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Compatibility of *B. toyoi* and colistin in post-weaned piglets medicated diets J. Morales[1], C. Piñeiro[1], G. Jiménez[2] and A. Blanch[3]. [1]PigCHAMP Pro Europa, S.A., Spain; [2]Rubinum, S.A., Spain; [3]Andersen, S.A., Spain.

ABSTRACT

The objective of the study was to assess the compatibility of a probiotic (*Bacillus cereus* var *toyoi*) and an antibiotic (Colistin) in piglets diets. The probiotic is a feed additive to stabilise gut flora and Colistin is a polypeptide antibiotic which is used as a therapeutycal treatment. For the study, 336 piglets were used, divided in 24 pens of 14 piglets each one. A randomised 2 x 2 factorial design was used to evaluate both main effects: the diet inclusion of *B. toyoi* and of Colistin, resulting in 4 treatmentes (T1-control, T2-*B.toyoi*, T3-Colistin and T4-*B.toyoi* + Colistin). *B. toyoi* supplemented piglets were selected from *B.toyoi* supplemented sows during gestation and lactation. Productive performance (individual average daily gain, and daily feed intake and feed efficiency per pen), and clinical signs (mortality, presence of diarrhoeas and faeces consistence) were controlled. Furthermore, faeces were sampled by pen (5 per treatment) in order to evaluate different microbiological populations: *Lactobacillus* and *Coliforms*. The presence of *B.toyoi* spores was also evaluated, and at weaning, spores of *B. toyoi* were already observed in piglets from sows supplemented with *B. toyoi*. Significant differences were observed in productive performance during the experimental period. Colistin promoted a higher daily feed intake (273 vs 253 g/d; $P<0.01$) and average daily gain ($P<0.05$). Furthermore, the combination of both products, *B. toyoi* and Colistin, showed a synergetic effect on productive performance. In particular, piglets supplemented with both products, grew faster (183 vs 156 g/d) than control group. The feed intake, which seemed to be the most important effect of additive, was improved (287 vs 254 g/d in *B. toyoi* + Colistin supplemented piglets vs others, respectively; $P<0.05$). Increasing feed intake at post-weaning period may promote a better health and growth at later ages. Differences were not observed among experimental treatments in terms of mortality, presence of diarrhoeas and faeces consistency. In faeces, at 35 d of life a tendency was observed by the probiotic administration ($P=0.08$), and *Lactobacillus* population was higher in *B. toyoi* supplemented piglets. The *Lactobacillus*/*Coliforms* ratio tended to be higher in *B. toyoi* group (T2). And also, an improvement in the ratio of the two bacteria was observed in Colistin group supplemented with *B. toyoi* (T4). Regarding the *B. toyoi* population in faeces, no significant difference was observed at 35 d of life between *B. toyoi* group (T2) and *B. toyoi* + Colistin group (T4), suggesting that this probiotic is fully compatible with the use of Colistin. In conclusion, the supplementation of *B. toyoi* in post-weaning diets in combination with Colistin, may have a synergetic effect on growth and feed intake during this critical period. Furthermore, *B. toyoi* can contribute to increase *Lactobacillus* population in faeces and also to improve the low population of *Lactobacillus* caused by the Colistin supplementation.

OBJETIVES

The aim of this study was to evaluate the compatibility of a probiotic, *Bacillus cereus* var. *toyoi*, with Colistin in piglet diets during the time from weaning to 40 day of age, based on productive performance, digestive pathologies, mortality and *Lactobacillus* and *Coliforms* populations evolution in faeces samples.

MATERIAL Y METHODS

Experimental animals

Three hundred and thirty-six piglets LW x LD-LW (7.9 ± 0.19 kg BW), half boars and half female, were used at weaning (28 d of age). Any piglet that presented symptoms of disease was discarded. Selected pigs were identified individually, weighed and distributed in blocks by sex and body weight among the feeding treatments.

Experimental facilities, housing and lodgings

The trial was carried out at SAT Carraturégano, a 700 sow's farm placed at Cantalejo, Segovia (Spain). The animals were housed in a clean disinfected wean-to-finish barn, containing identical rooms with 10 pens of 14 piglets per cage, which measured 2.9 m x 3.4 m (0.714 m^2/animal). Each cage had individual feeder and drinker. Environmental conditions during the trial (temperature and ventilation) were automatically controlled, according to the age of the animals.

Experimental design

Experimental piglets were randomly distributed in a 2 x 2 factorial arrengment with two main effects: the inclusion in diet of *B. toyoi* (0 or 1000 ppm) and of Colistin, resulting in 4 treatments (T1 – negative control, T2 – *B. toyoi*, T3 – colistin and T4 – *B. toyoi* + colistin). Piglets were distributed in blocks by initial weight and sex at the beginning of the trial. T1 and T3 piglets (fed with no *B. toyoi* supplemented diet) were selected from non-supplemented sows, while T2 and T4 piglets (fed with *B. toyoi* supplemented diet) were selected from *B. toyoi* sows (sows fed with 500 ppm of *B.toyoi* supplementation during gestation and lactation periods). Colistin in treatments T-3 and T-4 was only included in diet from 28 to 35 d of life.

Samplings, records and determinations

Pigs and feed were weighed at weaning (28) and 40 d of age, to calculate performance parameters (daily gain, feed intake, feed efficiency). Days of scour, injected animals, deaths and removed animals were registered every day. The consistency of the faeces was assessed by pen at 28, 35 and 40 d of age (0 normal, 1 soft, 2 diarrhoea). Finally, faeces were sampled at 28 and 35 d of age to identify and quantify *Lactobacillus* and *Coliforms* populations by direct culture assays (5 samples per treatment). *B. toyoi* in faeces at d 28 and 35 in treatments T-2 and T-4 were also quantified.

Statistical analysis
The resulting data were subjected to analysis of variance using the GLM procedure of SAS (1996) for a factorial arrangement of treatments. Factors taken into account were the diet supplementation of *B. toyoi* and of Colistin. Data were presented as least square means. Sex and room were included as block effects and initial body weight as a covariate. Days of scour and losses were analysed by the CATMOD procedure of SAS.

RESULTS AND DISCUSSION

At weaning, *B. toyoi* was detected in the faeces samples of piglets from *B. toyoi* supplemented sows (T-2 and T-5) at 10^5 level. At this time, *B. toyoi* had not been administered to those piglets yet, suggesting that those piglets ate the sow's feed and faeces containing *B. toyoi*. Then, bacteria population in piglets gut was different at post-weaning, before solid diet administration.

4.1 Productive performance
Productive performance data are presented in Table 3.

Table 3 – Body weight (kg), average daily gain (ADG; g/d), feed intake (FI; g/d) and feed efficiency (FE; g/g) of experimental piglets (28-40 d of age).

TTO	*B. toyoi*	COLISTIN	BODY WEIGHT		ADG	FI	FGR
			d 28	d 40			
1	**0**	**0**	8.12[a]	9.76[b]	156[b]	253[b]	1.68
2	**1000**	**0**	8.05[a]	9.90[ab]	168[ab]	252[b]	1.49
4	**0**	**120**	8.05[a]	9.99[ab]	176[ab]	258[b]	1.55
5	**1000**	**120**	7.25[b]	10.09[a]	183[a]	287[a]	1.64
	SEM[1]		*0.193*	*0.110*	*9.20*	*5.63*	*0.104*
	P[2] *B. toyoi*		*	*NS*	*NS*	*	*NS*
	P COLISTIN		*	*	*	**	*NS*
	P B. toyoi X COLISTIN		*	*NS*	*NS*	*	*NS*

[1]Standard error of the mean: n=84 for body weight and average daily gain and n=6 for feed intake and feed:gain ratio.
[2]Probability: *NS*, *P*>0.10; *, *P*<0.05; **, *P*<0.01.

Differences among experimental treatments were observed at the initial body weight (28 d of age), being piglets from T4 the lowest. Hence, initial body weight was included as a covariate to analyse productive performance.

Colistin showed an important effect on post-weaning performance. Colistin supplemented piglets showed a higher feed intake (273 vs 253 g/d; P<0.01) and average daily gain (P<0.05) than control group, and consequently they reached a higher body weight at 40 d of age (10.0 vs 9.8; P<0.05).

The addition of 1000 ppm of *B. toyoi* to post-weaning diets showed numerical differences (176 vs 166 g/d; P=0.26) on average daily gain. Furthermore, the combination of both, *B. toyoi* and Colistin, showed a synergetic effect on average daily gain and piglets from T4 grew faster than the rest of

experimental treatments (T4:183, T1:156, T2:168, T3:176 g/d) being significantly higher than piglets from the control group (T1; P<0.05). This additive effect was especially important in feed intake (287 vs 254 g/d; P interaction *B. toyoi* x Colistin<0.05), suggesting synergetic effect of both products when they are administered together. Probably, compatibility of both products improves with favourable bacteria gut environment. *B. toyoi* was detected at weaning, and it might promote a more favourable environment for Colistin action. As a result, increasing feed intake in this critical moment might assure a better health and growth along the growing and fattening periods.

4.2 Clinical signs

Only 7 deaths and removed animals (2.0%) were registered: 1 due to diarrhoea (T2) and 6 due to meningitis (1 from T1; 3 from T2; 1 from T3 and 1 from T4). No significant differences were observed among experimental treatment.

No important signs of diarrhoea were observed during the experimental period (28-40 d of age), and individual treatments were not required.

Faeces consistence was assessed at 28, 35 and 40 d of age (Table 4).

Table 4 – Faeces consistence per pen at 28, 35 and 40 d of life (0, normal; 1, soft and 2, diarrhoea) in piglets supplemented with *B. toyoi* (TOYO; 0 or 1000 ppm) and Colistin (COLIST; 0 or 120 ppm).

TTO	TOYO	COLIST	Day 28 of life			Day 35 of life			Day 40 of life		
			0	**1**	**0**	**1**	**2**	**2**	**0**	**1**	**2**
1	**0**	**0**	6	0	0	4	2	0	5	1	0
2	**1000**	**0**	5	1	0	5	1	0	6	0	0
4	**0**	**120**	5	1	0	6	0	0	6	0	0
5	**1000**	**120**	5	1	0	4	2	0	4	2	0
	P TTO		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

Faeces consistence was normal in all experimental pens throughout the experimental period. Furthermore, supplementation of *B. toyoi* and/or Colistin in post-weaning piglets diets did not promote significant differences in faeces consistence.

Lactobacillus and total Coliforms population analyses in faeces

Faeces microbiology results are shown in Table 5.

Table 5 – *Lactobacillus* (Lacto) and total *Coliforms* (Colif) populations (expressed as log of bacteria concentration; CFU/g) in faeces at 28 and 35 d of life in piglets supplemented with *B. toyoi* (TOYO; 0 or 1000 ppm) and colistin (COLIST; 0 or 120 ppm).

TTO	TOYO	COLIST	Day 28 of life		Day 35 of life		Day 35 of life
			Lacto	Colif	Lacto	Colif	Ratio of Lacto/Colif
1	**0**	**0**	7.54^{b}	6.97	8.49^{ab}	4.97	1.73
2	**1000**	**0**	8.31^{a}	5.97	8.58^{a}	4.76	1.81
4	**0**	**120**	8.02^{ab}	7.63	8.13^{b}	4.83	1.72
5	**1000**	**120**	8.26^{a}	7.97	8.55^{a}	4.28	2.11
	SEM		*0.181*	*2.831*	*0.137*	*0.349*	*0.152*
	P TTO		*	*NS*	*0.12*	*NS*	*NS*

At weaning day, *Lactobacillus* and total *Coliforms* populations in all groups were observed at the level of 1.6 x 10^8 and 2.5 x 10^7 CFU/g in faeces, respectively. Among experimental treatments, piglets from sows supplemented with *B. toyoi* (T2 and T4) showed higher *Lactobacillus* population than control piglets. One week later, at 35 d of life, *Lactobacillus* population increased and differences among experimental treatments were kept. In particular, piglets supplemented with *B. toyoi* (T-2) showed the highest *Lactobacillus* population. On the other hand, Colistin administered alone (T-4) showed lower population of *Lactobacillus* than control group at 35 d of life without a statistical significant. However, piglets supplemented with Colistin and *B. toyoi* showed higher population of *Lactobacillus* than control piglets and piglets supplemented with only Colistin as well, suggesting that *B. toyoi* improved *Lactobacillus* population.

Coliforms population did not differ among experimental treatments although it was numerically lower in the experimental groups (T2, T3 and T4) than in the control group (T1) showing *B. toyoi* + Colistin group (T4) the lowest value. On the other hand, the ratio of *Lactobacillus/Coliforms* (1.73; 1.81; 1.72; 2.11 in T-1, T-2, T-4 and T-5, respectively) tended to be higher in *B. toyoi* than in the control piglets and it was the highest in *B. toyoi* + Colistin supplemented piglets (T2 and T4) among the all experimental treatments. These results suggest that B. toyoi could contribute to stabilize a gut flora, which is an important factor of improvement of productive parameters.

B. toyoi population in faeces

Results on *B. toyoi* concentration in faeces at weaning (28 d) and at 35 d of life are shown in Table 6.

Table 6 – *Bacillus toyoi* faeces concentration (expressed as log of *B. toyoi* concentration) at 28 and 35 d of life in piglets supplemented with *B. toyoi* (1000 ppm) and with the supplementation or not of colistin (COLIST; 0 or 120 ppm).

TTO	TOYO	COLIST	Day 28	Day 35
2	**1000**	**0**	4.92	5.94
5	**1000**	**120**	5.02	5.93
	SEM		*0.218*	*0.060*
	P TTO		*NS*	*NS*

Differences in faeces *B. toyoi* concentration were not observed by the addition of Colistin (P=0.86 at 35 d of life). These results demonstrate that *B. toyoi* is fully compatible with Colistin.

5. CONCLUSIONS

Under these trial conditions we can conclude:

1. *B. toyoi* is detected at weaning in piglets from sows supplemented with *B. toyoi*.

2. The supplementation of *B. toyoi* and Colistin in post-weaning diets promoted a higher productive performance in this period (28-40 d of age) than the control group, respectively.

3. The combination of *B. toyoi* and Colistin in these diets had a synergetic effect on the growth rate and, especially, on feed intake in this critical period.

4. A low *Lactobacillus* population and a ratio of Lactobacillus/Coliform by the supplementation of Colistin, is improved by the supplementation of *B. toyoi*.

5. This probiotic is fully compatible with the use of colistin, since the population of *B. toyoi* in faeces showed no differences by the supplementation of Colistin.