

BENEFICIAL EFFECT OF BACILLUS SP. PB6 ON BROILER HEALTH STATUS AND GUT MICROBIOME IN COMMERCIAL POULTRY FARMS.

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INTRODUCTION

Performance enhancement	Immune stimulation
	<i>Bacillus</i> sp. PB6

						Result	S				
Averag	e total II	H scorin	g of all far	ms				1	Total lesion scoring of all farms		
Bird ID	DYSBIOSIS										
	OVE	RALL	CRANIAL				CAUDAL	3	*		
	Ballooning	Undigested	Inflammation	Flaccid	Abnormal	Thickness/	Same 4				

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Antimicrobial activity

Microbiome modulation

MATERIAL & METHODS

Set-up:

- 5 commercial broiler farms in Belgium
- Ross 308 broilers
- Commercial feed
- Capacity: between 28,000 and 65,000 broilers per house



		feed		content	Fragility	parameters as for cranial	2		*
10.00 8.00	00 Day 10		10.0	0 Day		1	*	*	
6.00			6.0	0 a	h		0	**	*
4.00 2.00	a 1.07	b 0.3	4.0 7 2.0 0.0	0 3.28 0 1	D 2.08			CONTROL	PB6
0.00	CONTRO		DRG	CONTROL	PR6		TMLS	1.62a	0.65b
CONTRO			0	CONTROL	r DO		SEM	0.10	0.10

Effect of PB6 on microbial diversity in the ileum at day 28

<u>Alpha-diversity</u> (Observed richness, Pielou evenness and Shannon diversity index)







<u>Beta-diversity</u> (Principle coordinate analysis using Bray-Curtis on relative abundance of microbiota data)



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DNA extraction and microbiota analysis of intestinal content of Ileum by 16S rRNA amplicon sequencing DNA extraction and microbiota analysis of intestinal content of Ileum by 16S rRNA amplicon sequencing

- Intestinal health (IH) scoring: monitored based on 10 different parameters for dysbiosis (0 in absence, 1 for presence; maximum score of 10)
- <u>Coccidiosis scoring</u>: coccidiosis was scored for three *Eimeria* species (*E. acervulina, E. tenella and E. maxima*). The scores for each species ranged from 0 (no lesions) to 3 (severe lesions).
- The intestinal health and coccidiosis scoring were assessed for treatment differences using the FitModel platform of JMP 18. Differences were considered significant at P<0.05.



The supplementation of *Bacillus* sp. PB6 to the drinking water of broilers **improved gut health** and **affected the ileal microbial composition**.

- Alpha-diversity was significantly higher in PB6 broilers compared to control broilers.
- The microbial composition was significantly different between PB6 broilers and control broilers.
 The PB6 broilers exhibited a greater abundance of Bacteroidota and a reduced abundance of Proteobacteria compared to the control birds.
 On days 10 and 28, PB6-treated broilers have more beneficial bacteria as Lachnospiraceae, Ruminicoccaceae, Butyricicoccaceae and Oscillospiraceae compared to control birds.



Adding PB6 induced a very specific microbiome. Beta-dispersion test for the homogeneity assumption (p=0.67)* and PERMANOVA test (p=0.001)*

*ASV sequences and abundance were loaded into QIIME2. Mitochondrial and chloroplast sequences were removed. Within QIIME2, the final microbial community data was evaluated for sufficient sequencing depth with alpha rarefaction curves, investigated for alpha (within-sample) and beta (between-sample) diversity after rarefaction to 4915, 30 960, 41 966, and 29 557 counts using phyloseq v1.44.0. Alpha-diversity measures were calculated with Observed richness, Pielou evenness and Shannon diversity indices. Statistical significance of the difference in alpha diversity metrics between treatment groups was tested using Wilcoxon Rank Sum Tests. Significance of the beta diversity was tested by Permutational Multivariate Analysis of Variance (PERMANOVA) with 999 permutations, preceded by a beta-dispersion test to test the assumption of similar multivariate dispersion.

Effect of PB6 on the composition of the ileum microbiome

Relative abundance of most prominent phyla



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