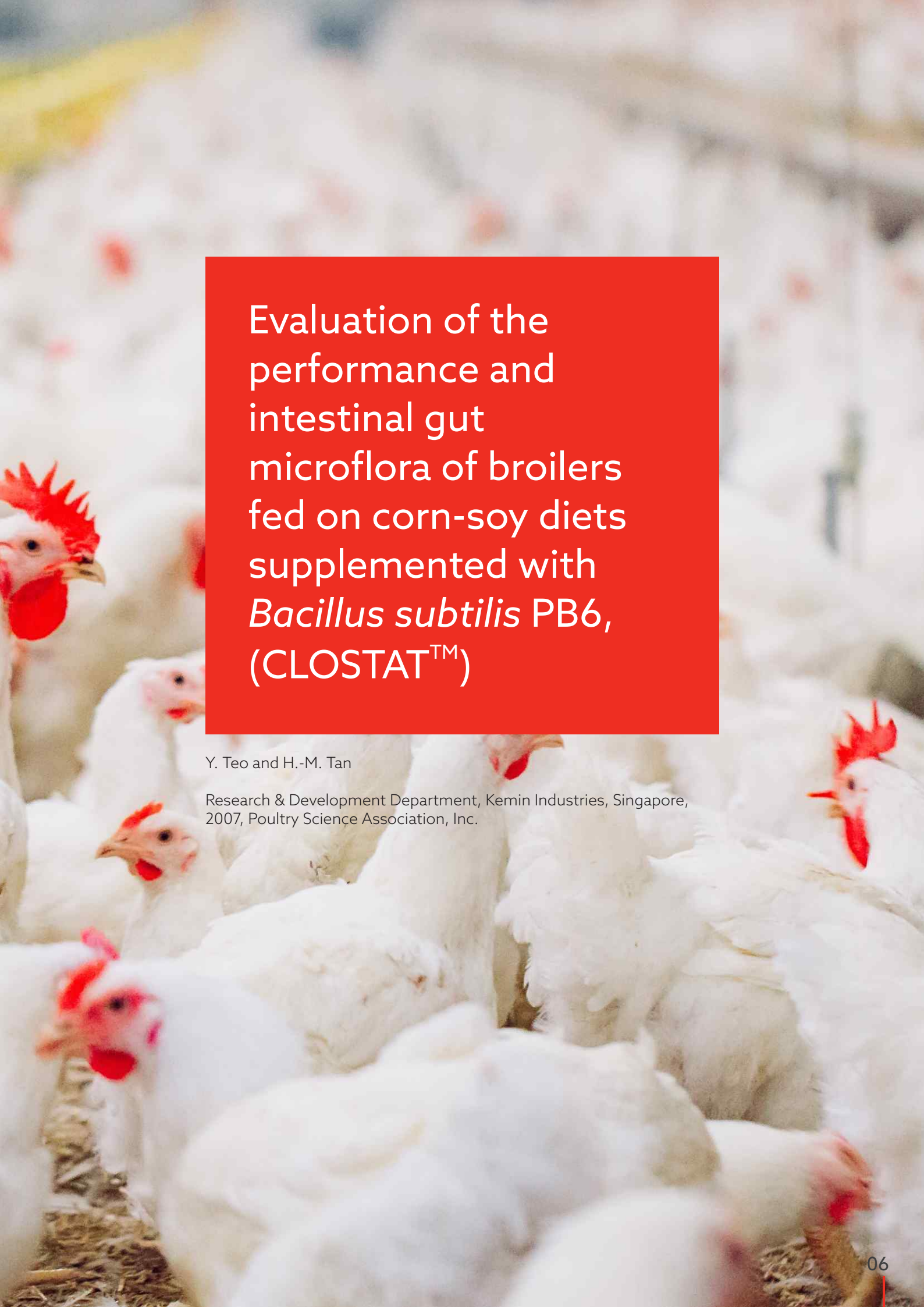
a-c Values with different superscripts within each group, differ significantly (P<0.05)
FCR = feed conversion ratio
Broilers were challenged with *E. coli*.

Summary

Unchallenged group when challenged, showed a shift in Bodyweight and FCR,

- The challenged group treated with CLOSTAT™ showed the highest body weight than the non-challenged negative control group and other challenged groups.

The body weight and FCR of 42-day old infected broilers treated with CLOSTAT™ improved significantly by 6.9% and 15 points, respectively, when compared to those infected with *E. coli* in the negative control group.



Evaluation of the performance and intestinal gut microflora of broilers fed on corn-soy diets supplemented with *Bacillus subtilis* PB6, (CLOSTAT™)

Y. Teo and H.-M. Tan

Research & Development Department, Kemin Industries, Singapore,
2007, Poultry Science Association, Inc.

Evaluation of the performance and intestinal gut microflora of broilers fed on corn-soy diets supplemented with *Bacillus subtilis* PB6, (CLOSTAT™)

Intestinal microflora is recognized as a key element in the health and well being of livestock. The beneficial bacteria as an AGP replacement is slowly gaining momentum and steadily develop as probiotics. Essentially, isolated bacteria will out compete harmful bacteria and also aid in the intestinal health of host animals. One of these probiotics is a strain of *Bacillus subtilis* PB6, a bacterium that inhibits the other gram-positive pathogen bacteria *Clostridium perfringens* responsible for deteriorating changes in the intestine.

In this study, four hundred broiler chickens were assigned to forty pens in a three-tier electrically heated brooder. The broilers were fed with three dietary treatments, with one hundred birds assigned to each treatment course. The treatment courses were as below.

- The negative control (no supplement -ation of any kind),
- Treatment course 1, a dose of *Bacillus subtilis* PB6 at 10^8 cfu/MT, and
- Treatment course 2, a dose of *Bacillus subtilis* PB6 at 10^9 cfu/MT.

At the end of the 42 days, the treatments were assessed for weight gain, feed intake, feed conversion ratio, and mortality (Table 1). Immune response and measurements were done with the analysis of ileal digesta & blood analysis. A high degree of phagocytosis was observed in the *Bacillus subtilis* PB6 supplemented group as compared to the antibiotic and negative control groups. The increase in invitro phagocytosis toward *E. coli* correlates to a decrease in ileal E. coli counts (Table 2).

Table 1: Influence of *Bacillus subtilis* PB6 on the weight gain, feed intake, and FCR of male broilers (1 to 42 d post hatching)

Treatment	Weight gain (g/bird)	Feed intake (g/bird)	FCR(g/g)	Mortality (died/total)
Negative control	2,742[a]	4,388[a]	1.626[a]	9/100
Bacillus subtilis PB6 (10^8cfu/MT)	2,776[a]	4,391[a]	1.619[ab]	4/100
Bacillus subtilis PB6 (10^9cfu/MT)	2,649[b]	4,138[b]	1.599[ab]	3/100
Pooled SEM	29.4	57.5	0.012	–
Significance, P - "value"	0.02	0.003	0.006	–
LSD, P <0.05	83.2	162.7	0.034	–

[a,b]Values with different superscripts in the same column are significantly different (P < 0.05).

Each mean value represents an average of 10 replicate pens of 10 birds each during 1 to 21 d post hatching and 10 replicate pens of 8 birds each during 22 to 42 d post hatching.

Table 2: Influence of *Bacillus subtilis* PB6 on ileal populations (cfu/g) of *Lactobacillus, Bifidobacterium, Clostridium, Escherichia,* and *Salmonella cultures,*

Treatment	*Lactobacillus Species*	*Bifidobacterium Species*	*Clostridium species*	*Escherichia coli*
		(cfu/g)		
Negative control	$2.7 X10^8$	$7.1 X10^5$	$5.9 X10^4$	$8.7 X10^5$
Bacillus subtilis PB6 (10^8cfu/MT)	$1.7 X10^8$	$5.2 X10^5$	$4.8 X10^2$	$1.2 X10^5$
Bacillus subtilis PB6 (10^9cfu/MT)	$5.6 X10^8$	$3.3 X10^5$	$1.5 X10^3$	$1.4 X10^4$
Pooled SEM	0.40	0.39	0.62	0.70
Significance, P - "value"	0.88	0.69	0.10	0.06
LSD, P <0.05	—	—	—	—

Each mean value represents an average of 10 birds. Ileal digesta samples were taken on d 21.
All ileal digesta samples were negative for Salmonella.
Values are not significantly different (P < 0.05).

Table 3: Influence of *Bacillus subtilis* PB6 on immune response in male broilers

Treatment	Phagocytosis
Negative control	6.59
Bacillus subtilis PB6 (10^8cfu/MT)	11.82
Bacillus subtilis PB6 (10^9cfu/MT)	8.85
Pooled SEM	2.81
Significance, P - "value"	0.09
LSD, P <0.05	8.26

Each mean value represents an average of 10 birds. Ileal blood samples were taken on d 21, and heterophils were stained with trypan blue and analyzed by flow cytometry for phagocytic activity toward Escherichia coli.

Summary

Broilers fed on a diet containing both 10^8 and 10^9 cfu/MT of *Bacillus subtilis* PB6 in the finisher phase had a better performance than control feed.
When the cells of *Bacillus subtilis* PB6 were fed to the experimental animals, beneficial microorganisms such as *Lactobacillus species* began to recolonize within the intestinal tract (to restore the normal microbial flora following extensive antibiotic usage or illness).

Compared with negative control, broilers supplemented with *Bacillus subtilis* PB6 feed tend to have lower ileal populations of *Clostridium perfringens* and E. coli.

Effect of *Bacillus subtilis* PB6, a natural probiotic on colon mucosal inflammation and plasma cytokines levels in inflammatory bowel disease

R Selvam, P Maheswari, P Kavitha

Research & Development Department, Kemin Industries, India
Indian Journal of Biochemistry & Biophysics, February 2009.

KEMIN INTESTINAL HEALTH

Effect of *Bacillus subtilis* PB6, a natural probiotic on colon mucosal inflammation and plasma cytokines levels in inflammatory bowel disease

The microbial environment of the intestine plays a major role in the development of inflammatory bowel disease (IBD). Hence, a specific targeting of the microbiota presents an option for therapeutic interventions. The pathophysiology of IBD involves the production of diverse lipid mediators: namely, eicosanoid, lysophospholipids, and platelet-activating factor, in which phospholipase A2 (PLA2) is the key enzyme. Thus, it has been postulated that control of lipid mediators' production by inhibition of PLA2 would be useful for the treatment of IBD.

The present study tests this hypothesis by examining the therapeutic effect of a novel natural probiotic, *Bacillus subtilis* PB6 (ATCC- PTA 6737). *B. subtilis* PB6 secretes surfactin (cyclic lipopeptides), which has anti-bacterial potential. These surfactins inhibit PLA2, a rate-limiting enzyme involved in the arachidonic acid associated inflammatory pathway and downregulates the inflammatory response by regulating the eicosanoid and cytokine pathways. With this concept, an animal trial was conducted in a rat model of 2, 4, 6-trinitrobenzene sulfonic acid (TNBS) induced colitis.

The oral administration of *Bacillus subtilis* PB6 suppressed the colitis that was measured by mortality rate, changes in weight gain, colon morphology, and the levels of plasma cytokines. The animals treated orally with PB6 at 1.5×10^8 cfu/kg thrice daily from day 4 to 10 significantly improve gross pathology of the colon and regain the colon weight to normal ($p< 0.05$), compared to TNBS-induced positive control. The plasma levels of pro-inflammatory cytokines (TNF Alfa,IL-1 beta, IL-6 and IFN gamma) are also significantly lowered ($p<0.05$) and anti-inflammatory cytokine (IL-10 and TGF Alfa) significantly ($p<0.05$) increased after the oral administration of *Bacillus subtilis* PB6 on day 11. The present study supports the concept that PB6 inhibits PLA2 by the secreting surfactin. In a clinical investigation, it is found to be well tolerated by all the healthy volunteers.

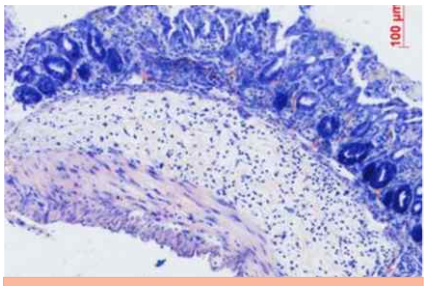
Summary

Bacillus subtilis PB6 reversed the inflammatory changes induced by the use of TNBS, better than the anti-inflammatory drug Prednisolone.

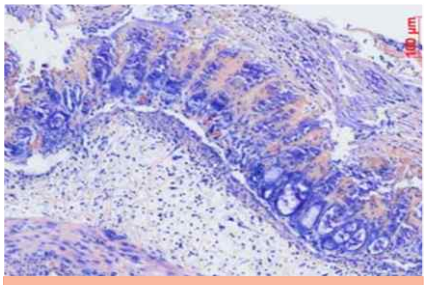
Bacillus subtilis Pb6 administration lowered down the serum level of inflammatory messengers, SAA, and IL, almost similar to the normal or no challenge conditions.

Histomorphology and serum anti-inflammatory activity of CLOSTAT™

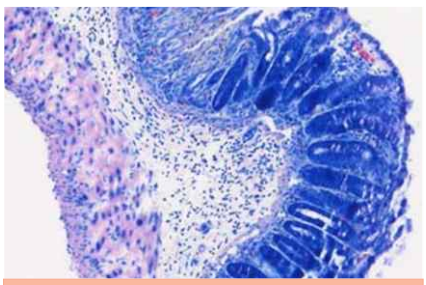
Buffer and no TNBS
No Inflammation & normal villi



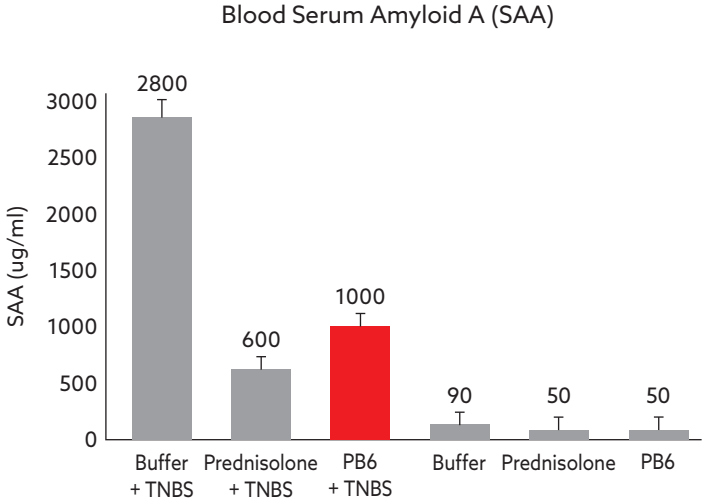
Prednisolone + TNBS
Profuse Inflammation



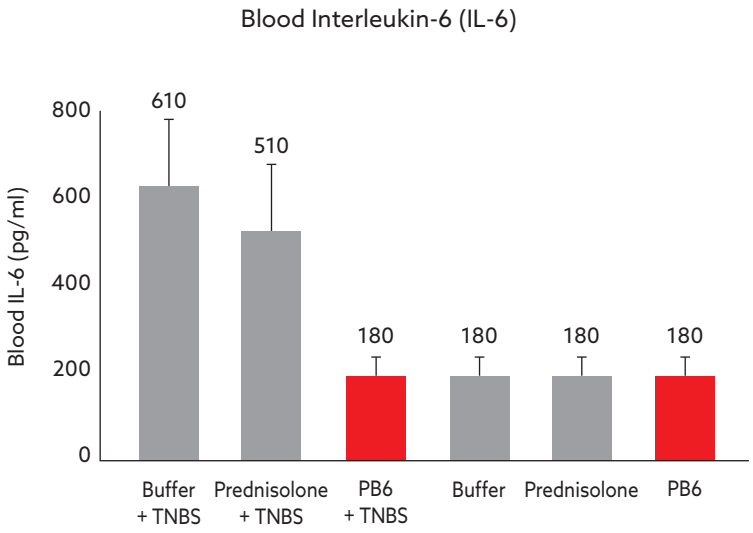
Buffer + TNBS Profuse Inflammation, & distorted villi



Pb6 + TNBS Controlled inflammation, Recovery in villi morphology



Serum Amyloid A is an acute phase protein. Its serum levels increase as a reaction to inflammation. Elevated levels of S AA may indicate the progression of certain diseases.



IL-6 is an interleukin that acts as a pro-inflammatory cytokine (PB6 & Prednisolone group indicated no inflammation)

Effect of dietary supplementation of *Bacillus subtilis* PB6 (CLOSTAT™) on performance, immunity, gut health and carcass traits in

T. Melegy, N.F. Khaled, R. El-Bana, and H. Abdellatif

Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Cairo University,
Journal of American Science, 2011;7(12).

Effect of dietary supplementation of *Bacillus subtilis* PB6 (CLOSTAT™) on performance, immunity, gut health and carcass traits in broilers

Probiotics are bacterial cell preparations that enhance the microbiome of a living subject in a way that stimulates beneficial gut activity and suppresses harmful activities. They have grown in popularity as a movement to ban antibiotic growth promoters (AGPs), which has gained strength due to fears of cross-species antibiotic-resistant pathogens. One such probiotic is *Bacillus subtilis* PB6, commercially named CLOSTAT™.

Bacillus subtilis PB6 has many mechanisms of action, one of which is to inhibit the *Clostridium perfringens* through bactericidal action and also through competitive exclusion, which removes pathogenic bacteria. Nine hundred Cobb-500 broilers were randomly assigned into two experimental groups to assess the effects of the supplementation of PB6 on the performance, intestinal bacterial count, immunity, and carcass traits in broiler chickens. Group 1 was a positive control, and Group 2 was supplemented with CLOSTAT™.

The results showed that the CLOSTAT™ supplemented group had a significantly better final body weight and feed conversion ratio. Microbial analysis of fecal samples was carried out by counting the total bacterial population and clostridial population and observed a marked reduction in both the faeces of the experimental group when compared to the control group. Dietary supplementation with *Bacillus subtilis* PB6 could improve the performance, immune response, dressing percent and have an antimicrobial effect against *Clostridium perfringens*.

Table 1: Effect of dietary supplementation of CLOSTAT™ on broiler performance

Parameter	Control group[1]	CLOSTAT™ supplemented group	P value
Initial body weight (g)	48.64± 2.1	48.56 ±1.9	0.1800
Final body weight (g)	1810.93 ± 47.8	1902.23 ± 20.2	0.0020
Body weight gain (g)	1762.29 ± 44.4	1853.67 ± 17.2	0.0010
Total feed consumed/chick (g)	3421.43 ± 96.8	3432.85 ± 59.6	0.7310
FCR (Feed: gain)	1.94± 0.025	1.85± 0.024	0.0004
Mortality %	3.56 ± 0.31	2.67 ±0.22	0.0220

[1]Control group was fed with the basal diet.

CLOSTAT™ supplemented group was fed the basal diet fortified by 500 g CLOSTAT™ 2×10^7cfu/g/Ton of feed. Data is presented as mean ± SD for 6 replicates of 75 chicks per pen in each group. The level of significance was set at ($P< 0.05$).

Table 2: Effect of dietary supplementation of CLOSTAT™ on total aerobic bacterial count (cfu/g) in fecal samples collected from broilers at different times.

Age	Control group*	CLOSTAT™ supplemented group	P value
21 d	$1.20 \times 10^9 \pm 8.3$	$4.32 \times 10^8 \pm 3.2$	0.0410
28 d	$1.68 \times 10^9 \pm 8.7$	$4.44 \times 10^8 \pm 2.7$	0.0060
35 d	$1.86 \times 10^9 \pm 6.1$	$4.39 \times 10^8 \pm 2.9$	0.0004
40 d	$1.60 \times 10^9 \pm 4.8$	$4.28 \times 10^8 \pm 2.9$	0.0005

*Control group was fed with the basal diet.

CLOSTAT™ supplemented group was fed the basal diet fortified by 500 g CLOSTAT™ 2×10^7cfu/g/Ton of feed. Data is presented as mean ± SD for 6 replicates of 75 chicks per pen in each group. The level of significance was set at ($P < 0.05$).

Summary

Dietary supplementation of *Bacillus subtilis* PB6 improved the performance, dressing yield, and immune response.

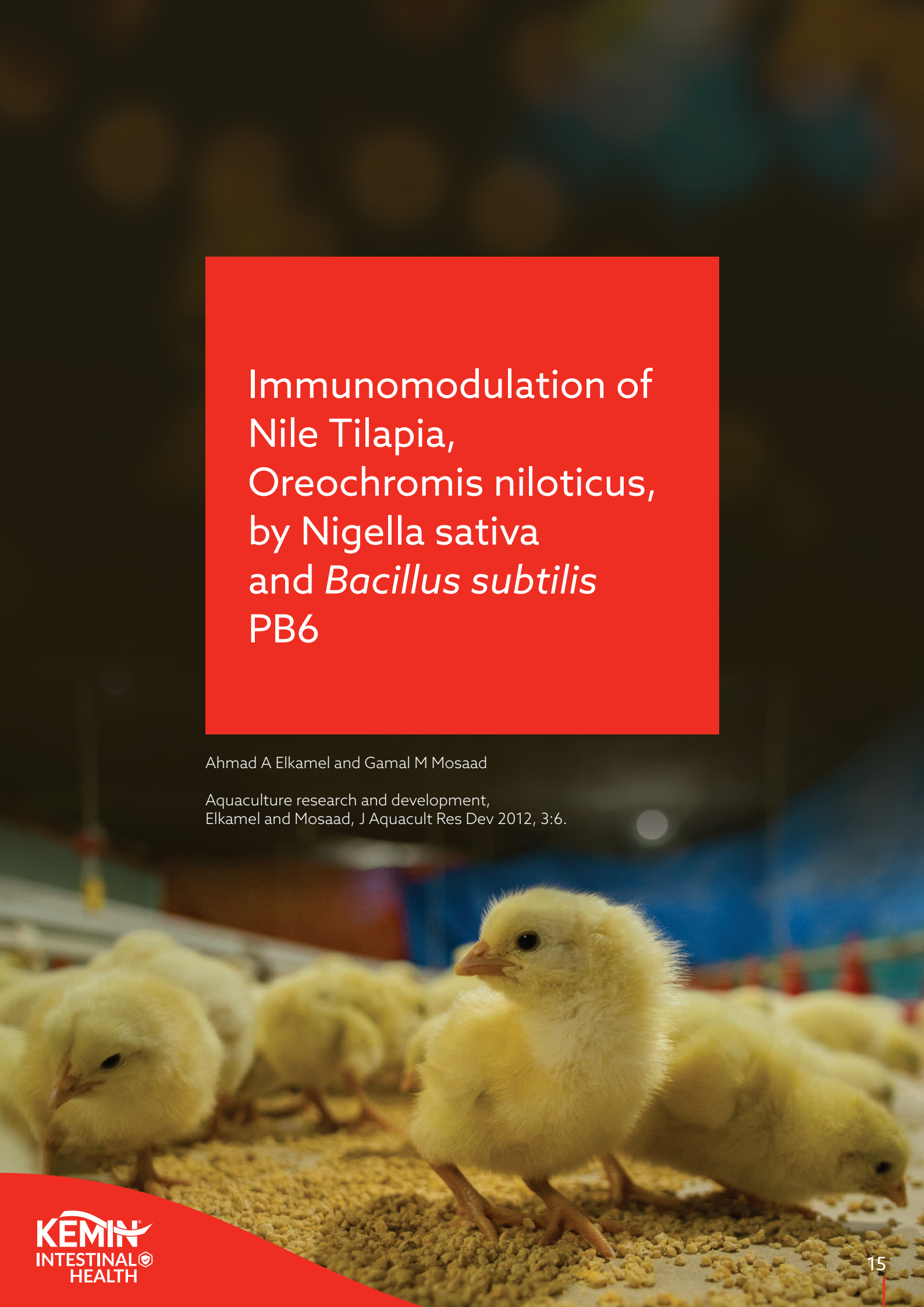
Bacillus subtilis PB6 has an antimicrobial effect against *Clostridium perfringens* in broiler chicks and maintains the overall healthy gut microflora.

Mortality was less over the control, which may be due to the overall balanced microbiota, better gut health, and gut integrity.

Immunomodulation of Nile Tilapia, Oreochromis niloticus, by Nigella sativa and *Bacillus subtilis* PB6

Ahmad A Elkamel and Gamal M Mosaad

Aquaculture research and development,
Elkamel and Mosaad, J Aquacult Res Dev 2012, 3:6.



KEMIN INTESTINAL HEALTH

Immunomodulation of Nile Tilapia, Oreochromis niloticus, by Nigella sativa and *Bacillus subtilis* PB6

The aim of this study is to investigate the modulation of the immune system of Nile tilapia, Oreochromis niloticus, by the single or combined action of black cumin seeds, Nigella sativa, and *Bacillus subtilis* PB6 (CLOSTAT™) as feed additives.

In the current experiment four diet regimes were formulated and used to feed fish for 30 successive days,

- basic (control), without any feed additives,
- basic diet with 0.04% of CLOSTAT™,
- basic diet with 3.0% of Nigella sativa,
- basic diet with 0.04% CLOSTAT™ and 3.0% Nigella sativa.

Half of the fish were used to investigate some of the immune parameters as serum globulins, white blood cell counts, and phagocytic activities and phagocytic indices. The other half of fish was subjected to infection challenge with Aeromonas hydrophila to investigate the disease resistance ability of the fish received the feeding additives.

Results showed that the serum globulins have significantly increased in fish received the combination diet, while white blood cells have significantly increased in fish fed with nigella or combination ration.

Phagocytic activities and indices of the fish fed the combination ration were significantly higher than those of the control groups. The mortality rate of fish fed with nigella or combination ration and challenged with A. hydrophila were significantly lower than those that received the basic diet. The current study clearly demonstrated that black cumin, CLOSTAT™ or both can be used to modulate the immune system of Nile tilapia to the favor of resistance to diseases.

Effect of four diet regimes, used to feed Nile tilapia, Oreochromis niloticus, on the white blood cells (WBCs) count, globulin level, phagocytic activity, phagocytic index, and cumulative mortality per- cent of fish challenged with Aeromonas hydrophila.

Table: Effects of CLOSTAT™ & Immunomodulation

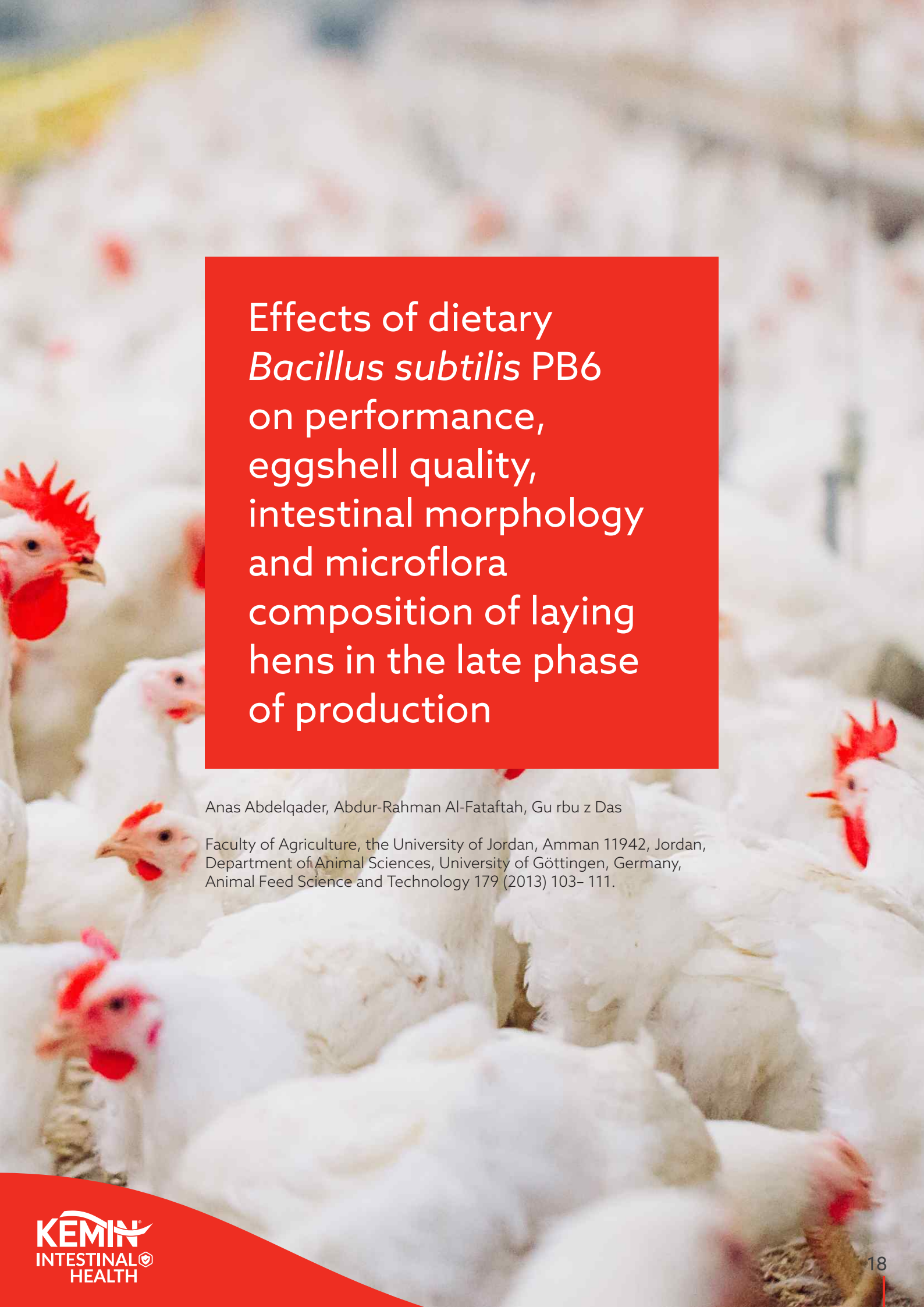
	Basic (Control)	CLOSTAT™	Nigella	Combination
WBCs	36.2 ± 3.86^{a}	39.41 ± 4.78^{ab}	40.22 ± 4.41^{b}	44.53 ± 5.11^{c}
Globulin (g/dl)	4.18 ± 1.01^{a}	4.63 ± 1.20^{ab}	4.88 ± 1.74^{ab}	5.89 ± 1.05^{b}
Phagocytic activity	24.4 ± 2.10^{a}	26.20 ± 1.89^{a}	30.74 ± 2.33^{b}	37.35 ± 3.21^{c}
Phagocytic index	1.01 ± 0.02^{a}	1.02 ± 0.12^{a}	1.09 ± 0.25^{ab}	1.21 ± 0.47^{b}

Summary

Stimulation of the immune system of Nile tilapia as a result of feeding of black cumin or combination diets have positively impacted the immune resistance of fish.

WBC numbers, globulin proteins and the phagocytic activities of fish phagocytes indicated that the that dietary supplementation of CLOSTAT™, black cumin, and combination of these two enhanced the overall immune response of Nile tilapia.

The modulation of the fish immunity has greatly enhanced the resistance of challenged fish to A. hydrophila. Indicated by the significant decrease in mortalities in fish received the Nigella or combination diets.



Effects of dietary *Bacillus subtilis* PB6 on performance, eggshell quality, intestinal morphology and microflora composition of laying hens in the late phase of production

Anas Abdelqader, Abdur-Rahman Al-Fataftah, Gu rbu z Das

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Department of Animal Sciences, University of Göttingen, Germany,
Animal Feed Science and Technology 179 (2013) 103– 111.

KEMIN INTESTINAL HEALTH

Effects of dietary *Bacillus subtilis* PB6 on performance, eggshell quality, intestinal morphology and microflora composition of laying hens in the late phase of production

Eggshell quality is an important parameter in poultry industry, influencing the economic profitability. Strength of eggshell is a vital defense line against physical damage and consequently penetration of pathogenic microbes. Eggshell quality is affected by many factors such as diseases, nutritional status, heat stress, and age which leads to great economic losses.

One of the main concerns is a decrease in eggshell quality as the hen ages, due to an increase in egg weight without an increase in the amount of calcium carbonate deposited in the shell. The gut microflora plays a major protective function to keep the integrity of the intestinal mucosa, which influence the Calcium absorption

This study was performed to investigate the potential improvement of laying hen's performance and eggshell quality in the late phase of production by dietary inclusion of *Bacillus subtilis* PB6. Eighty Lohmann White hens were distributed into two treatment groups with five replicates per treatment and four hens per replicate. The treatment groups were given different diets based around a basal diet. The control group had only the basal diet. Treatment group two was given a basal diet plus 2.3 $X10^{11}$ cfu/MT *Bacillus subtilis* PB6.

The results from the trial show that dietary supplementation of *Bacillus subtilis* PB6, improved feed conversion ratio, egg performance, eggshell quality, and calcium retention compared with the control group.

Summary

Egg production, egg weight, egg mass and feed conversion were improved with dietary supplementation of *Bacillus subtilis* PB6.

Control group excreted more Calcium than *Bacillus subtilis* PB6 fed groups, however total Ca intake was similar among all experimental groups, apparent Ca retention was improved in *Bacillus subtilis* PB6 fed groups compared with the control.

The percentage of unmarketable eggs was also lower in treatment group. Tibia density, ash, and Ca content increased in the *Bacillus subtilis* PB6 treatment group as well.

Table 1: Effects of dietary treatments on laying hens' productive performance and eggshell quality

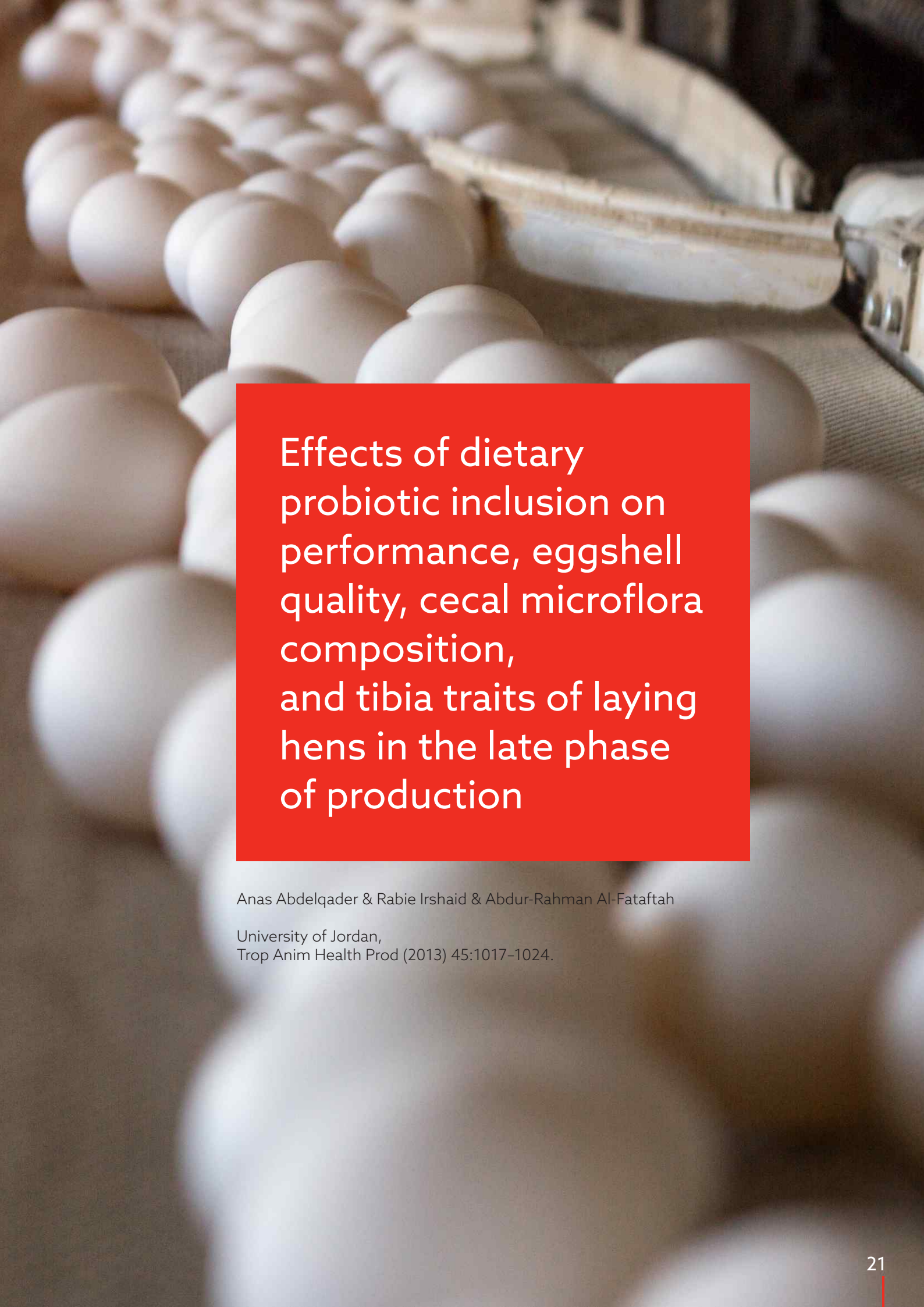
Parameters	Control	*Bacillus subtilis* PB6
Productive performance		
Body weight 64-week of age (g)	1700	1699
Body weight 75-week of age (g)	1714	1715
Egg production(%)	70.0^{z}	74.4^{x}
Egg weight (g)	61.0^{z}	64.8x
Egg mass (g/hen/day)	42.1^{y}	48.0^{x}
Feed intake (g/d)	110.8	110.2
Feed conversion (kg/kg)	2.8^{y}	2.3x
Eggshell quality traits		
Eggshell weight (% of egg weight)	6.9^{z}	8.4^{y}
Eggshell thickness (mm)	0.33^{z}	0.36^{y}
Eggshell density (mg/cm^{2})	72.2^{z}	75.5^{x}
Unmarketable eggs (%)^{b}	8.4^{x}	3.5^{y}

^{x,y,z} means in the same row with different letters are significantly different at P<0.05; NS: not significant (P>0.05).

Table 2: Effects of dietary treatments on laying hen's apparent calcium retention, tibia traits, and pH of excreta, intestinal digesta, and cecal content

Parameters	Control	*Bacillus subtilis* PB6
Calcium		
Calcium intake (g/d)	4.80	4.77
Calcium excretion (g/d)	3.13^{x}	2.03^{y}
Calcium retention (g/d)	1.67^{y}	2.73^{x}
Calcium in eggshell (g)	1.73^{z}	2.07^{y}
Calcium balance (g/d)	-0.07^{y}	0.67^{x}
Tibia traits		
Density (g/cm^{3})	1.18^{z}	1.25^{yz}
Ash (mg/g)	536.0^{y}	550.8^{y}
Calcium (mg/g of ash)	313.1^{z}	335.7^{yz}
pH		
Excreta	7.0^{x}	6.9^{x}
Intestinal digesta	6.9^{x}	6.7^{xy}
Cecal content	7.1^{x}	6.8^{x}

^{x,y,z} means in the same row with different letters are significantly different at P<0.05; NS: not significant (P>0.05).



Effects of dietary probiotic inclusion on performance, eggshell quality, cecal microflora composition, and tibia traits of laying hens in the late phase of production

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University of Jordan,
Trop Anim Health Prod (2013) 45:1017–1024.

Effects of dietary probiotic inclusion on performance, eggshell quality, cecal microflora composition, and tibia traits of laying hens in the late phase of production

The integrity of the eggshell is a vital natural defense mechanism that protects the egg from physical damage and microbial invasion. Eggshell strength impairs the late phase of egg production, and there is an increase in incidences of cracked egg percentage. Aged layers are less responsive to calcium than younger ones. The gut microflora has a protective function to keep the intestinal integrity constant and adding beneficial bacteria to the diet helps to recover the intestinal integrity. *Bacillus subtilis* PB6 was used extensively in this regard. The primary objective of those applications is investigating the use of probiotics to improve the performance, eggshell quality, cecal microflora composition, and tibia traits of laying hens in the late phase of production.

To investigate the use of probiotics, ninety-six white laying hens were split into three treatment groups and fed with three different diets as below.

- Basal diet
- Basal diet plus 2.3 X 10^{11} cfu / MT of *Bacillus subtilis* PB6
- Basal diet plus 4.6 X 10^{11} cfu / MT of *Bacillus subtilis* PB6

The hens were fed with the diet for ten weeks, and the assessment metrics were egg production, egg weight, egg mass, eggshell weight, and eggshell thickness.

The results showed that across all those metrics, CLOSTAT™ exhibited the maximum statistically significant increase than any treatment group and has the lowest number of average unmarketable eggs percentage wise with the largest increase in tibia weight, density, and ash content.

To conclude, it is possible to improve egg performance and eggshell quality by dietary inclusion of *Bacillus subtilis* PB6.

Summary

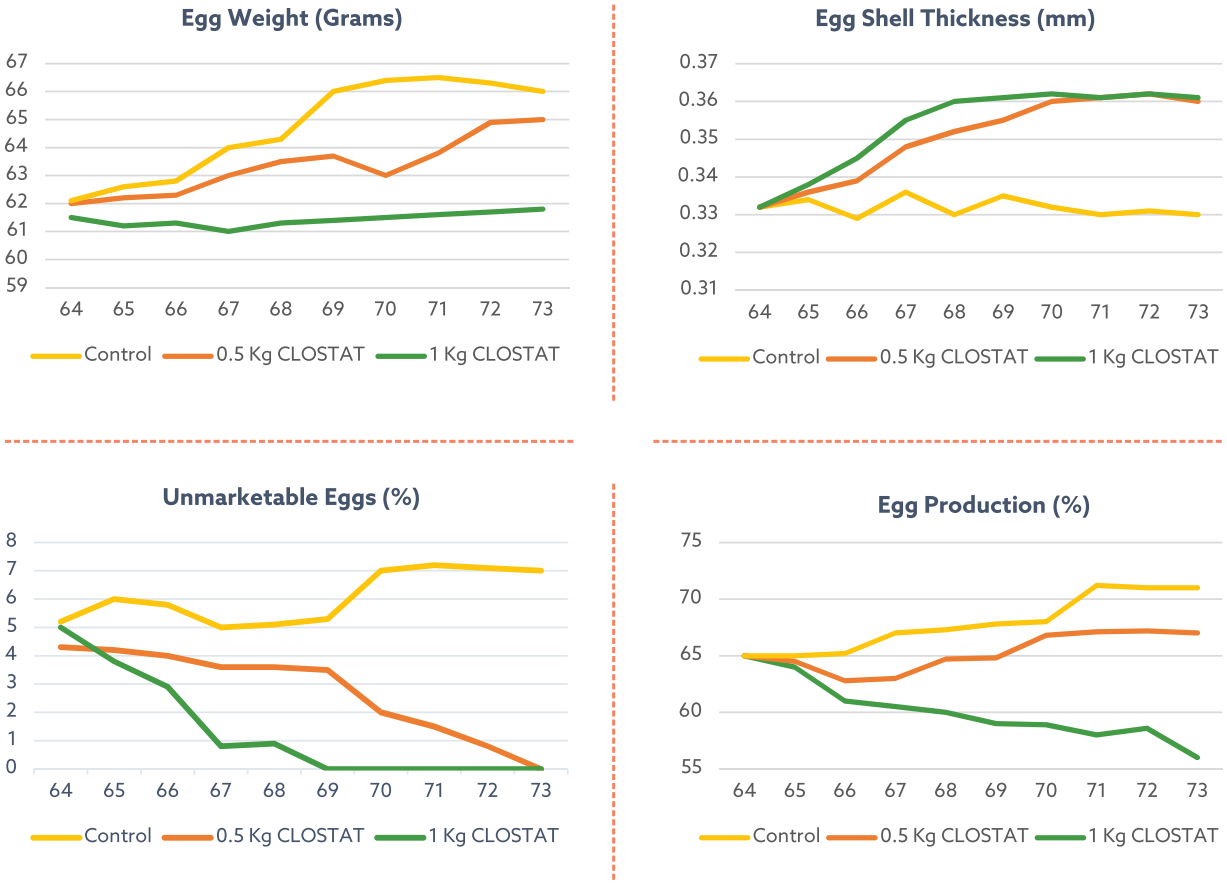
At the late phase of production, the laying hens limit nutrient absorption efficiency due to poor gut health. CLOSTAT™ has been involved directly in the stabilization of the intestinal microflora ecosystem and recovery of gut health and mucosal integrity, thus enhances the absorption.

The positive effects of CLOSTAT™ on eggshell quality attributes to the enhancement of Calcium absorption and availability due to microbial stability. CLOSTAT™ improved gut health, which directly improved birds' health and performance.

CLOSTAT™ increased fermentation rate and short-chain fatty acids (SCFA). This SCFA stimulates intestinal epithelial cell proliferation and villus height, which increases the absorption efficiency.

The late phase of production with *Bacillus subtilis* PB6 significantly improved egg production, egg weight, egg mass, eggshell thickness, eggshell weight, and eggshell density and reduced the percentage of unmarketable eggs.

Results for illustration



Graphs illustrating different improved egg quality parameters

Bacillus subtilis PB6 based probiotic CLOSTAT™ improves intestinal morphology and microbiological status of broiler chickens under *Clostridium perfringens* challenge.

Abudabos, A.M., A.H. Alyemni and Marsha. B.A. Al, 2013.

Department of Animal Production, College of Food and Agricultural Sciences, King Saud University,
International Journal of Agriculture Biology, 15: 978–982.

KEMIN INTESTINAL HEALTH

Bacillus subtilis PB6 based probiotic CLOSTAT™ improves intestinal morphology and microbiological status of broiler chickens under *Clostridium perfringens* challenge.

CLOSTAT™ provides a strong and flexible alternative against growth promoting antibiotic feed additives. This is in current trends of livestock production in general and poultry production in specific. In order to put an end to the use of AGPs and increase the use of non-antibiotic feed additives, such as probiotics, out of which CLOSTAT™ is a choice.

In this study, one hundred Ross 308 chickens were obtained and separated into four groups split between twenty cages. Each group was given a different treatment course of feed with a different composition.

- T1, the control, was given an unmedicated diet of corn-soybean meal mash based on a typical starter and finisher diet cycle.
- T2, the infected control was given the same diet but with the addition of *Clostridium perfringens* as a contaminated meal.
- T3, was the same as T2 with an addition of 100 grams/MT of Enramycin, a growth promoting antibiotic.
- T4, was the same as infected control with an addition of 1 X 10^9 cfu/MT of *Bacillus subtilis* PB6 present in CLOSTAT™.

On day 16 and on day 30, five birds from each treatment group were selected. The subjects were euthanized before having their small intestines weighed, measured, and then dissected. It was found that in the birds treated with CLOSTAT™, the morphological status of the small intestine and the cumulative FCR value were improved numerically in Treatment Group 4. Treatment Group 4 also had a higher level of livability rate than all but the control group. Levels of *Clostridium perfringens* were considerably lower inside the gut of T4 relative to the other groups.

Table: Bodyweight gain (BWG), feed Intake (FI) and feed conversion ratio (FCR) of broiler chickens given experimental diets at different ages,

	Treatment					
	Normal Control	IC + Enramycin 100 gram	Infective Control (IC)	IC + CLOSTAT™ 1 X 10^9 cfu/MT	SEM	P
	Cumulative					
Intestine weight (g/cm)	0.28	0.21	0.27	0.24	±0.02	NS
Ileal Villus height (µm)	4509	3765	3804	4053	±234.9	NS
BWG (g)	1345.3	1312.3	1384.6	1340.9	±35.5	NS
Feed (g)	2127.4	2114.5	2084.8	1998.3	±46.1	NS
FCR (g:g)	1.583	1.611	1.511	1.492	±0.03	0.007
Livability (%)	100	92	92	96	±6.0	NS
BPI	297	262	295	302	±20	NS

IC – Infected control, BPI- Broiler Performance Index

Summary

Bacillus subitlis PB6 was able to reverse the changes in challenged diet, like damage to mucosa due to inflammation and prevent loss of nutrient absorption.

Antibiotic supplementation caused low villi height; well explained by the suppressing effect of antibiotic on the beneficial bacteria in the gut, *Lactobacillus* and *Bifidobacteria*.

CLOSTAT™ reduced significantly the bacterial count of *Clostridium perfringens* & improved the morphological status of the small intestine.

The effect of the probiotics *Bacillus subtilis* PB6 on the selected indicators of the table eggs quality, fat and cholesterol

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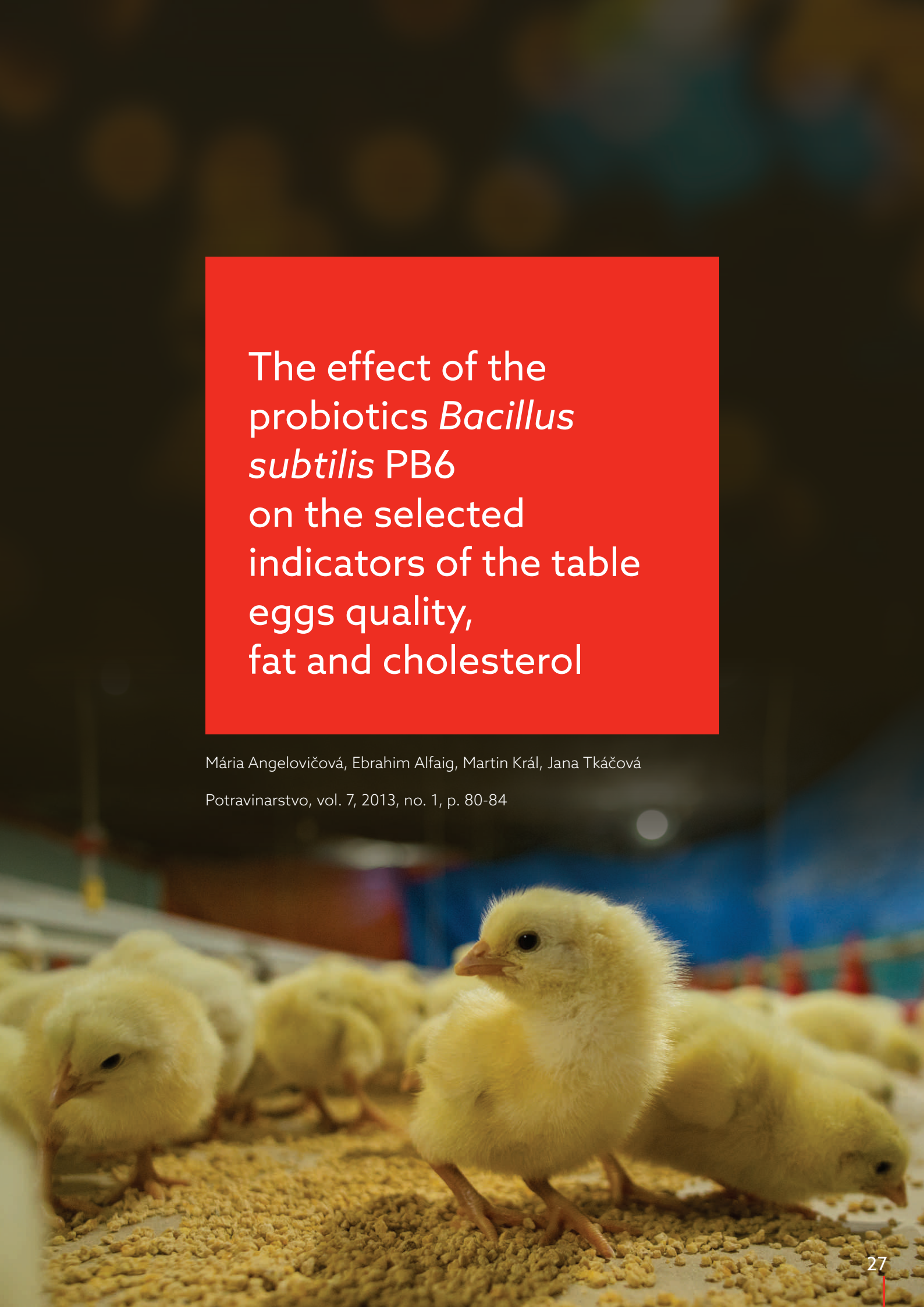
Potravinarstvo, vol. 7, 2013, no. 1, p. 80-84



The effect of the probiotics *Bacillus subtilis* PB6 on the selected indicators of the table eggs quality, fat and cholesterol

Feed additives target animals intended for food production. The probiotics used as a feed additive increases the microbial equilibrium of the gut. This study aimed to determine the effect of dietary probiotics *Bacillus subtilis* PB6 on egg weight, egg mass weight, egg fat content, and cholesterol content in egg yolk in laying hens (ISA Brown) during two experiments.

The probiotics were supplied to the laying hens for 42 days as a preparation period before egg sample collection. A total of 36 ISA Brown laying hens were divided into two treatment groups.

- The control group laying hens were fed a basal diet without probiotics,
- Treatment group was supplemented with bacteria *Bacillus subtilis* PB6 min $2.3 X10^{11}$ cfu/MT.

The egg samples for the 1st and 2nd experiments were collected six days after the hens reached the age of 34 and 61 weeks, respectively.

Bacillus subtilis PB6 supplementation significantly ($P<0.05$) increased the cholesterol content in egg yolk expressed as g/pc.

Summary

Supplementation of CLOSTAT™ saw a tendency to increase the weight gain of eggs as well as egg mass.

CLOSTAT™ has increased the fat content in the egg and decrease the cholesterol content of the table eggs.

Effects of direct-fed probiotics on broiler performance and susceptibility to oral Salmonella challenge

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Journal of Food, Agriculture & Environment Vol.12(2):30-34.2014.

Effects of direct-fed probiotics on broiler performance and susceptibility to oral Salmonella challenge

Salmonella enterica serotypes, Enteritidis, and Typhimurium are the most common serotypes causing salmonellosis in broiler chicken leading to food safety issues. Chicken challenged with Salmonella shows a significant decline in growth performance. Historically, Antibiotic Growth Promoter supplements to poultry feed combat potentially pathogenic organisms. However, given the recent rise in drug-resistant strains of pathogenic bacteria and the dangers of a cross-species pathogen with drug-resistant properties, there has been a broad push towards a new measure called Probiotics. Probiotics are naturally occurring strains of competing bacteria that have specific action and remove harmful bacteria like *Clostridium perfringens* or Salmonella through different mechanisms of action, including competitive exclusion.

This study intends to examine the usage of *Bacillus subtilis* PB6, a probiotic strain of bacteria known as CLOSTAT™, as an alternative to in-feed antibiotics on broiler performance during and after being challenged with Salmonella. The birds were treated with four different treatment courses.

- First group: a positive control unmedicated and unchallenged by Salmonella,
- The second group: an infected control unmedicated and challenged by Salmonella,
- The third group: challenged & treated with a probiotic known as *Bacillus toyonensis* 1g/kg diet, and
- The fourth group: challenged & treated with the probiotic known as CLOSTAT™ 1g/kg diet.

The results of the study were that the chickens that received CLOSTAT™ were able to resist and eliminate the negative effects of the bacterial challenge as well as improve the cumulative feed conversion ratio. The results suggest that COSTAT™ had a positive influence on the performance of broiler chickens and could serve as an effective alternative to AGPs in the diet of broiler chickens.

Table: Feed Intake, Body weight gain and feed conversation ratio of broiler chickens given experimental diets (d 0 to 35)

Treatment	Feed (g)	Body weight gain (g)	Feed conversion (g:g)
Control	3107.2[a]	1843.2[a]	1.668[b]
Infected Control	2891.2[b]	1658.9[c]	1.768[a]
Challenged + *Bacillus toyonensis*	2765.7[b]	1696.2[bc]	1.632[bc]
Challenged +CLOSTAT™	2831.7[b]	1765.2[abc]	1.605[bc]
SEM±	48.6	41.41	0.032
P value	***	*	**

Each mean is based on 10 replicates of birds,
[abc] Means in the column with different superscripts differ significantly. *P<0.005, *P<0.001, ***<P<0.0001.

Summary

CLOSTAT™ was able to eliminate the negative effects of the bacterial challenge, as the poor performance was associated with broilers infected with a pathogenic strain of Salmonella.

CLOSTAT™ under the condition of this trial had a positive influence on the performance of broiler chickens and could serve as an alternative to AGPs in broiler diet.

Cumulative performance results show bird fed with CLOSTAT™ had better ability to convert the feed consumed, better than the control as well as infected control and higher profitability.

Effect of different probiotics on breast quality characteristics of broilers under Salmonella challenge

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Italian Journal of Animal Science 2014; volume 13:3189

KEMIN INTESTINAL HEALTH

Effect of different probiotics on breast quality characteristics of broilers under Salmonella challenge

In modern world there is increase in a concern about development of antibiotic resistant pathogenic bacteria in livestock. The concern is that these antibiotic resistant bacteria could in theory jump across species. The drug resistant properties of pathogen makes it impossible to effectively treat in humans, thus an alternative is required. One that can protect livestock and fight the dangerous pathogens like *Clostridium perfringens* and Salmonella pathogenic bacteria that exist in the intestines and gut of the animal.

Probiotics have been shown to prevent the colonization of the gut by pathogenic bacteria through the different mechanisms of action including competitive exclusion. Additionally, other studies have shown that probiotics improve the performance of broiler chickens by promoting a higher rate of growth & increasing the feed conversion efficiency.

To carry out this study two hundred, one-day-old Cobb 500 chicks were allocated in four experimental treatments for forty-two days. Ten cages of birds received four courses of treatments.

- T1, positive control
- T2, negative control (NC)
- T3,supplemented with antibiotics (Neoxywal),
- T4, supplemented with probiotic CLOSTAT™

Overall, the results showed that probiotic supplementation CLOSTAT™ has improved the parameters of the muscle characteristics, also reduced the extent of the destruction of muscle fibers that are caused by homogenization.

Summary

CLOSTAT™ group showed minimum cooking loss %(CL). Cooking loss is directly correlated with loss of juiciness during the cooking. Least cooking loss is a property of best meat quality & least shrinkage.

CLOSTAT™ group showed minimum damage to myofibrils, myofibrillar fragmentation is the extent of destruction of myofibrils caused by homogenization.

Higher Water holding capacity (WHC) refers to CLOSTAT™ group it is an indication of better ability of muscles to bind water under specific conditions. Better WHC% means lesser CL%.

Table 1: Physical properties of breast muscle meat of broilers fed experimental diets

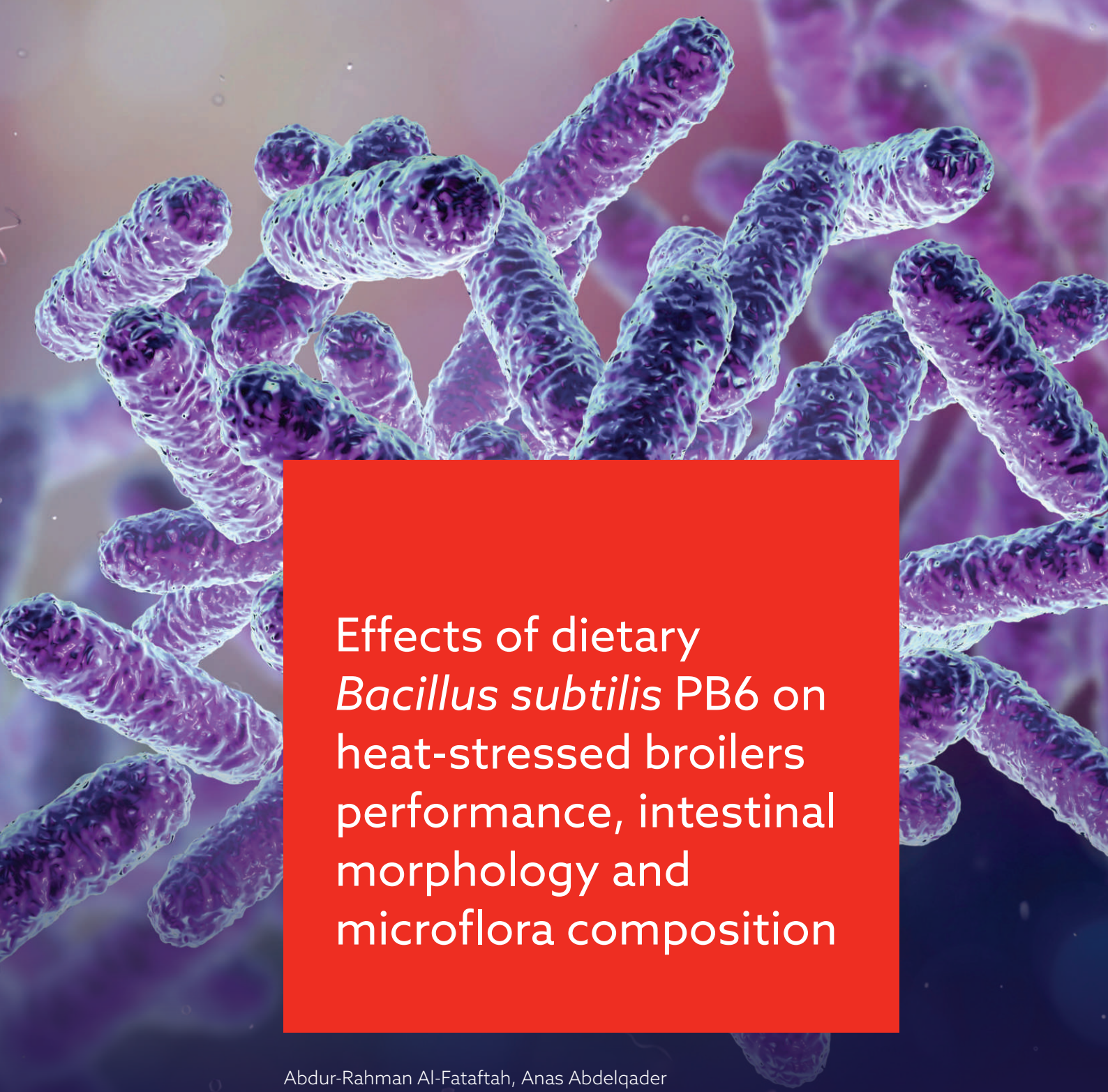
	Cooking Loss % (CL)	Drip Loss %	Water Holding Capacity% (WHC)	Myofibrillar fragmentation %	Shearing force, kgf
Group					
T1 –Positive Control	18.7[C]	1.78	36.5	112.2[a]	2.71
T2 –Negative Control	23.3[b]	1.86	33.5	101.1[a]	2.81
T3 –NC + Neoxywal	33.8[a]	2.25	37.4	101.2[a]	3.31
T4 –NC + CLOSTAT™	18.2	2.10	38.1	51.1[b]	3.20
Probability					
SEM	1.19				
Significance	***	ns	ns	ns***	ns

[abc] means in the column with differ significant.

Table 2: Texture profile analysis in breast meat of broilers

	Cooking Loss %	Drip Loss %	Water Holding Capacity %	Myofibrillar fragmentation %
Group				
T1 –Positive Control	18.7[C]	1.78	36.5	112.2a
T2 –Negative Control	23.3[b]	1.86	33.5	101.1a
T3 –NC + Neoxywal	33.8[a]	2.25	37.4	101.2a
T4 –NC + CLOSTAT™	18.2[c]	2.10	38.1	51.1 b
Probability				
SEM	1.19			
Significance	***	ns	Ns	ns***

[abc] means in the column with differ significant.



Effects of dietary *Bacillus subtilis* PB6 on heat-stressed broilers performance, intestinal morphology and microflora composition

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Department of Animal Production, Faculty of Agriculture, The University of Jordan, Animal Feed Science and Technology 198 (2014) 279–285.

Effects of dietary *Bacillus subtilis* PB6 on heat-stressed broilers performance, intestinal morphology and microflora composition

Heat stress is a problem for all forms of life in tropical or sub-tropical regions across the globe. From India to the Middle East to Central America heat stress is regularly responsible for the deaths of livestock and economical damage. Heat stress inhibits the expression of livestock's maximum genetic potential and can result in poor performance as well as high mortality. Given the unique physiological challenges faced by broiler chickens when they encounter conditions outside of their thermoneutral zone it then becomes clear that a mitigation measure is needed.

One such mitigation method is the supplementation of probiotics to improve thermotolerance and to help in the recovery of intestinal walls and cells damaged by exposure to high heat conditions. However, the data in this area are somewhat lacking. The objective of this study was to assess the potential of Bacillus subtilis PB6 to counter the negative effects of heat stress on chicken performance, intestinal villus-crypt system, and intestinal microbiome balance.

One-day old Hubbard male broilers (480 Nos) were randomly distributed into four treatment groups separated into a two by two factorial arrangement based on diet and temperature.

- Group 1, had a normal diet and thermoneutral conditions,
- Group 2, had a normal diet and lived in heat stress conditions,
- Group 3, had diet supplemented with *B. subtilis* PB6 in thermoneutral condition, and
- Group 4, had diet supplemented with *B. subtilis* PB6 in heat stress conditions.

As broilers are more susceptible to heat stress than are slower growing domestic fowl, the present study confirmed the adverse effects of heat stress on broilers performance, intestinal epithelial morphology and intestinal microflora balance. The results of the study showed that *Bacillus subtilis* PB6 reversed and decreased feed to gain ratio, mortality, and viable counts of Clostridium and Coliform bacteria.

Bacillus subtilis PB6 had additional positive effects in heat-stressed broilers as revealed by improvement in the areas of final body weight, average daily gain, villus height, villus surface area, epithelial intestinal cell area and beneficial intestinal bacteria counts. Broiler thermotolerance was not improved but conditions arising from exceeding that tolerance were mitigated.

The inclusion of *B. subtilis* PB6 was effective, partially in overcoming the adverse effects of higher than thermoneutral conditions. Dietary inclusion of *B. subtilis* could improve the performance of heat-stressed broiler chickens by improving the intestinal epithelial characteristics and microflora balance. however heat-stress associated mortality was decreased. Protecting the intestinal microflora environment and epithelial cells structure would be an approach to partially ameliorate the adverse effects of heat stress on broilers health and performance.

Table 1. Effect of *Bacillus subtilis* PB6 on the morphology of duodenum & ileum of broilers

Parameters	Thermoneutral		Heat stress	
	Basel diet	*Bacillus subtilis* Pb6	Basel diet	*Bacillus subtilis* Pb6
Villus height (µm)				
Duodenum	1552.8^{y}	1823.4^{w}	1289.0^{z}	1713.3^{x}
Ileum	523.0^{y}	658.6^{x}	408.6^{z}	655.8^{x}
Crypt depth (µm)				
Duodenum	315.9^{x}	304.5^{y}	296.0^{z}	320.8^{x}
Ileum	122.1^{x}	108.2^{y}	92.0^{z}	130.3^{x}
Duodenum	382.6^{y}	482 .1^{x}	271.5^{z}	479.0^{x}
Ileum	110.4^{y}	186.3^{x}	86.0^{z}	184.7^{x}
Duodenum	220.5^{y}	248.4^{x}	187.3^{z}	248.7^{x}
Ileum	162.4^{y}	198.2^{x}	127.8^{z}	196.6^{x}

^{w,x,y,z} Means with different letters in the same row are significantly different at P<0.05; NS: not significant (P>0.05). SEM, pooled standard error of the mean; Temp, temperature effect.

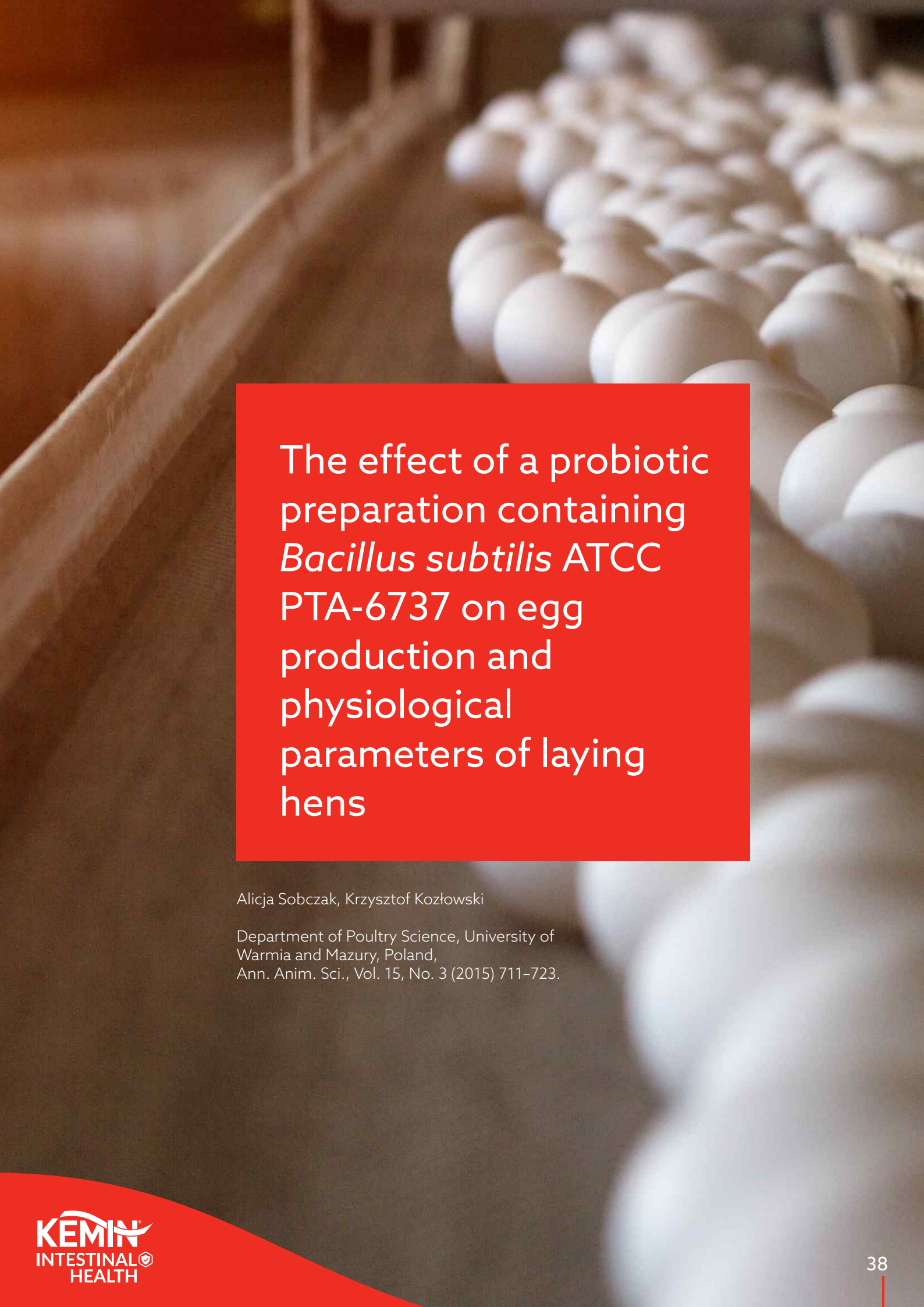
Table 2. Effect of *Bacillus subtilis* PB6 on the intestinal microflora of broilers

Parameters	Thermoneutral		Heat stress	
	Basel diet	CLOSTAT™	Basel diet	CLOSTAT™
Lactobacillus	9.04^{y}	10.25^{w}	7.06^{z}	9.68^{x}
Bifidobacterium	7.22^{y}	8.57^{w}	6.14^{z}	8.36^{x}
Clostridium	4.11^{yz}	4.03^{z}	5.78^{x}	4.13^{y}
Coliforms	5.72^{y}	4.74^{z}	6.88^{x}	4.80^{z}

^{w,x,y,z}Means with different letters in the same row are significantly different at P<0.05; NS: not significant (P>0.05). SEM, pooled standard error of the mean; Temp, temperature effect.

Summary

Dietary inclusion of *Bacillus subtilis* PB6 improves the performance of heat-stressed broiler chickens by improving the intestinal epithelial characteristics and microflora balance. CLOSTAT™ protects the host from pathogen colonization by competing for epithelial binding sites and by producing antimicrobial bacteriocins.



The effect of a probiotic preparation containing *Bacillus subtilis* ATCC PTA-6737 on egg production and physiological parameters of laying hens

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Ann. Anim. Sci., Vol. 15, No. 3 (2015) 711–723.

KEMIN INTESTINAL HEALTH

The effect of a probiotic preparation containing *Bacillus subtilis* ATCC PTA-6737 on egg production and physiological parameters of laying hens

Probiotics enhance the gut microbiome of a living organism in a way that stimulates beneficial gut activity and suppresses harmful pathogenic activities. The movement to ban antibiotic growth promoters (AGPs) is getting stronger due to fears of cross species antibiotic resistant pathogens. However due to the increasing interest in probiotics, scientists have been attempting to find other applications of commercially available probiotics such as CLOSTAT™

The goal of this study was to assess the effect of a probiotic preparation containing *Bacillus subtilis* PB6 on the egg production of physiological parameters of laying hens. To that end a total of two hundred and eighty-eight Lohmann Brown laying hens were randomly divided into two treatment groups.

- Group 1, control diet without supplementation
- Group 2, treatment diet with supplementation of CLOSTAT™

Over the course of the study the number and weight of eggs laid, feed intake, feed conversion, egg quality, the fatty acid profile and cholesterol content of yolk lipids, and selected blood biochemical parameters of hens were measured.

Eggs laid by the experimental group showed significantly higher levels of yolk color and albumen quality as well as improvements in shell thickness and breaking strength when compared to the control group.

The conclusion of the study was that a probiotic preparation containing *Bacillus subtilis* PB6 had a beneficial influence on selected performance parameters of laying hens, egg quality and cholesterol content.

Table 1: Production parameters of laying hens

Item	Group		SEM	P
	Control	CLOSTAT™		
Egg weight (gm)	61.3 ± 0.7	61.8 ± 1.3	0.242	0.346
Egg mass (gm/hen)	56.5 ± 1.2	57.5 ± 1.2	0.282	0.419
Laying rate (%)	92.2 ± 1.2	92.2 ± 1.0	0.250	0.963
FCR (gm feed/gm egg mass)	2.19 ± 0.10	2.18 ± 0.09	0.017	0.919

[a,b] Values in rows with different letters differ significantly ($P<0.05$).

Table 2. Effect of dietary *Bacillus subtilis* ATCC PTA-6737 on egg quality

Item	Group		SEM	P
	Control	CLOSTAT ™		
Shell thickness (mm)	0.355 ± 0.008[b]	0.365 ± 0.008[a]	0.002	0.007
Shell strength (N)	45.12 ± 2.30[b]	47.63 ± 2.78[a]	0.572	0.025
Yolk color (points)	7.83 ± 0.83[b]	9.01 ± 0.71[a]	0.194	0.001
Haugh units	70.45 ± 3.45[b]	72.95 ± 2.59[a]	0.656	0.043
Egg composition (%)				
Yolk	23.78 ± 0.61	23.54 ± 0.40	0.106	0.244
Albumen	66.43 ± 0.68	66.42 ± 0.44	0.114	0.992
Shell	9.79 ± 0.18[b]	10.04 ± 0.15[a]	0.043	0.001

[a,b] Values in rows with different letters differ significantly (P<0.05).

Summary

CLOSTAT™ with *Bacillus subtilis* PB6 added to feed for laying hens contributed to an improvement in eggshell quality parameters. The beneficial influence increased the eggshell thickness.

CLOSTAT™ increased the rate of fermentation and the production of short-chain fatty acids, (SCFA), which reduces the luminal pH which increases calcium solubility and absorption.

Increased SCFA production by the supplementation of CLOSTAT™ stimulate the intestinal epithelial cell proliferation and villus height. Due to these morphological changes absorption efficacy increases, as a result more nutrients including calcium can be assimilated thus the improvement in shell quality.

Effect of *Bacillus subtilis* PB6 on growth and gut microflora in *Clostridium perfringens* challenged broilers

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Ameliorative effect of *Bacillus subtilis* PB6 on growth performance and intestinal architecture in broiler infected with Salmonella

Probiotics are viable bacteria that enhance the intestinal microbiota of a living subject in a way that stimulates beneficial gut activity and suppresses harmful activities. They have grown in popularity as the movement to ban antibiotic growth promoters (AGPs). Probiotics has gained strength due to fears of cross species antibiotic resistant pathogens. One such probiotic is *Bacillus subtilis* PB6 or CLOSTAT™.

The effect of *Bacillus subtilis* PB6 or CLOSTAT™ as a probiotic is significant. *Bacillus subtilis* PB6 has shown to produce a bacteriocin, effective against *Clostridium perfringens*, which is potentially pathogenic for both humans and animals. Primary studies have evaluated the effect of *Bacillus subtilis* PB6 on intestinal health improvement in broiler chickens against *Clostridium perfringens*.

The trial included three hundred and eighty-four Hubbard one day old chicken of average weight procured from a hatcher. Chicken were sexed and then allotted randomly to four separate groups,

- T1, positive control,
- T2, negative control,
- T3, with 5×10^8 cfu/MT of *Bacillus subtilis* PB6
- T4, with 1×10^9 cfu/MT of *Bacillus subtilis* PB6

Twenty-four birds in each replicate with an equal number of males and females.

The parameters recorded were body weight gain, feed intake, mortality, feed conversion ratio, and feed efficiency. T4, Treatment group had 7.2% higher feed intake than the infected control and a significantly lower amount of clostridial bacteria in the gut.

Additionally, high levels of *Bacillus subtilis* PB6 produced a positive effect by increasing Lactobacillus count which led to better digestion and metabolism, hence the increase in feed intake. Both levels of probiotic, T3 and T4 showed comparable feed conversion ratios but the treatment course with higher amounts of probiotic showed better feed conversion ratios in later stages of development.

Highest overall FCR values were observed in the sixth week of the study in negative control. Significant improvement in FCR towards the end of the rearing period corresponds with other studies related to CLOSTAT™.

Table 1. Effect of probiotic supplementation performance of broiler from day 1-42

Parameters	T1 (Positive Control)	T2 (Negative control)	T3 (5 X 10^8 cfu/MT)	T4 (1 X 10^9 cfu/MT)	Probability
Body Weight (g)	2210[a]	2054[b]	2113[ab]	2216[a]	0.02
Weight Gain (g)	2168[a]	2014[b]	2071[ab]	2176[a]	0.02
Feed Intake (g)	3686[a]	3464[b]	3529[ab]	3712[a]	0.05
FCR (g)	1.701	1.722	1.704	1.706	0.96
Feed Efficiency	0.588	0.582	0.587	0.586	0.98

[a, b] Means in the same row with different letters are significantly different at P<0.05; NS: not significant (P>0.05).

- T1-Positive control-Basal diet without *C. perfringens* challenge.
- T2-Negative control-Basal diet with CLOSTA™ with *C. perfringens* challenge
- T3-Basal diet with *C. perfringens* challenge & CLOSTAT™ 5 X 10^8 cfu/MT
- T4-Basal diet with *C. perfringens* challenge & CLOSTAT™ 1 X 10^9cfu/MT

Summary

The study shows linear improvement in feed intake with increasing levels of *Bacillus subtilis* PB6 diet. Reversal of challenges of *Clostridium perfringens* on performance.

The inclusion of CLOSTAT™ in T4, T3 shown numerous increases in the count of Lactobacillus and beneficiary bacteria that reduces challenges of *C. perfringens* infection.

Bacillus subtilis PB6 interfere with the colonization and persistent of bacterial pathogen in the chicken associated with subclinical enteritis.

Ameliorative effect of *Bacillus subtilis* PB6 on growth performance and intestinal architecture in broiler infected with Salmonella

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KEMIN INTESTINAL HEALTH

Ameliorative effect of *Bacillus subtilis* PB6 on growth performance and Intestinal architecture in broiler infected with Salmonella

Globally, salmonellosis in broiler chickens causes huge economic loss. Antibiotics and antibiotic growth promoters are widely explored and used to improve feed efficiency and reduce the spread of harmful microbes. They have produced promising results as additives and have resulted in drug resistance which also leaves potentially dangerous residues in eggs and meat. The current study examines the effect of *Bacillus subtilis* PB6 on the health of chickens challenged with *Salmonella*.
Six hundred Ross 308 broiler chickens were allocated to four treatments, each with ten replicates. The four treatments were detailed as below.

- Negative control with a base diet.
- A positive control is the base diet that has been infected with Salmonella.
- T1, is *Salmonella* infected diet and treated with Avilamycin, an AGP.
- T2, is *Salmonella* infected diet with *Bacillus subtilis* PB6 at a 2 x10^7 cfu/g of feed.

The results of the treatments throughout the trial revealed that the feed conversion ratio was lower in T2 compared to other treatments. Similarly, the performance efficiency factor (PEF) was significantly higher in T2, *Bacillus subtilis* PB6 group compared to the positive control group. Dietary supplementation with *B. subtilis* PB6 improved growth and intestinal health by reversing the negative effects of Salmonellosis.

Table 1. Growth performance

Treatment	FI (g)	BW (g)	FCR (g:g)	PFE
Negative control	437.0^{ab}	346.9^{a}	1.259^{d}	196.6^{a}
Positive control	426.8^{a}	282.9^{c}	1.511^{a}	134.6^{d}
T1-Avilamycin	391.4^{b}	281.9^{c}	1.390^{b}	150.5^{bcd}
T2 - CLOSTAT™	440.8^{a}	321.5^{ab}	1.374^{bc}	171.3^{b}

^{a,b,c,d} Means within a column differ significantly ($p<0.01$), T1 Infected + Avilamycin, T2 Infected plus *Bacillus subtilis* PB6.

Table 2. Villi Morphology

Treatment	Villus Height (um)	Villus Width (um)
Negative control	439[c]	76.7[a]
Positive control	425[c]	64.1[7b]
T1-Avilamycin	544[b]	73.9[a]
T2 -CLOSTAT™	614[a]	57.6[bc]

[a,b,c,] Means within a column differ significantly ($p<0.01$), T1 Infected + Avilamycin, T2 Infected plus Bacillus subtilis PB6.

Summary

Villi height was significantly higher in CLOSTAT™ fed group than Non infected control, the effect of CLOSTAT™ supplementation restores the villus dimensions. Thus, better broiler performance & higher growth rate due to high nutrient absorption & less loss of nutrients.

Supplementation with CLOSTAT™ showed reversed effect of salmonella virulence on gut health of broilers over other AGPs treated groups.

Dietary probiotic CLOSTAT™ of group T2 has shown enhanced commensal growth and restored the homeostasis in the intestinal microbiome resulting in better performance parameters.

Bacillus subtilis PB6 based probiotic supplementation plays a role in the recovery after the necrotic enteritis challenge

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Bacillus subtilis PB6 based probiotic supplementation plays a role in the recovery after the necrotic enteritis challenge

As commercial broiler birds reared under intensive conditions, the vulnerability towards infectious diseases like Necrotic Enteritis (NE) has increased due to higher predisposing factors. Though antibiotic growth promoters (AGPs) like antibiotics treat one of the most devastating diseases, they couldn't reverse the gut morphological changes brought by *Clostridium perfringens*. Probiotic supplementation, being the nearest choice as AGP alternative, shows that *Bacillus subtilis* PB6 is an active microbial that had cidal action on pathogen causing necrotic enteritis. The current investigation evaluates the ability & effectiveness of *Bacillus subtilis* PB6 supplementation on birds' recovery from NE, cecal microflora composition, and short-chain fatty acid (SCFA) production, the performance of chicken, histomorphometric measurements of ileum when challenged for NE. The trial consisted of four groups.

- T1, Negative control - unchallenged.
- T2, Positive control challenged with *Clostridium perfringens* - challenged.
- T3, Group supplemented with *Bacillus subtilis* PB6 – unchallenged.
- T4, NE challenged birds supplemented with *Bacillus subtilis* PB6.

The samples and data were collected and noted at the culling age of 40. Livability percentage was less in T2 challenged group. The T4 group showed a reversal in mortality with the addition of *Bacillus subtilis* PB6 & total body weight, feed consumption, production efficiency factor (PEF) was calculated & discussed in the Table 1.

Table 1. Performance of all four groups

		FI, g	BW, kg	FCR, g	PEF	Livability %
T1	Negative control	2569	1.934ab	1.329^{b}	415.8^{ab}	100^{a}
T2	Positive control challenged	2555	1.800^{b}	1.441^{a}	329.7^{b}	93b
T3	Supplemented with Bacillus subtilis PB6 –unchallenged	2573	2.009^{a}	1.372^{b}	419.3^{a}	100^{a}
T4	NE challenged birds supplemented with Bacillus subtilis PB6 -challenged	2558	1.845^{b}	1.417^{a}	334.2^{ab}	97^{ab}
	SEM ±	63.2	0.048	0.013	27.2	1.172
	P-Value	NS	0.042*	0.0008	0.06^{NS}	0.0018**

^{abcd} *Means in the column with different superscripts differ significantly. NS, not significant.*

Intestinal lesion scores indicate that group T2 had higher lesion score than group T4, both challenged for NE, however supplementation of *Bacillus subtilis* PB6 indicates recovery in group T4. The lesion scores were discussed in table.

Table 2. Intestinal lesion scoring

Treatment group	Macroscopic NE Lesion Scores (Points)		
	Duodenum	Jejunum	Ileum
T2 : Positive Control, *C. perfringens* challenged	1.86[a]	1.21[a]	1.07[a]
T4 : *B.subtilis*, *C. perfringens* challenged	0.86[b]	0.40[b]	0.50[b]
SEM[1]	± 0.188	± 0.158	± 0.108
p-Value	0.0001***	0.0001***	0.0001***

SEM: standard error of the mean, [ab] Means values within a column with different superscripts are significantly different *, P <0.05;**, P <0.01; ***, P <0.001, NS, not significant

Cecal contents were collected at age 40, cecal content both *Bacillus subtilis* PB6 fed group T3 & group T4 showed increase in SCFAs and cecal rise in acetic acid and butyric acid. There was a significant increase in cecum propionic acid with *C. perfringens* challenge T2.

The tissues from ileal mid-section analyzed for histological studies by calculating villus surface area with villus height & villus width. T4 group showed recovery of NE changes, and lowered inflammatory changes of intestine.

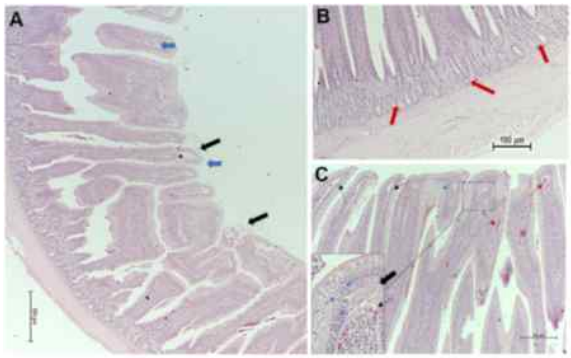


Fig 1 (T2, challenged without CLOSTAT™)

Ileum photomicrograph of NE challenge (T2), Without *Bacillus subtilis* PB6, (A) illustrating disorganized villi with fusion (blue arrows), (B) crypt hyperplasia (red arrows). (C) Edema spreading throughout the structure of the lamina propria (black arrows). Noteworthy is a marked separation of the epithelial cells from the basement membrane (thin black arrows). Several cells in the lamina propria show signs of necrotic cell death.

Ileum photomicrograph of broilers under NE challenge and *B. subtilis* PB6 probiotic supplementation (T4), (A) Illustrating some separation and presence of necrosis coagulation on villus superficial tips (black arrow). (B) and (C) Less inflammation and edema observed through the structure of the lamina propria, Broad and thickened villus tips showing relatively tall organized intestinal villi (blue arrows).

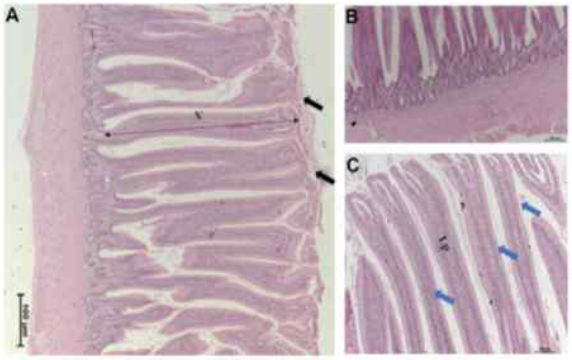


Fig 2 (T4, Challenged + CLOSTAT™)

Summary

B. subtilis PB6 supplementation has shown increased broilers productivity, including improved body weight, reduced FCR, especially in broilers challenged with *C. perfringens*.

The supplementation with *B. subtilis* PB6 reduced NE intestinal lesion scores, attributed to the production of anti-clostridial factors produced by *B. subtilis* PB6.

B. subtilis PB6 supplementation did assist the birds in dealing with NE outbreak long term recovery.

Scientific opinion on the safety and efficacy of *Bacillus subtilis* PB6 as a feed additive for chickens for fattening

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)

2009, European Food Safety Authority (EFSA), Parma, Italy

Scientific Opinion on the safety and efficacy of *Bacillus subtilis* PB6 as a feed additive for chickens for fattening

Introduction

Bacillus subtilis PB6 is the trade name for a feed additive based on viable spores of a strain of *Bacillus subtilis*. It intends to be used in feed for chickens for fattening. The active agent identified as *Bacillus subtilis* is a species considered by EFSA to be suitable for QPS assessment. The sensitivity to antibiotics and the absence of toxigenic potential qualified the strain for QPS status. Consequently, the additive is presumed safe for chickens for fattening, the consumer, and the environment.

Three studies done in two European countries provide to support the efficacy of *Bacillus subtilis* PB6. A significant improvement was observed in one or more zootechnical parameters (final body weight, feed to gain ratio) in all three studies. Therefore, the FEEDAP Panel considers that evidence of the efficacy of *Bacillus subtilis* PB6 in chickens for fattening has been provided at the minimum recommended dose.

The compatibility of *Bacillus subtilis* PB6 with the coccidiostats decoquinate, diclazuril, salinomycin sodium, narasin / nicarbazin, and lasalocid A sodium has been demonstrated.

Characterization of the active agent

Bacillus subtilis PB6 was isolated from the intestinal tract of a chicken that was not subjected to any genetic modification. The strain is deposited at the American Type Culture Collection with the accession number ATCC PTA-6737.

Stability and homogeneity

The stability of *Bacillus subtilis* PB6 (1.74 x 10^{10} cfu/g) was studied when stored at 5° C and 25° C. There are no statistical differences in cell counts over one year of storage.

Stability to pelleting was examined at different pelleting temperatures (ranging from 70 to 95 °C). The viability of *Bacillus subtilis* PB6 was not affected by the highest pelleting temperatures (90 – 95°C). Moreover, the bacilli count remained stable for three months of storage after pelleting.

The stability of *Bacillus subtilis* PB6 in premixtures was studied for six months when stored at room temperature. There is no significant decrease in bacterial counts during the storage period.

Compatibility with coccidiostats

The compatibility of *Bacillus subtilis* PB6 with various coccidiostats was studied using the stepwise approach proposed by the FEEDAP Panel (EFSA, 2008c). Three chemical coccidiostats (decoquinate, robenidine, and diclazuril) and six ionophores (asalocid A sodium, narasin, salinomycin sodium, maduramycin ammonium, monensin sodium, and semduramycin sodium) and one combined product (narasin/nicarbazin) were evaluated for their minimum inhibitory concentrations (MIC) against *Bacillus subtilis* PB6. The MIC values of decoquinate and diclazuril were above four times the maximum authorized concentration in the feed, therefore, compatibility is assumed with these two coccidiostats. Incompatibility could not be excluded for the remaining coccidiostats.

An in vivo study was performed to investigate the compatibility of *Bacillus subtilis* PB6 with salinomycin sodium, narasin/nicarbazin, and lasalocid A sodium. No adverse effects from a 100-fold overdose with the additive were observed on the performance, health, or the results of the macroscopic evaluation of chickens for fattening.

Efficacy

The applicant has provided three studies carried out in two European countries.

Trial 1

A total of 960 one-day-old male Ross 308 chickens were allocated to three dietary treatments (eight replicates of 40 birds per treatment) for 42 days (feeding ad libitum). The treatments resulted from the supplementation of a wheat-soy-based diet with *Bacillus subtilis* PB6 at 0, 1 x 10^7, and 5 x 10^7 cfu/kg feed confirmed by analyses. Feed intake and body weight were determined on day 42.
Birds receiving *Bacillus subtilis* PB6 showed a significantly higher weight gain and improved feed to gain ratio than the control group (Table 1).

Table 1: Performance data of chickens for fattening (42 d) treated with Bacillus subtilis PB6

Bacillus subtilis pb6 (cfu kg)	Weight gain (kg/bird)	Feed intake (kg/bird)	Feed / gain (kg/bird)
0	2.61[a]	4.70	1.80[a]
1x10^7	2.71[b]	4.64	1.72[b]
5x10^7	2.73[b]	4.61	1.69[b]

[a, b]Means in a column with different superscripts are statistically different (P<0.05)

Trial 2

The second study was done in conjunction with the tolerance study. Mean mortality (3.1%) was not affected by treatments. Results showed that birds fed the product irrespective of the dose had a significantly higher body weight than control birds. The overall results of the trial are shown in Table 2.

Table 2: Performance data of chickens for fattening (42 d) treated with *Bacillus subtilis* PB6

Bacillus subtilis PB6 (cfu kg)	Weight gain (kg/bird)	Feed intake (kg/bird)	Feed / gain (kg/bird)
0	2.44[a]	4.70	1.93
1x10^7	2.52[b]	4.77	1.91
5x10^7	2.49[b]	4.77	1.93
5x10^9	2.49[b]	4.80	1.93

[a,b]Means in a column with different superscripts are statistically different (P<0.05)

Trial 3

A total of 960 one-day-old male Ross 308 chickens were distributed into three treatments (eight replicates of 40 chickens per pen). The treatments resulted from the supplementation of a wheat-soy-based diet with *Bacillus subtilis* PB6 at 0, 1 x 10^7, and 5 x 10^7 cfu/kg (confirmed by analysis).

The trial lasted for 42 days. Bodyweight and feed intake were determined at day 42. Chickens receiving the additive showed a significantly higher weight gain at both dosages. The feed to gain ratio was also significantly improved at 5 x 10^7 cfu/kg (Table 3).

Table 3: Performance data of chickens for fattening (42 d) treated with *Bacillus subtilis* PB6

Bacillus subtilis pb6 (cfu kg)	Weight gain (kg/bird)	Feed intake (kg/bird)	Feed / gain (kg/bird)
0	2.69[a]	4.71	1.675[a]
1x10^7	2.80[b]	4.77	1.70[ab]
5x10^7	2.81[b]	4.74	1.69[b]

[a, b]Means in a column with different superscripts are statistically different (P<0.05)

Summary

The active agent identified as *Bacillus subtilis* PB6 is a species considered by EFSA to be suitable for QPS assessment. The sensitivity to antibiotics and the absence of toxigenic potential qualified the strain for QPS status.

A significant improvement of zootechnical parameters was observed in three studies, where the chickens for fattening fed with *Bacillus subtilis* PB6 at 1 x 10^7 cfu/kg feeding stuff. Therefore, the FEEDAP Panel considers that evidence of the efficacy of *Bacillus subtilis* PB6 has been demonstrated at the minimum recommended dose.

The compatibility of *Bacillus subtilis* PB6 with the coccidiostats decoquinate, diclazuril, salinomycin sodium, narasin/nicarbazin, and lasalocid A sodium has been demonstrated & no adverse effects from a 100-fold overdose with the additive were observed on the performance, health.

Scientific opinion on the safety and efficacy of *Bacillus subtilis* PB6 as a feed additive for laying hens and minor poultry species for laying

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)

2015, European Food Safety Authority (EFSA), Parma, Italy

Scientific opinion on the safety and efficacy of *Bacillus subtilis* PB6 as a feed additive for laying hens and minor poultry species for laying

Introduction

Bacillus subtilis PB6 is the trade name for a feed additive based on viable spores of a single strain of *B. subtilis*.

Characterization of the Active Agent

The strain is deposited in the American Type Culture Collection with the accession number ATCC PTA-6737.

Efficacy for laying hens

Four trials, each of 168 days duration, were carried out in laying hens, Birds at the start of the trial were between 18 & 21 weeks of age, and the breeds used were Hyline (trial 1) or Lohmann brown (trials 2, 3, and 4).

Birds were allocated either to a control group given only the basal diet (based on wheat/soybean in trial 1, wheat / corn / soybean in trials 2 and 3, and corn / soybean in study 4,

Individual or pen body weights of birds were recorded at the start and end of each study and routinely monitored for general health status. Feed intake was recorded and used to calculate the feed to egg ratio. The number of eggs produced, percentage laying rate, mean egg weight, and daily or total egg mass determined. A summary of the results for the whole period studied is shown in Table 1.

Table 1: Summary of the performance results of the trials made with laying hens

Trial No	Total number of animals No of replicates per treatment x No of birds per replicate	Treatment (CFU/kg feed)	Laying rate (%)	Average egg weight (g)	egg mass or daily egg mass (g)[a]	Feed intake (g/bird)	Feed to egg ratio
1[b]	324 36x3	0 $1x10^7$ $1x10^8$	91.1 90.5 92.4*	63.5 62.9* 62.7*	4700 4603 4751	126.0 125.5 125.4	2.26 2.28 2.24
2[c]	432 8x18	0 $1x10^7$ $1x10^8$	90.8 93.2* 94.0*	62.6 63.0 63.4	57.1 58.9* 59.8*	113.2 114.6 114.1	2.02 1.97 1.93*
3[d]	324 9x18	0 $1x10^8$	85.9 85.2	56.3 56.8*	49.4 49.5	11104 111.9	2.75 2.74
4[e]	288 9x16	0 $1x10^8$	92.2 92.2	61.3 61.8*	57.0 57.4	118.1 119.1*	2.90 2.18

(a): Average egg mass per phase (28 days) and replicate in trial 1 and daily egg mass in trials 2, 3 and 4.]

Summary

All four trials showed some evidence of a beneficial effect on egg production. The laying rate significantly improved at the recommended dose of 1×10^8 CFU/kg feed in two of the four trials

The other beneficiary effects of CLOSTAT™ from the trial considered are,

- A significant average egg weight increased in two studies.
- Overall decrease in incidences of cracked eggs,
- Better shell stability when measured against plate pressure test,
- Significant increase in shell thickness,
- Lowers over all soft shell eggs formation,
- Improvement in total egg mass with CLOSTAT™
- Better Feed to Egg ratio.

Therefore, the FEEDAP panel concludes that *Bacillus subtilis* PB6 shows the potential to be efficacious in laying hens.

Bacillus subtilis PB6 demonstrates growth inhibition toward equine-specific bacterial pathogens

Meredith L. Burke, Sally A. Moore
2017, Journal of Equine Veterinary Science.

KEMIN INTESTINAL HEALTH

Bacillus subtilis PB6 demonstrates growth inhibition toward equine-specific bacterial pathogens

Typically, the bacteria associated with enterocolitis and acute diarrhea in horses and neonatal foals are Salmonella, *Clostridium perfringens*, and Clostridium difficile. Clostridium spp. are spore-forming toxin-producing gram-positive bacteria that are common inhabitants of the intestines of the equine. The use of antibiotics causes a shift in microbial populations leading to the overgrowth and subsequent toxin production of the said bacteria. Probiotics have gained interest to prevent diarrhea in horses and foals. However, to date, only a few published clinical trials showed positive effects of probiotics in equine.

This study aimed to evaluate *Bacillus subtilis* PB6 as a potential probiotic for equine by screening for antagonistic activity against Six bacterial pathogens isolated from equine patients. The antimicrobial activity of PB6 was evaluated using two different in vitro methodologies.

- A streak line assay resulted in measurable zones of clearing between the growth of PB6 and Clostridium difficile, *Cl. perfringens*, *Rhodococcus equi*, and Streptococcus equi.

- A broth microdilution assay using cell-free supernatant from PB6 culture, which demonstrated the inhibition of Salmonella typhimurium and R. equi growth. The results indicate the potential of PB6 to be a beneficial probiotic for use in the equine industry.

Fig 1: CLOSTAT™ demonstrates the clear zone of Inhibition against Cl. perfringens.

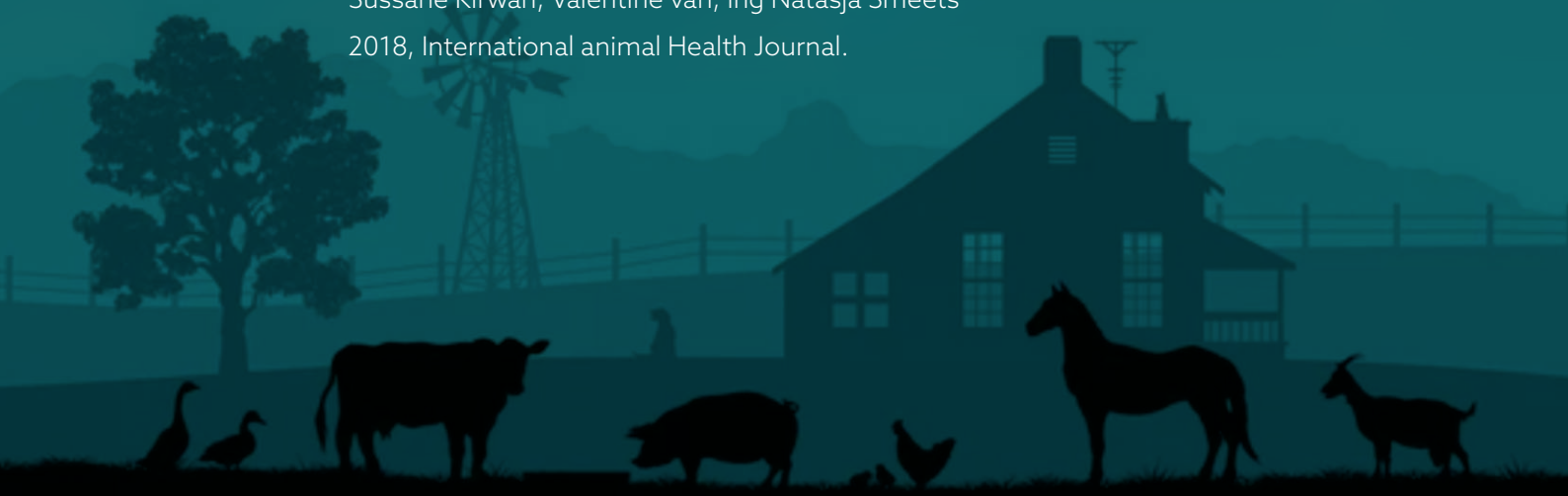
Summary

In the cross-streak assay, zones of b-hemolysis surrounded *Bacillus subtilis* PB6 on blood agar plates, a further indication of the presence of inhibitory molecules, which gives a clear indication that *Bacillus subtilis* PB6 has anti-*Clostridium perfringens* activity.

Reduction of antibiotic use in livestock; identifying targets and potential alternatives

Sussane Kirwan, Valentine van, Ing Natasja Smeets

2018, International animal Health Journal.



Reduction of antibiotic use in livestock; identifying targets and potential alternatives

Reduction in antibiotic usage has been a necessity of the current decade. As a need to diminish the long-term negative effects, the present study aimed at testing a hypothesis of a specific strain, *Bacillus subtilis* PB6, to reduce antibiotic use in commercial farming.

The trial was conducted for a period of 27 weeks, with a commercial turkey integrator. The data for 27 weeks of treatment was collected and compared with the last 3 years incidences of enteritis, colibacillosis, and ornithobacterium (ORT) infection along with the necessity & use of antibiotic treatment. The data collected under similar conditions of rearing and housing, breed of turkey, and management.

The application of *B. subtilis* PB6, 3×10^8 cfu per Liter (CLOSTAT™) was made from hatch to slaughter age via drinking water. The decision of antibiotic for treatment was left to technical in-charge, depending upon severity.

The data collected showed the effect of the application of *B. subtilis* PB6, which significantly reduced the application of antibiotics grouped under colistin by 44%. The use of Tylosin, fluoroquinolone, doxycycline, and fluoroquinolones decreased by 51%. However, the usage of beta-lactams decreased by only 13%, which is less significant than the first two.

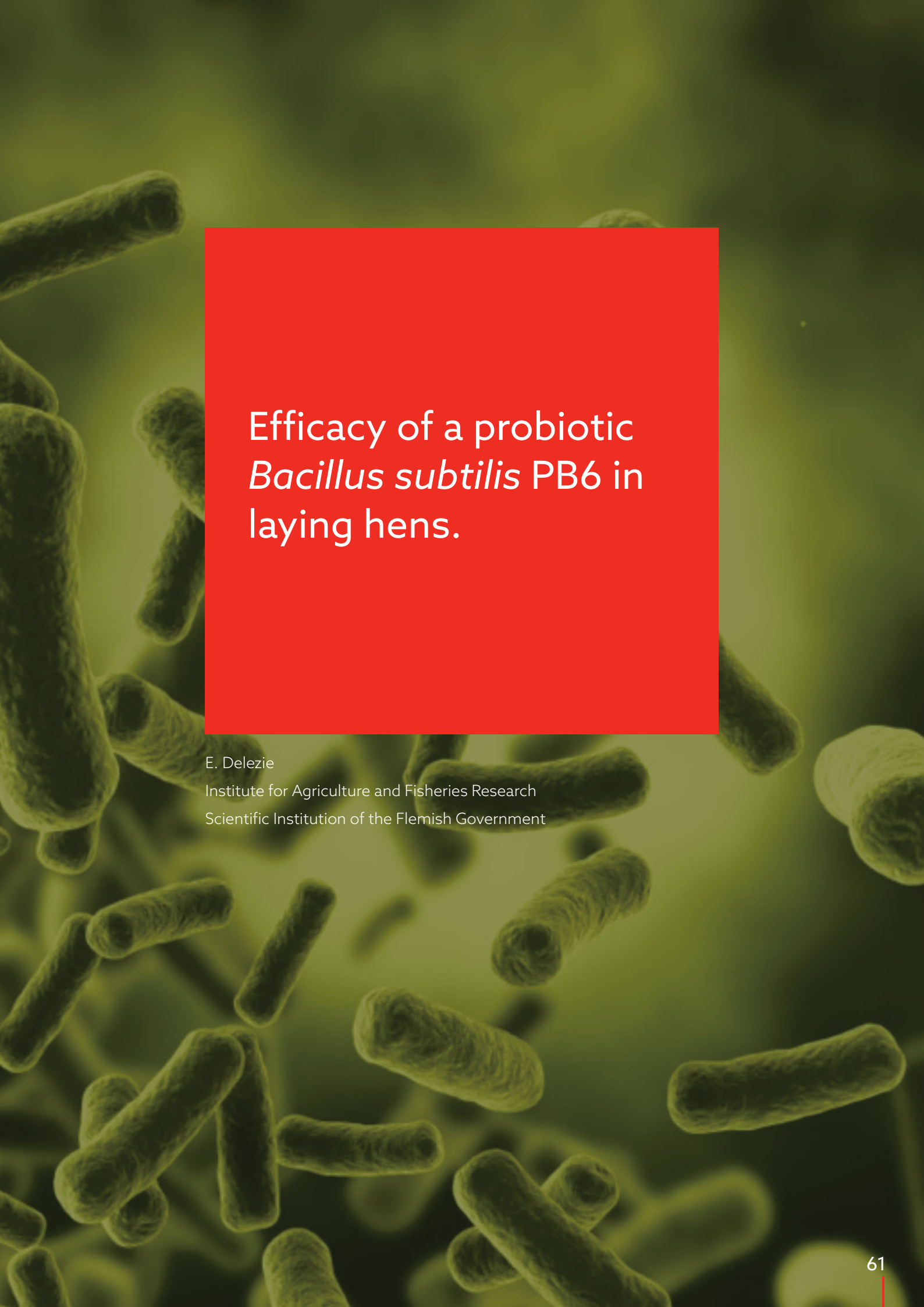
Based on the diseases recorded on weekly basis, the data suggests that the incidences of enteritis reduced by 38%, colibacillosis by 34%, and ORT by 38%.

Summary

The present field study tries to prevent disease rather than using antibiotics to cure it on occurrence and reduce the overall use of Antibiotics to raise the Livestock.

The usage of CLOSTAT™, has overall positive effect on well being of animal, those fallen less sick or responded better to the treatment.

The treatment with *B. subtilis* PB6 indeed resulted in antibiotic reduction usage over a period of time. Overall, the trial presented here clearly indicated that it is possible to use an active microbial (*B. subtilis* PB6) to reduce therapeutic antibiotic use in turkey.



Efficacy of a probiotic *Bacillus subtilis* PB6 in laying hens.

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Efficacy of a probiotic *Bacillus subtilis* PB6 in laying hens.

Bacillus subtilis PB6 (ATCC PTA-6737) is a naturally occurring proprietary strain of bacteria. It's currently authorized for use in chickens for the purposes of fattening by way of competitive exclusion of harmful bacteria such as *Clostridium perfringens*. The objective of this study was to provide additional efficacy data for registration of *Bacillus subtilis* strain ATCC PTA-6737 for use in laying hens.

The trial began with four hundred and thirty-two Lohmann brown hens separated into three dietary treatment groups with eight replicates of eighteen medium laying hens each. The duration of the trial was one hundred and sixty-eight days with six periods of 28 days. The treatment groups were given letter designations and separated.

- Treatment A: Basal diet,
- Treatment B: Basal diet + *Bacillus subtilis* PB6 (1×10^7 cfu/kg feed),
- Treatment C: Basal diet + *Bacillus subtilis* PB6 (1×10^8 cfu/kg feed).

The results showed that birds given the *Bacillus subtilis* PB6 performed significantly better than the control group in every metric. Resulting in notably higher egg laying rate, daily egg mass, and better feed conversion as well as showing a significantly lower percent average of unmarketable or broken eggs. A positive effect was also noticed for bird mortality as mortality in the supplemented group was half what it was in the control group.

Table 1. The effect of dietary treatment on mean hen weight and growth.

Treatment	Body weight at the begin of the experiment(g)	Body weight at the end of the experiment(g)	Growth (g/a)
A	1743	1988	240
B	1740	2024	284
C	1762	2003	241
SEM	0.012	0.020	0.018
P-value	NS	NS	NS

Summary

Both the CLOSTAT™ supplemented groups did result in higher laying rate and laying egg mass as compared to the control diet.

A significant improvement in feed conversion ratio for egg was observed as compared to other 2 groups. This shows the economic importance with the addition of CLOSTAT™.

The Incidence of cracked eggs was significantly lower when laying hens were given supplemented with CLOSTAT™.

Table 2 : The effect of dietary treatment and period on laying rate (%), egg weight (g), daily egg mass (g), daily feed intake (g/a) and feed conversion

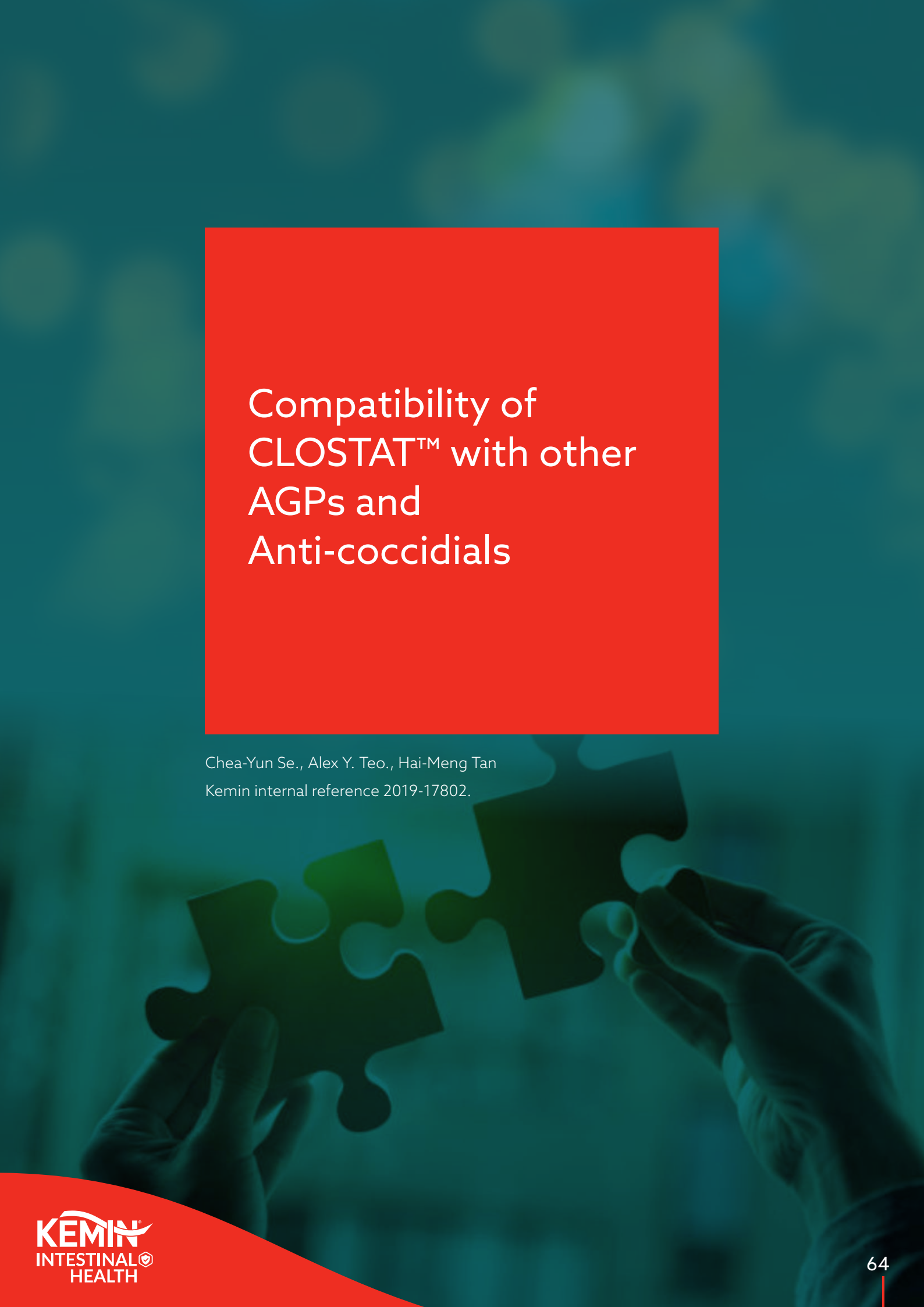
	Treatment	Laying rate,%	Egg weight, gm	daily egg mass, gm	daily feed intake, gm	FCR
Mean Value						
Treatment	A	90.84[b]	62.61	57.06[b]	113.24	2.02[b]
	B	93.19[a]	63.04	58.93[a]	114.60	1.97[ab]
	C	**94.01[a]**	**63.43**	**59.79[a]**	**114.12**	**1.93[a]**
	SEM	0.64	0.28	0.59	0.39	0.02
Period	1	78.65[b]	57.30[d]	45.11[c]	111.93[b]	2.50[c]
	2	96.30[a]	62.54[c]	60.22[b]	115.75[a]	1.92[b]
	3	94.62[a]	63.71[cb]	60.31[b]	113.19[ab]	1.88[ab]
	4	96.21[a]	63.87[cb]	61.46[ab]	113.49[ab]	1.85[ab]
	5	96.05[a]	64.64[b]	62.09[ab]	115.74[a]	1.87[ab]
	6	95.00[a]	66.45[a]	63.13[a]	113.97[ab]	1.81[a]
	SEM	0.64	0.28	0.59	0.39	0.02
ANOVA						
Period		<0.0001	<0.0001	<0.0001	0.029	<0.0001
Treatment		0.0017	NS	0.0010	NS	0.0019
Period x Treatment		NS	NS	NS	NS	NS

[a,b]means with the same letter are not significantly different from each other at P ⩽ .05

Compatibility of CLOSTAT™ with other AGPs and Anti-coccidials

Chea-Yun Se., Alex Y. Teo., Hai-Meng Tan

Kemin internal reference 2019-17802.



Compatibility of CLOSTAT™ with other AGPs Anti-coccidials

The development of CLOSTAT™ provides an alternative solution to use antibiotics to treat necrotic enteritis in poultry. However, the prevalence of other intestinal infections in chickens, like coccidiosis and salmonellosis require the use of other coccidiostats and antibiotics in the feed industry. As a result, our current studies examined the compatibility of CLOSTAT™ with various antibiotics and coccidiostats commonly used in the feed industry.

Results from our studies showed that CLOSTAT™ containing a proprietary strain of *Bacillus subtilis* PB6 is compatible with the AGPs manufacturers' recommended dosages in poultry. It shows that *Bacillus subtilis* PB6 was not affected using Avilamycin, Flavomycin, and Bacitracin at the feed industry dosages (Table 1).

Viability of *B. subtilis* were also tested in the presence of Tylosin (44 ppm), Doxycycline (40 ppm), Enrofloxacine (50 ppm), Salinomycin (60 ppm) and Narasin (80 ppm), respectively in a feed system simulating the conditions of the gastrointestinal tract of chicken. *B. subtilis* PB6 was found to be compatible with these AGPs as there was little or no change in the bacterial count in dosed feed.

Table 1: Minimum inhibitory concentrations (MIC) of antibiotics against CLOSTAT™

Antlbiotics	Recommended dosage in Poultry (ppm)	MIC (ppm)
Avilamycin (10%)	10 to 20	20
Flavomycin (8%)	1 to 20	>20
Bacitracin (15%)	4 to 50	120

Viability of *B. subtilis* were also tested in the presence of coccidiostats like Maduramicin, Semduramicin, Monensin, Salinomycin & Narasin. The Minimum inhibitory concentrations (MIC) of coccidiostats, doesn't inhibit *Bacillus subtilis* PB6. Table (2).

Table 2: Minimum inhibitory concentrations (MIC) of *Coccidiostats* against CLOSTAT™.

Antlbiotics	Recommended dosage in Poultry (ppm)	MIC (ppm)
Maduramicin (1%)	5	>5
Semduramicin (5%)	25	>25
Monensi (10%)	100 to 120	>120

Figure 1: shows the compatibility study of CLOSTAT™ against Salinomycin

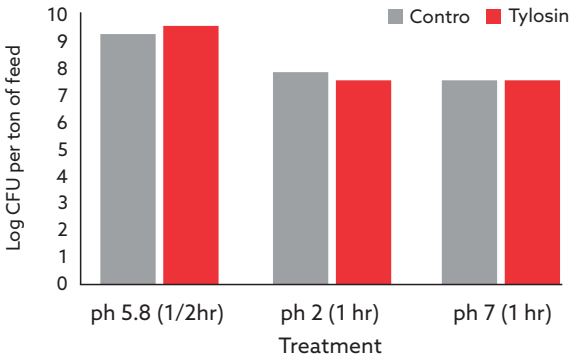


Figure 2: shows the compatibility study of CLOSTAT™ against Tylosin

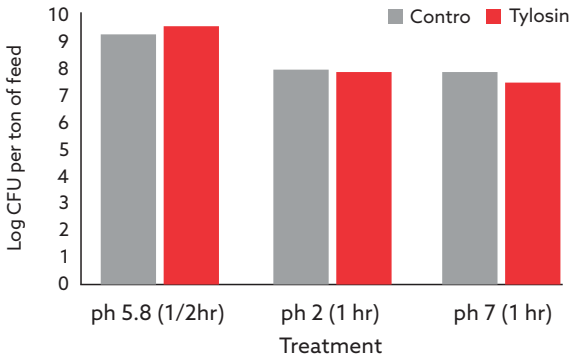


Figure 3: shows the compatibility study of CLOSTAT™ against Enrofloxacine

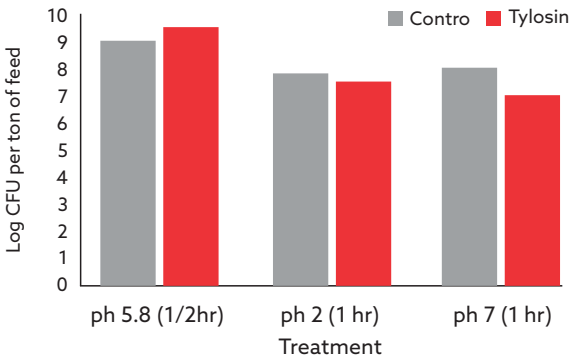
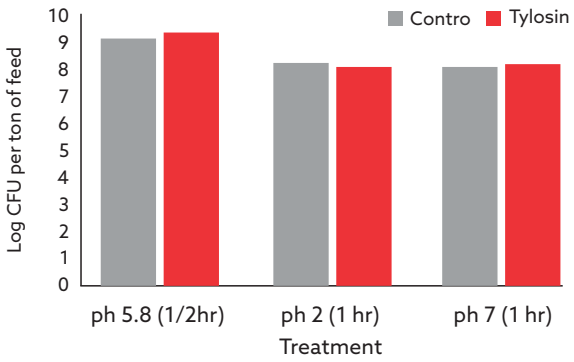


Figure 4: shows the compatibility study of CLOSTAT™ against Doxycycline



Role of *Bacillus subtilis* PB6 in inhibiting the quorum sensing of *Clostridium perfringens*

J-Xi Ng, Tricia Lim and BoonFei Tan

Kemin internal reference 2020-1551.

KEMIN INTESTINAL HEALTH

Role of *Bacillus subtilis* PB6 in inhibiting the quorum sensing of Clostridium perfringens

Quorum sensing (QS) is a mechanism by which bacteria regulate gene expression in response to fluctuation in cell density. *Clostridium perfringens* influences biofilm formation and production of net B toxins, responsible primarily for avian necrotic enteritis. The Agr-type QS system is found in Staphylococcus aureus, *Clostridium perfringens*, Enterococcus faecalis and Listeria monocytogenes. Whereas Fengycin is a cyclic antimicrobial peptide naturally produced by *Bacillus subtilis* PB6 and has been shown to prevent Agr-type quorum sensing of S. aureus by blocking the interactions between autoinducing peptides (AIP) and its receptor proteins.

A wild type *Clostridium perfringens* characterized based on sequencing of netB gene was used in this study. The amount of fengycin produced by *Bacillus subtilis* PB6 grown overnight in Tryptic soy agar with 0.6% yeast extract (TSAYE) was determined using a modified HPLC method.

Effect of fengycin on biofilm formation: *Clostridium perfringens* was cultured in TSBYE broth containing increasing levels of fengycin, after 72-hr incubation, biofilm was stained using crystal violet dye, and quantified.

Bacillus subtilis PB6 can prevent quorum sensing of *Clostridium perfringens* through the production of fengycin, a lipopeptide naturally produced during growth. The inhibition of quorum by fengycin can result in reduced pathogenesis of *Clostridium perfringens* including prevention of toxin production, and formation of biofilm. QS inhibition by *Bacillus subtilis* PB6 is effective in preventing necrotic enteritis and biofilm formation of *Clostridium perfringens*.

Fengycin inhibits biofilm formation by *C. perfringens* without killing the cells
• *Bacillus subtilis* PB6 produces >3 ppm fengycin in overnight culture.

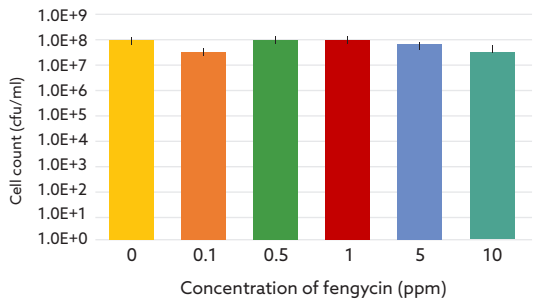


Figure 1: Shows increasing level of fengycin having no lethal effect on *C. perfringens* cultures

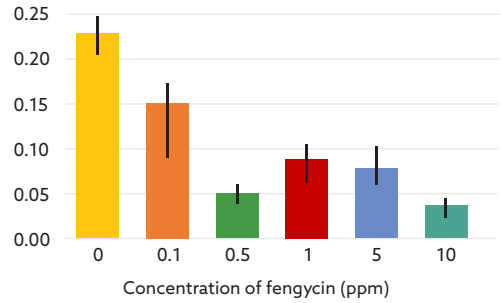


Figure 2: shows increasing level of fengycin having a negative impact on biofilm formation in culture incubated for 72 hours.

Summary

Gram positive pathogen *Clostridium perfringens* has ability to communicate through chemical messenger to each other to express the virulence. The virulence once expressed induce the disease & causes bird to lose the performance.

Bacillus subtilis PB6 present in CLOSTAT™ has ability to secrete cyclic antimicrobial peptide fengycin, which downregulates the genes expression of *Clostridium perfringens*. Intercepting the Quorum Sensing actions & having a negative impact on biofilm production.

Efficacy of CLOSTAT™ against commonly used antimicrobial solution against isolates of field *Clostridium perfringens* strains

Ruchi Majila, Nagendran N & Venket Shelke

Kemin internal reference 2019-17410.

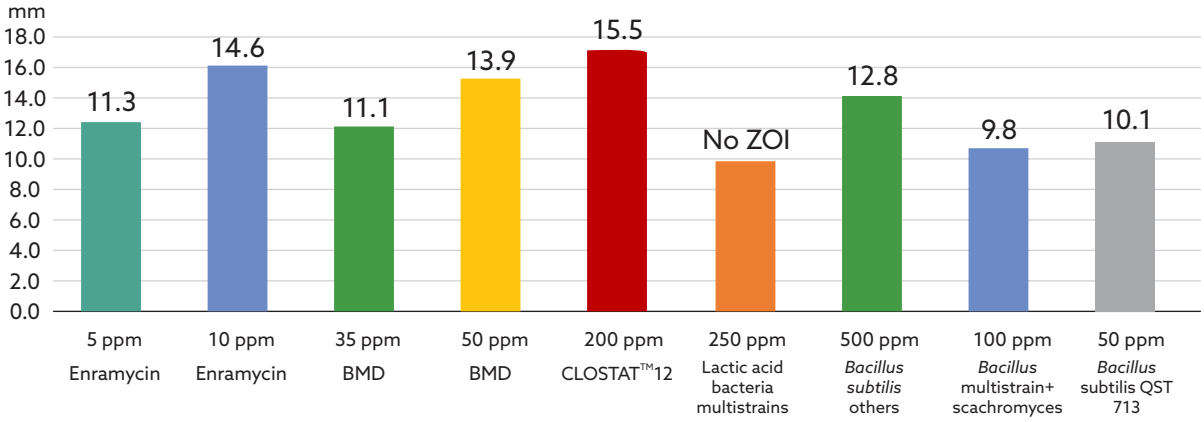
KEMIN INTESTINAL HEALTH

Efficacy of CLOSTAT™ against commonly used antimicrobial solution against isolates of field *Clostridium perfringens* strains

The virulence of field *Clostridium perfringens* strain can be different when we compare to the lab strain. With the hypothesis that the response of available solutions for Necrotic Enteritis could be different for the strains of *Clostridium perfringens* found in field. Kemin South Asia Pvt Ltd team had worked on isolates of *Clostridium perfringens* with the help of standard biochemical tests for the intestinal content samples collected from various geographical locations covering India. These isolates were checked for the efficacy of few commonly used AGPs, Probiotics and CLOSTAT™ against it by Disc Diffusion Method (DDM).

In this test, discs containing antibiotics /probiotics were placed on an agar plate where field isolate of *Clostridium perfringens* had been placed, and the plate was incubated anaerobically overnight at 37° C. If an antibiotic / probiotic stops the bacteria from growing or kills the bacteria, there will be an area around the disc where the bacteria have not grown enough to be visible. This is called a Zone of Inhibition (ZOI). Bigger the Zone of Inhibition, better the efficacy of AGP/ probiotic against *Clostridium perfringens*.

India Comparitive field trial



Summary

CLOSTAT™ had shown maximum Zone of Inhibition against *Clostridium perfringens* in comparison to probiotics available in poultry industry including other strains of *Bacillus*; other AGP such has shown up to 30% less efficacy and other probiotics has shown 60% less efficacy.

Utilization of a direct-fed microbial *Bacillus subtilis* PB6 to improve performance of nursing piglets: Comparison of sow, piglet, and combination treatment regimens

Jarod Hanson, Perry Harms, Matt Culbertson, Joe Hahn,
Kemin internal reference 2016-00015.

Utilization of a direct-fed microbial (*Bacillus subtilis PB6*) to improve performance of nursing piglets: Comparison of sow, piglet, and combination treatment regimens

In swine enteric diseases there are 2 highly pathogenic strains, named *Clostridium perfringens* and Clostridium difficile. They severely effect the piglets especially prior to weaning conditions. The infections by these opportunistic bacteria can result in decreased livability and overall poor performance of piglets. The use of direct-fed microbial (DFM) materials has become increasingly popular as feed additives which interact with both the commensal and opportunistic bacteria of the tract and may provide an opportunity for intestinal balance.

The goal of this study was to evaluate performance responses with commercial application of PB6 in piglets. Trial utilized three hundred and sixty sows and their respective litters separated into two treatment groups of one hundred and eighty sows each. The two groups were the control group and the treated group, with a replication over two consecutive weeks.

The control group was fed a standard ration as specified by the farmer (Murphy-Brown), treated sows were fed the control diet with the addition of CLOSTAT™ via top dress application during the gestation period. Metrics measured in the piglets included birth weight, wean weight, average daily gain, wean age, and percent pre-wean attrition.

- Treatment 1 (control sows) received no oral direct fed microbial,
- Treatment 2 (treated sows) received no oral direct fed microbial,
- Treatment 3 (control sows) received a 1 ml oral dose of (CLOSTAT™ liquid) at 1 day of age,
- Treatment 4 (treated sows) received a 1 ml oral dose of (CLOSTAT™ liquid) at 1 day of age.

The results indicated that the combined treatment in the feed to the sow and oral gavage application to the piglet was significant. Total wean weights were significantly higher in the treated groups as was average daily gain. Mortality and attrition were both lower and overall, the conclusion of the study is that application of PB6 during gestation and lactation can have significant and positive effects in nursing piglets.

Table: Effect of Probiotic Supplementation on Weaning piglets, ADG per day and % attrition Explained.

Description	Treatment group	Piglet wean weight (pounds)	ADG (pounds/day)	% attrition
Sow control/ piglet control	1	10.46[a]	0.4121[a]	25.4[c]
Sow treated/ piglet control	2	10.63[a]	0.4210[a]	19.21[bc]
Sow control/ piglet treated	3	11.08[b]	0.4460[b]	23.05[bc]
Sow treated/ piglet treated	4	11.08[b]	0.4459[b]	18.6[a]

Means with different letters within columns differ significantly ($P < 0.05$)

Summary

The total wean weights were significantly greater in all treated groups as compared to non-treated piglets. It was due to inhibition of *Clostridium perfringens* & *Clostridium difficile* along with minimum inflammatory changes inside the gut of piglets.

The average daily gain (ADG) was also significantly different in the all treated groups as compared to non-treated piglets. CLOSTAT™ helped to decrease the pathogenic effects of the *Clostridium*.

The attrition percent was significantly different in all the treated groups as compared to the non-treated sows, due to *Bacillus subtilis* PB6 efficacy controls overall *Clostridium* infection, lowering down the morbidity & mortality.

Use of *Bacillus subtilis* PB6 as a potential antibiotic growth promoter replacement in improving performance of broiler birds

Sathishkumar Jayaraman, Partha Das, Prakash Saini,

Kemin internal reference 2017-00185.

Use of *Bacillus subtilis* PB6 as a potential antibiotic growth promoter replacement in improving performance of broiler birds

The danger of a drug resistant disease is too high, that's why there is need for use of antibiotic growth promoters / Antibiotics to be replaced or removed from food animal industry soon. The current consensus is that probiotics, competitively exclude harmful bacteria in the guts of livestock, is the most promising method of implementation.

One such probiotic available is CLOSTAT™ which is the brand name for a strain of *Bacillus subtilis* PB6. CLOSTAT™ is a useful bacterium that's been shown to prevent necrotic enteritis, due to its cidal action on *Clostridium perfringens*, and improve both gut health and general performance in broiler.

The goal of the study was to assess these claims and potential of *Bacillus subtilis* PB6 as a replacement for antibiotic growth promoters.

To that end a thirty-five-day trial was conducted with 240 VenCobb 400 broiler chickens which were divided into four treatment groups after which three were challenged orally with *Clostridium perfringens*.

- Negative control,
- Treatment with CLOSTAT™,
- Treatment with antibiotic BMD and
- Treatment with antibiotic Avilamycin.

The results were that the group treated with PB6 had a significantly higher body weight gain, improved FCR and lower levels of harmful bacteria *Clostridium perfringens*. The results are clear that supplementation with *Bacillus subtilis* PB6 provided equal or better broiler performance as compared to antibiotic growth promoters.

Table 1: Villus histomorphometry of jejunum of birds at 35 days of trial

Groups	Villus height	Crypt depth	V/C ratio
Control (unchallenged)	$864.30^{b} \pm 21.82$	$60.03^{a} \pm 3.09$	$14.69^{b} \pm 0.79$
Challenged+CLOSTAT™	$1238.22^{a} \pm 110.47$	$65.37^{a} \pm 1.41$	$18.94^{a} \pm 1.64$
Challenged+BMD	$687.009^{b} \pm 11.88$	$61.54^{a} \pm 1.91$	$11.26^{c} \pm 0.90$
Challenged+Avilamycin	$771.17^{b} \pm 48.61$	$63.16^{a} \pm 1.58$	$12.36^{bc} \pm 0.90$

^{a,b,c} Groups that are significantly different from each other within column at $P<0.05$ are indicated by different indices.

Table 2: Influence of different treatments on body weight (grams)

Groups	Week 1	Week 2	Week 3	Week 4	Week 5
Control	138.83 ± 0.47^{a}	341.62 ± 5.28^{c}	717.62 ± 5.12^{a}	$1,140.07 \pm 17.49^{b}$	$1,591.55 \pm 26.39^{b}$
B. subtilis PB6	132.10 ± 0.99^{c}	391.12 ± 4.02^{a}	726.88 ± 4.43^{a}	$1,229.88 \pm 13.98^{a}$	$1,702.05 \pm 20.49^{a}$
BMD	134.50 ± 0.68^{b}	393.50 ± 2.67^{a}	685.40 ± 2.66^{c}	$1,198.07 \pm 14.30^{a}$	$1,693.34 \pm 22.49^{a}$
AVL	139.32 ± 0.90^{a}	379.32 ± 3.24^{b}	697.30 ± 3.15^{b}	$1,143.75 \pm 14.71^{b}$	$1,586.12 \pm 23.84^{b}$

AVL – Avilamycin; BMD - bacitracin methylene disalicylate.

[a,b,c] Groups that are significantly different from each other within column at P < 0.05 are indicated by different indices. All data are represented as mean ± standard error

Table 3: Effect of different treatments on FCR of birds during the trial.

Groups	Week 1	Week 2	Week 3	Week 4	Week 5
Control	0.92.83 ± 0.01[c]	1.26 ± 0.02[a]	1.34 ± 0.01[c]	1.48 ± 0.03[a]	1.51 ± 0.03[a]
B. subtilis PB6	1.00 ± 0.01[a]	1.11 ± 0.01[b]	1.37 ± 0.01[b]	1.40 ± 0.02[b]	1.49 ±0.2[b]
BMD	0.95 ± 0.01[b]	1.10 ± 0.01[b]	1.43 ± 0.01[a]	1.41 ± 0.02[b]	1.52 ± 0.02[a,b]
AVL	0.92 ± 0.01[c]	1.12 ± 0.01[b]	1.36 ± 0.01[b,c]	1.40 ± 0.02[b]	1.53 ± 0.03[a,b]

AVL – Avilamycin; BMD - bacitracin methylene disalicylate.

[a,b,c] Groups that are significantly different from each other within column at P < 0.05 are indicated by different indices.

All data are represented as mean ± standard error

Summary

The effect of the different treatments on villus height showed a general trend that *Bacillus subtilis* PB6 facilitated the growth of villi compared to the negative control, BMD, and Avilamycin.

The results from this study demonstrated that supplementation with *Bacillus subtilis* PB6 provided better broiler performance as compared to BMD and Avilamycin.

Bacillus subtilis PB6 (CLOSTAT™) supplementation improved FCR by 3 and 4 points compared to BMD and Avilamycin, respectively. This beneficial effect of CLOSTAT™ could be attributed to various reasons such as proliferation of beneficial bacteria in the gut, reduction of pathogenic bacteria, improved gut health, and increased villus histology.

Bacillus subtilis PB6 improves intestinal health of broiler chickens challenged with *Clostridium perfringens*-induced necrotic enteritis

Sathishkumar Jayaraman, Gokila Thangavel, Hannah Kurian, Ravichandran Mani, Rajalekshmi Mukkalil, and Haridasan Chirakkal.
Kemin internal reference 2016-00026

Bacillus subtilis PB6 improves intestinal health of broiler chickens challenged with *Clostridium perfringens*-induced necrotic enteritis

Necrotic Enteritis is an enterotoxemic disease caused by the bacterium *Clostridium perfringens*, endemic disease to poultry. Necrotic Enteritis deteriorates the intestinal wall, increasing FCR and lowering body weight gain and frequently causing morbidity & mortality in poultry. The current study tested the effect of a strain of the *Bacillus subtilis* PB6 on broiler birds with induced necrotic enteritis. The study was conducted using two hundred and sixteen Cobb 400 broiler chicks split into three treatment groups of six replicates which each contained twelve birds. The three treatment groups were,

- Uninfected control,
- Infected control, and
- Infected group treated with *Bacillus subtilis* PB6.

The birds were analyzed for body weight gain, feed conversion ratio, number of intestinal lesion score, and intestinal *Clostridium perfringens* counts. Over the course of the thirty-five day experiment the infected control group showed thickened mucosa, increased rate of hemorrhages, intestinal lesioning, and ballooning of the intestine.

The experimental group showed significant reductions in *Clostridium perfringens* bacterium, considerably lower levels of intestinal lesions, and overall better intestinal health integrity as a result of the introduction and supplementation of *Bacillus subtilis* PB6 to the broiler poultry.

Table: Duodenal villus length and villi length to crypt depth ratio of broiler bird on d 7 Post infection

Treatment group	Villus height	Villi length /Crypt depth
Control	$1350.66^{ab} \pm 106.17$	$5.86^{ab} \pm 0.44$
Infected control	$1147.91^{b} \pm 128.37$	$4.44^{b} \pm 0.60$
Infection + CLOSTAT ™	$1149.62^{a} \pm 94.5$	$6.62^{a} \pm 0.70$
P - Value	0.04	0.01

[ab] Groups that are significantly different from each other within a column at $P<0.05$ are indicated by different superscripts (n=6), The results are reported in mean ± SE.

Summary

Supplementation with *Bacillus subtilis* PB6 controlled *Clostridium perfringens* induced Necrotic Enteritis in broiler birds & reversed the gut morphology changes. Improved the gut health and gut integrity.

The supplementation of *Bacillus subtilis* PB6 reduced FCR compared with the infected group, can act as a replacement for AGP.

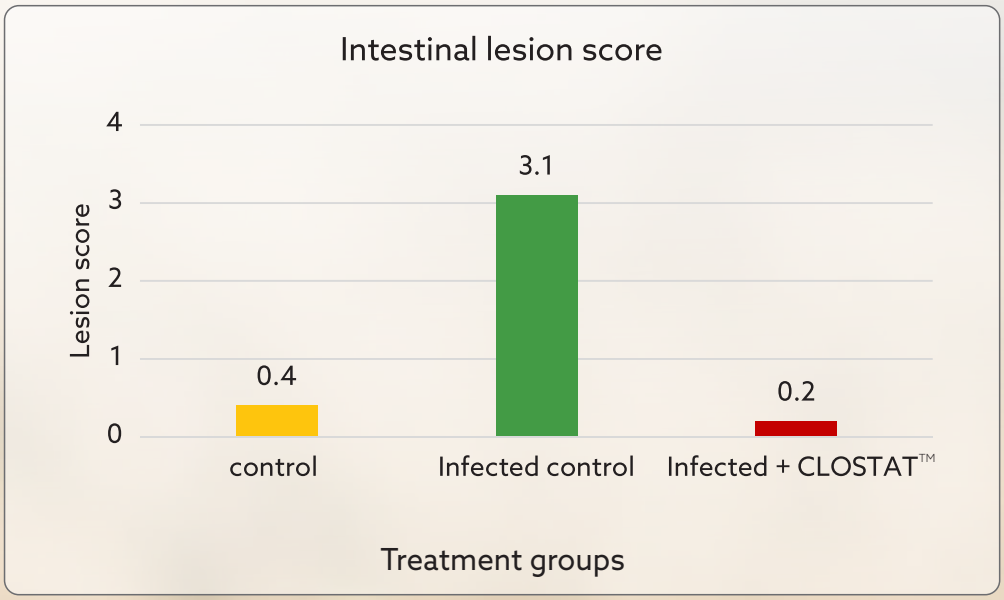


Figure: Intestinal lesion scores of controls, infected group, and infected group supplemented with *Bacillus subitlis* PB6 on day 28th of trial. Groups that are significantly different from each other at P>0.005 are indicated by different letters (a, b).

Efficacy of *Bacillus subtilis* Pb6, and *Bacillus* coagulans with phyto-compound against commonly used antimicrobial solution in commercial broilers

Vaibhav Bhamare, Sairam Kumar, Prabhakar Ramamoorthy, Rajkumar S, Chandrasekar S

Kemin internal reference 2020-6911

KEMIN INTESTINAL HEALTH

Efficacy of *Bacillus subtilis* PB6, and *Bacillus coagulans* with phyto-compound against commonly used antimicrobial solution in commercial broilers

In order to gain a complete control over gut health challenges an efficacy trial in commercial rearing conditions were carried out, where CLOSTAT™ 365 – 12 Dry was compared with 5 different and commonly used treatments for gut health like BMD, Enramycin as AGPs and other Probiotics Product A, Product B & Product C were fed to poultry bird till 28th day. The weekly body weight gain, feed consumption, FCR and mortality % were recorded in all the groups. A quantitative microbiome analysis was also done for TBS and Enterobacteriaceae. The least FCR 1.329 was recorded in CLOSTAT™ 365 12 Dry @ 200 g/MT group. The outcome of this trial concludes that CLOSTAT™ 365 – 12 Dry is a most reliable solution in order to replace the AGPs & other probiotics completely, with much better performance outcome.

Introduction

Aggravated predisposing factors has made Intestine of modern commercial broilers compromised due to the selection of trait for rapid growth over immunity. The uncontrollable factors such as change of season, higher virulence as well as higher incidences of C. perfringens in raw material, use of unreliable solutions due to changing consumer demands, could not able to keep Intestinal health in its ideal conditions challenging the performance as well as heavily impacting the economics of producer. The economic impact may be higher due to use of double dose, or variable dose of AGPs in seasonal challenges or higher water medication costs along with poor performance of the broiler bird. Also, the changed demand from end consumer has forced producer to look for alternatives as use of AGP's in poultry feed

The current study was conducted to evaluate the effect of Probiotic CLOSTAT™ 365 comparing to AGPs & other probiotics to provide most reliable solution to alternatives with higher impact on the performance of broilers and their gut health.

Method

A corn soya-based mash diet was fed ad libitum to the birds throughout the study period. Three feed formulations were prepared according to the phases of the life of the bird: pre-starter, starter, and finisher. The different treatment groups as shown in table were included in the study.
As per the different treatment groups different AGPs or pro-biotic were included in the control diet. Data was evaluated to compare the performance and determine the ROI at the end of trial.

Table 1: Groups considered for trial

Groups	DIET
GROUP 1	Control feed + BMD at dose of 500 g/ton
GROUP 2	Control feed + Enramycin at dose of 125 g/ton
GROUP 3	Control feed + CLOSTAT™ 365 - 12 Dry at dose of 200g/ton
GROUP 4	Control feed + Competitor probiotic A, at dose of 500g/ton
GROUP 5	Control feed + Competitor probiotic B, at dose of 500g/ton
GROUP 6	Control feed + Competitor probiotic C, at dose of 500g/ton

Parameters Studied

- Weekly body weight gain, & Feed conversion ratio (FCR)
- Intestinal lesion scoring of birds for dysbacteriosis on d 29 (Photographs of Intestinal lesions)
- Histopathology of the villi from Jejunum section if intestine

Weekly performance data of all the groups has been given in the table -2. Overall birds' performance in all the groups was as per current industry standards. The highest body weight & least FCR was recorded in CLOSTAT™ 365 groups. In entire 6 groups, BMD performed least in Body weight & FCR performance.

Table 1: Weekly performance Groups

week	Particular/ Groups	BMD	Enramycin	CLOSTAT™ 365	Probiotic A	Probiotic B	Probiotic C
1	Body weight (g)	211	199	210	205	208	204
	FCR	0.845	0.848	0.815	0.820	0.832	0.819
2	Body weight (g)	546	529	552	532	535	529
	FCR	1.085	1.075	1.060	1.069	1.090	1.067
3	Body weight (g)	1085	1071	1112	1075	1078	1080
	FCR	1.224	1.195	1.190	1.208	1.209	1.186
4	Body weight (g)	1646	1656	1724	1663	1655	1667
	FCR	1.385	1.348	1.329	1.350	1.352	1.332

Return on Investment (ROI)

The ROI was calculated based on the final weight and FCR on day 29 of the trial (Table 3). The ROI was found to be 1:16 for CLOSTAT™ 365. The production cost per Kg of live weight for CLOSTAT™ 365 was lower by 2.25 INR than BMD group, and 0.60 INR less compared to Enramycin.

Table 3: ROI of birds supplemented with different feed additives (29th Day)

Particulars	BMD	Enramycin	CLOSTAT ™ 365	Probiotic A	Probiotic B	Probiotic C
FCR (g:g)	1.385	1.348	1.329	1.350	1.352	1.332
Avg Body weight (kg)	1.65	1.66	1.72	1.66	1.65	1.67
Avg feed cost per kg (INR)	26.18	26.14	26.13	26.31	26.19	26.09
Kgs per MT of feed (kg)	722	742	752	740	740	750
EEF	410	438	448	440	422	439
Inclusion cost (INR)	120	130	130	170	160	90

Chick cost – 25 INR, Market rate of birds sold 70 INR, Inclusion cost of feed additives considered as std

Effect of dietary additives on Intestinal Lesion scoring for NE

Intestinal scoring was performed on 29th day of the trial. Two bird per replicate from each of the treatment groups were sacrificed by cervical dislocation for the intestinal scoring. The intestines of the birds were scored for Dysbacteriosis/bacterial enteritis. The scoring was done based on the severity of the lesions in the gut. The score was given on a scale of 0 to 5 based on the description and observations of Kemin Dysbacteriosis scoring system. Results are presented in table 4.

Table : 4 Effect of different feed additives supplementation on intestinal necrotic enteritis lesion score (0-5) of broilers on 29 d.

S.No	Diet	Average Lesion Score
1	BMD	0.34
2	Enramycin	0.25
3	CLOSTAT ™ 365	0
4	Probiotic A	0.17
5	Probiotic B	0.25
6	Probiotic C	0.42

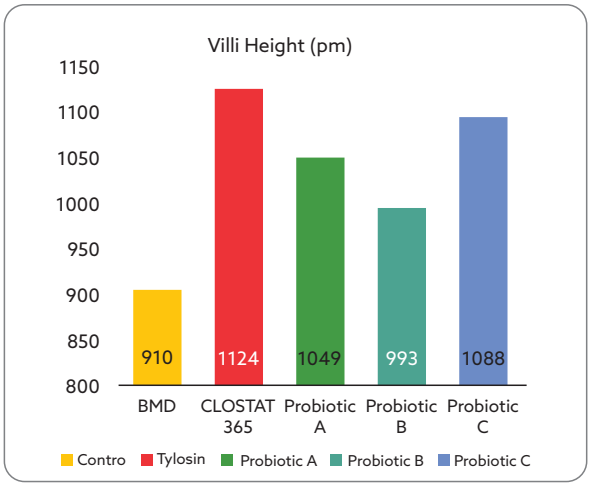
As per the table there were no enteritis lesions found in the intestine of the bird from CLOSTAT™ 365 group, the highest lesions could be seen in Probiotic C group followed by BMD.

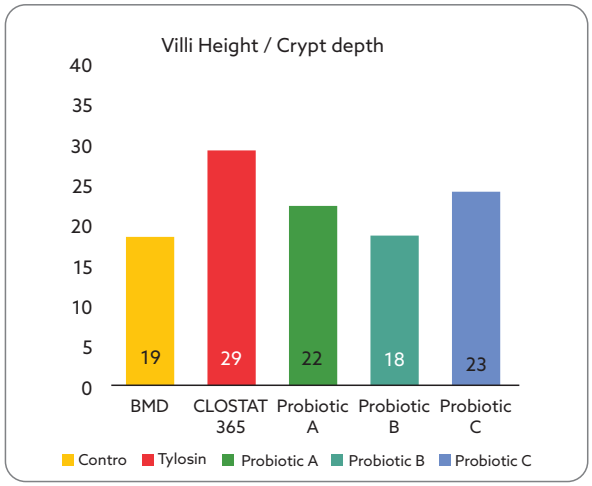
Histopathology of Intestine on 29th Day,

The length of the villi and depth of the crypt, and the ratio in between them is considered as the most important parameter for intestinal health and recovery. A high ratio is an indication of a long villus in which epithelium is sufficiently maturated and functionally active, in combination with a shallow crypt with constant cell renewal. In case of short villi & higher crypt depth, the effect on birds would be poor nutrient absorption, increased secretion in the gastrointestinal tract, and lower performance (Xu et al., 2003).

Table 3: ROI of birds supplemented with different feed additives (29th Day)

	Villi height (pm)	Crypt depth (pm)	Villi height Crypt depth ratio
BMD	910	49	19
CLOSTAT ™ 365	1124	39	29
Probiotic A	1049	47	22
Probiotic B	993	54	18
Probiotic C	1088	47	23





Jejunal villus histomorphometry of broiler birds on d 29 a) BMD Group, with stunted villi, b) CLOSTAT™ 365 group showing the matured & structured villi c) Probiotic A group with some distorted, damaged villi, along with cell hyperplasia.

Discussion

The trial demonstrates the efficacy of AGP's which are thought as only solution for NE management, also against other *Bacillus subtilis* containing other probiotics against the new CLOSTAT™ 365 Dry.

The results from this study demonstrate that supplementation of CLOSTAT™ 365 provides a much better broiler performance as compared to AGP's & Other probiotics. In 4th week CLOSTAT™ 365 significantly improved body weight (77 grams)and FCR (6 points) compared to BMD. EEF denotes the overall performance of a poultry flock. The formula considers data about flock mortality, live weight of the birds, and FCR to determine EEF. In this study, the group supplemented with CLOSTAT™ 365 had higher EEF value compared to other treatment groups (Figure 3) which is 448, almost 38 points higher than BMD group. The ROI was also as per efficiency indicating 16 times return than the cost & efficacy against BMD. The cost of production per kg of live weight is also found to be more economical for CLOSTAT™ 365 group compared to rest of 5 groups.

Conclusion

Increased predisposing factors has significantly impacted the producer economically and psychologically. The multiple effects like Enteritis, Dysbacteriosis & indigested feed always gives negative outcome, and more economical burden. Antibiotic growth promoters and various probiotics are used in poultry diets to control intestinal disorders and reduce the impact on producer. The results of this trial show that CLOSTAT™ 365 -12 dry can be used as an alternative to most commonly used AGPs like BMD, Enramycin, since it gives much better performance under similar conditions certainly it is the only reliable alternative to AGPs in market, as in the same performance trial the other 3 probiotics show inferior performance and inability to minimize intestinal disorders.

Summary

Bacillus subtilis PB6, along with *Bacillus* coagulans has shown better efficacy on villi, performance, lesion scoring of commercially reared birds.

The CLOSTAT™ 365, shows better performance over commonly used AGPs, BMD & Enramycin, also the other *Bacillus subtilis* containing probiotics did not show any efficacy in terms of performance and ROI.

Nil Bacterial enteritis lesion score proves that CLOSTAT™ 365 will work much better than other products which are used for controlling NE & gut health, in challenging periods.

Better Villi length & least Villi height to crypt ratio shows better nutrient absorption capacity, due to higher surface area available. Other product failed to protect villi from damage. That shows CLOSTAT™ 365 has action other than anti-clostridia, in the gut health.

Bacillus subtilis Pb6

The Active Microbial for Natural Solution

Scan to watch

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Common Challenges Faced by

Chicken gut

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POWER OF 8

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POWER OF 8

Better Nutrient Absorption

Improve Intestinal immunity

To Increase "SCFA" production

Reduce inflammation

Grow commensals

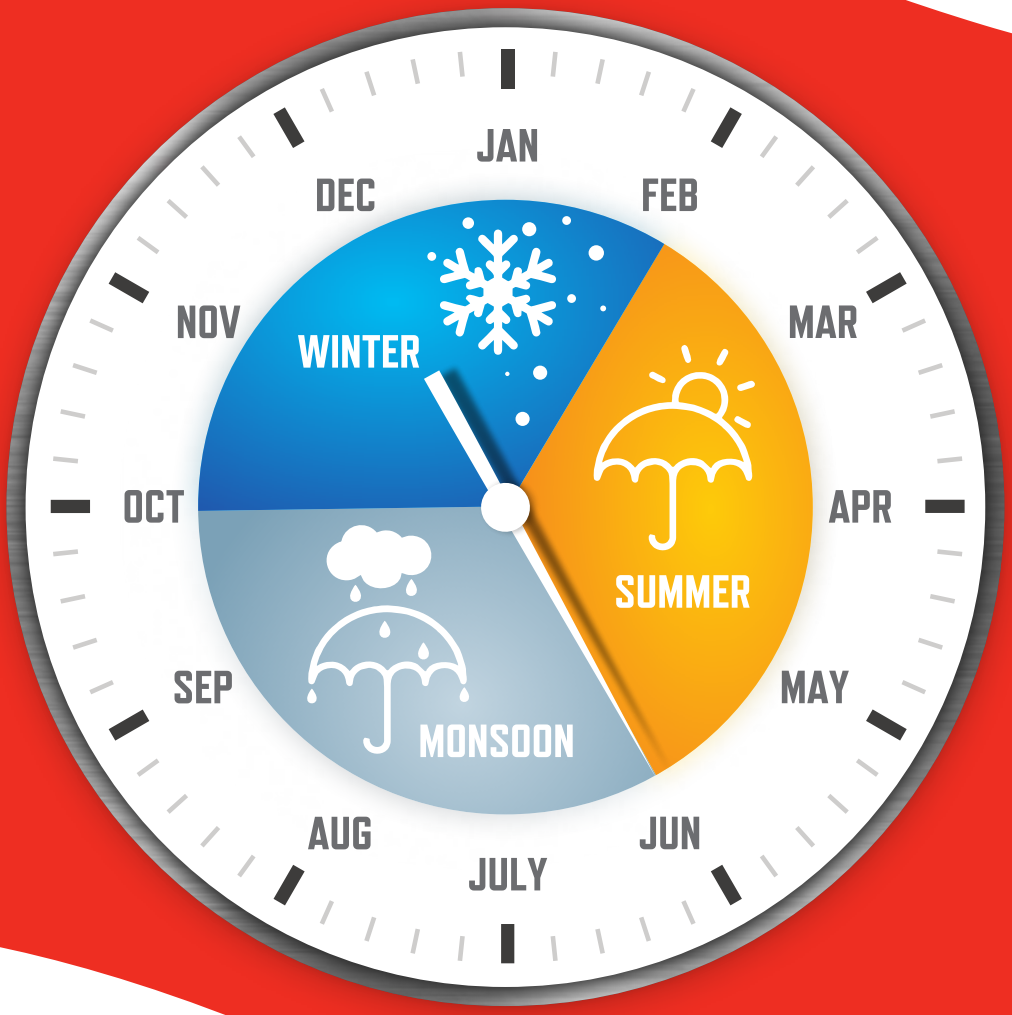
Reduce Intensity of Predisposing Factors

Cidal action on pathogens

Better Egg Quality Parameters

KEMIN INTESTINAL HEALTH

CLOSTAT™ 365



Annual Enteritis Prevention Program

Over the years, challenges on chicken gut health are intensified, thus it is critical to manage the gut health for effective control over enteritis.

KEMIN INTESTINAL HEALTH

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