THE EFFECTS OF THE PROBIOTIC TOYOCERIN IN FATTENING POULTRY

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Introduction

Under normal production circumstances broilers and turkeys hatch in hatcheries, thus a transfer of microorganisms, established in the intestinal tract of the parent animals, is not possible. It is therefore important to support the desired microflora when the microbial colonization of the intestinal tract begins and to counteract the multiplication of undesired germs. Just as during the rearing period of other species this can be done by means of probiotics. However, specific features of the feed production and the short stay of the chymus in the intestinal tract of fattening poultry must be considered.

The use of probiotic bacillus spores in poultry feed is ideal because they have an adventage due to their good heat stability which is important because relatively high temperatures occur during pelletizing of poultry feed. As mainly the vegetative form of bacillus spores is efficient, it must be guaranteed that they quickly germinate in the intestinal tract of poultry. The rapid germination rate of B. toyoi has been demonstrated in piglets (THELEN and PALLAUF, 1996), however, results in poultry are not yet available.

Therefore basic experiments have been carried out to examine the germination rate of B. toyoi in different sections of the intestinal tract of broilers.

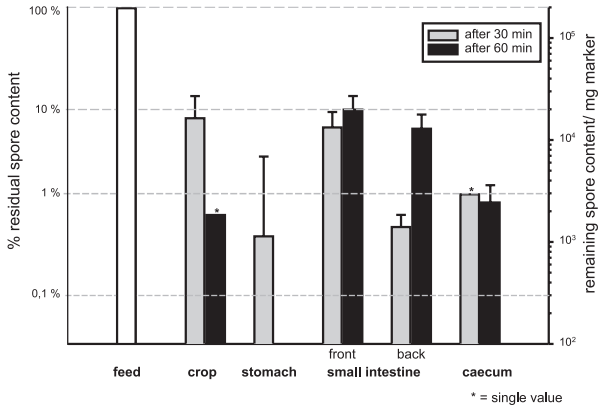
Does ToyoCerin germinate rapidly in the intestinal tract of broilers?

To examine the way of germination of Bacillus cereus var. toyoi, 3 weeks old broilers, which previously received feed without ToyoCerin, were fed with feed including the probiotic ($1x10^9$ cfu/kg) over a period of 30 minutes. The feed was marked with titanium dioxide in order to compare the quantity of ToyoCerin in the intestine measured after slaughter with the quantity administered. The broilers were slaughtered at different times after the ToyoCerin intake (30 min, 60 min, 120 min, 7 days; n = 3). The intestinal content was taken, under aerobic conditions, from single sections of the intestinal tract. For the determination of B. toyoi the intestinal contents were diluted before and after heat treatment (80°C, 15 min), dripped on a selective agar plate and incubated at 37°C for 18 hours. By means of heat treatment it is possible to distinguish between the total B. toyoi number (before heat treatment: spores + metabolic cells) and sporulated cells (after heat treatment).

The results (figure 1) demonstrate that the ToyoCerin spores ingested with the feed quickly germinate in the intestinal tract of broilers. Already after 30 minutes from intake only 10 % of the B. toyoi was available in sporulated form in the crop. After 60 minutes sporulated B. toyoi-germs were found in the crop in only 1 out of 3 broilers.

This means, that B. toyoi cells are already available as germinated, metabolic cells in the crop even if a part of those germinated ToyoCerin cells could be explained by the chymus backflow from the lower digestive tract.

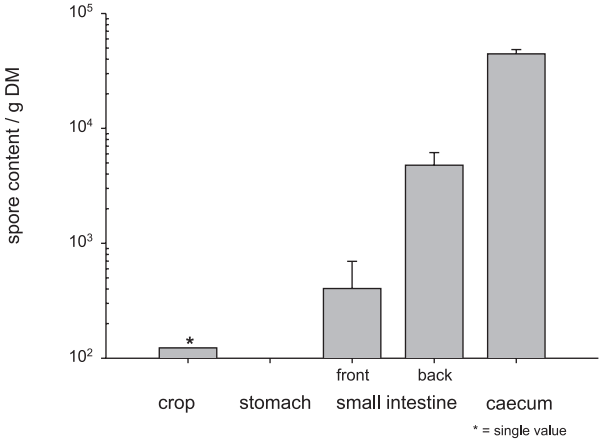
Figure 1: Germination of ToyoCerin in the intestinal tract of broilers (remaining spore content at different moments after feed intake (n = 3))



The spore content decreased progressively from the small intestine to the caecum. The reason for this may be the fact that the total chymus with ToyoCerin had not yet passed the intestinal tract at that moment. But it is remarkable that B. toyoi cells were already found in the appendix 30 minutes after completing intake. This would point to an intensive mixing by the chymus.

Examination of broilers which were slaughtered 2 hours after the intake showed that B. toyoi is no longer available in the crop. Thus the probiotic does not settle. That this may be valid for the whole intestinal tract can be seen from the spore contents measured one week after the ToyoCerin intake (Figure 2). At that point ToyoCerin-contents of any significance were only found in the caecum. Few or no spores were found at the beginning of the intestinal tract.

Figure 2: Occurence of ToyoCerin one week after administration (n = 3)



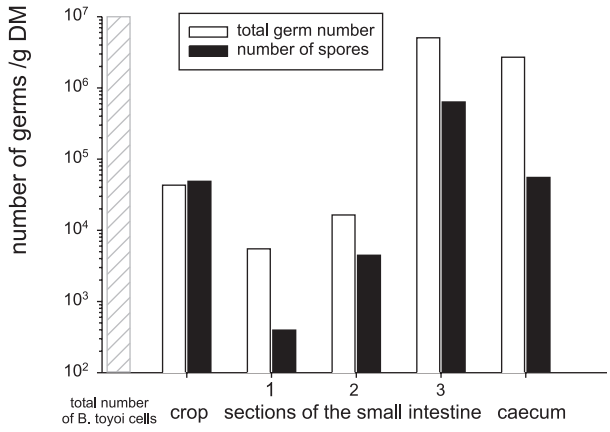
The results show that B. toyoi passes the intestinal tract with the chymus as a transient microorganism without settling in the intestinal flora. ToyoCerin, therefore, is only efficient in case of a continuous intake.

How do the ToyoCerin spores act in the intestinal tract?

Despite their quick germination, ToyoCerin spores were found in the whole intestinal tract, thus metabolically active cells probably produce endospores when passing through the digestive tract and these possibly germinate again. To provide this proof, germinated B. toyoi cells ($1x10^7$ cfu) were given as a single dose to 3-week-old broilers.

The broilers were slaughtered (n = 3) two hours after intake of the germinated metabolically active ToyoCerin cells and the intestinal content was taken from different segments of the intestinal tract. Figure 3 shows the total amount of ToyoCerin germs as well as the measured spore concentrations. Clearly the metabolically active B. toyoi cells in the crop again produced endospores 2 hours after administration. The ToyoCerin concentration measured directly after the passage through the stomach (small intestine 1) was less than in the following sections (small intestine 2, 3, caecum). At the end of the small intestine the B. toyoi concentration was nearly as high as the total administered quantity of metabolically active cells. A slight increase of the spores in this intestinal section is assumed.

Figure 3: Mode of action of metabolically active B. toyoi cells in the intestinal tract of broilers (2 hours after supplementation of 10^7 vegetative cells)



The following conclusion may be drawn from the results: B. toyoi re-sporulates after the first germination and possibly continues this cycle during its passage through the intestinal tract.

Do probiotics have a positive effect on performance in poultry fattening?

Different effects of probiotics in poultry are described in the literature (JIN et al., 1997; BARROW, 1992) but their detailed efficiency in poultry fattening is little known. The support of the desired microflora is probably the main reason for the better performance frequently measured after administration of probiotics. Different results of experiments already made could explain this, because the extent of the probiotic effect mainly depends on the steady state of the microflora.

Influence of ToyoCerin on the performance of broilers

As the formation of an intestinal flora, which is positively changed by probiotics, takes some time, and especially in view of the short fattening periods in broilers, it must be checked whether probiotics administered via the feed improve the performance.

The influence of different ToyoCerin concentrations on performance was tested in a fattening trial with 600 male broilers (Lohmann Meat). 25 broilers were kept in each of 24 pens for a period of 35 days and fed with a maize-wheat-soybean diet (see table 1). The diet contained 12.8 MJ ME/kg at 23.2 % crude protein, 2.5 % crude fibre and 6.8 % crude fat in the starter period (day 1 - 14) and from the day 15 on to the end of the fattening 12.9 % MJ ME/kg at 20.9 % crude protein, 2.4 % crude fibre and 7.1 % crude fat.

Table 1: Diet composition of the broiler trial (g/kg)

	Starter (1.-14.day)	Grower (15.-35. day)
maize	300	300
wheat	265.7	318
soya extract meal	300	260
fish meal	50	30
soybean oil	47	47
premix *	15	15
$CaCO_3$	10.8	14
monocalciumphoshate	8.7	11
L-Lysine	0.6	2.2
Dl-Methionine	2.2	2.8

* premix: 1,200,000 IU vit. A; 120,000 IU vit D_3; 4,000 mg vit. E; 200 mg vit. B_1; 600 mg vit. B_2; 2,500 mg niacin; 400 mg vit. B_6; 4,000 µg vit. B_{12}; 20,000 µg biotin; 1,800 mg pantothenic acid; 50,000 mg choline chloride; 75,000 mg Fe; 7,500 mg Mn; 10,000 mg Zn; 70 mg Co; 150 mg J

The performance data in table 2 indicate that the feed conversion was improved by ToyoCerin by 3.2 % (ToyoCerin 50); 1.9 % (ToyoCerin 100) resp. 2.5 % (ToyoCerin 100/20). This influence on the feed conversion in the ToyoCerin 50-group was statistically significant ($p<0.05$) in the second part of the trial (day 15 - 35). The improvement in feed conversion was depending on a decreased feed intake. The better feed conversion in the other supplementation-groups (ToyoCerin 100 resp. ToyoCerin 100/20) was achieved by a live weight increase of 1.8 % and 2 % respectively compared to the control.

Mortality occured in all trial groups and - except for the ToyoCerin 50 group (3.7 %) - losses were at a level of 5.7 % to 5.9 %.

The results show that the performance, which was already good in the control group without growth promoters and coccidiostats, could be improved by ToyoCerin. No significant influence of the dosage was detected. The

Table 2: Influence of different ToyoCerin concentrations on the performance of broilers

		Control	ToyoCerin 50*	ToyoCerin 100*	ToyoCerin 100 / 20**
ToyoCerin Starter	[cfu/kg]	0	0.5 x 10^9	1.0 x 10^9	1.0 x 10^9
Grower	[cfu/kg]	0	0.5 x 10^9	1.0 x 109	0.2 x 10^9
animals	[n]	137	140	135	136
1. - 14. Day weight gain	[g]	394 ± 20	395 ± 16	401 ± 20	400 ± 15
feed conversion ratio	[g/g]	1.410 ± 0.05	1.376 ± 0.069	1.359 ± 0.032	1.364 ± 0.081
15. - 35. Day weight gain	[g]	1354 ± 52	1353 ± 43	1378 ± 55	1383 ± 29
feed conversion ratio	[g/g]	1.746[a] ± 0.048	1.688[b] ± 0.045	1.719[ab] ± 0.050	1.705[ab] ± 0.054
1. - 35. Day weight gain	[g]	1747 ± 67	1747 ± 57	1779 ± 71	1782 ± 26
feed conversion ratio	[g/g]	1.670 ± 0.048	1.617 ± 0.045	1.638 ± 0.050	1.628 ± 0.054

Values with different letters differ significantly (p<0,05; Sheffe Test)

* mg ToyoCerin 10^{10}/kg feed

** mg ToyoCerin 10^{10}/kg feed - reduced dosage in grower feed

effect of the probiotic was mainly seen in improved feed conversion rate. The results of Richter, published in 1999, showing an improved feed conversion by 1.4 % - 4.8 % using ToyoCerin confirm this. But in contrast to the examination mentioned above, RICHTER (1999) found that increasing ToyoCerin dosages led to reduced mortality.

In a practical trial with 45.540 Cobb broilers at the beginning of 1999 (LAH, 1999) the feed conversion could be improved by 5.1 % with ToyoCerin (1 x 10^9 cfu/kg starter feed; 0,2 x 10^9 cfu/kg fattening feed) compared with the control group. This was mainly caused by the higher live weight of the broilers of the ToyoCerin group at the end of the 34 day-long fattening period. Here also mortality could be reduced from 2.02 % to 1.47 % with ToyoCerin. In contrast to the aforementioned institute trials, the feed of the practical trials contained one coccidiostat from the group of ionophores in both treatments. Thus ToyoCerin, combined with growth promoting substances, could also increase performance.

Influence of ToyoCerin on the performance of turkeys

In trials investigating the effeciency of probiotics in turkeys, conventional growth promoters, with performance promoting effects (ionophores) have been used. Some of these trials demonstrated that probiotics may have an additional effect on the performance. Already in 1988 (trials of Kartzfehn) the live weight could be improved by 2 % and the feed conversion rate improved by 2.5 % by means of a combination of ToyoCerin (1x10^9 cfu/kg) and Virginiamycin.

It would be interesting to know to what extent high performances could be maintained by probiotics without supplementation with conventional growth promoters, in view of the current dispute over the use of conventional growth promoters.

With this in mind, JEROCH and co-workers (1998) carried out a trial with different ToyoCerin dosages compared with a growth promoter (zinc-bacitracin) with 540 male turkeys (Big 6) over a period of 22 weeks. The birds (15) were placed in each of 36 pens and reared according to the recommendations of the breeder for a 6-phase-fattening (Informationen zur Putenmast, Editor Moorgut Kartzfehn). All turkeys received the same standard diet, which contained a substance to protect against coccidiosis (2.5 mg/kg halofuginon) and against histomoniasis (50 mg/kg nifursol) in phases 1 to 4.

The feed of the trial groups was identical apart from the different concentrations of the additive:

Control:	without growth promoter
Group ZBA:	P_1-P_2 : 50 mg/kg
	P_3-P_6 : 20 mg/kg
Group ToyoCerin 100:	P_1-P_6 : 1 x 10^9 cfu/kg
Group ToyoCerin 50:	P_1-P_6 : 0.5 x 10^9 cfu/kg
Group ToyoCerin 20:	P_1-P_6 : 0.2 x 10^9 cfu/kg
Group ToyoCerin 100/50/20:	P_1-P_2 : 1 x 10^9 cfu/kg
	P_3-P_4 : 0.5 x 10^9 cfu/kg
	P_5-P_6 : 0.2 x 10^9 cfu/kg

The results are shown in table 3. It is clear that, compared with the control, an improvement of the feed conversion rate of 4.3 % to 7.4 % could be achieved by means of all additive supplementations. The best results could be seen in the ToyoCerin 50 and the ToyoCerin 20 group. In nearly all groups the improved feed conversion was caused by an increased live weight as well as a reduced feed intake of 2 % to 4.5 %. Only in the ToyoCerin 100/50/20 group the live weight was not improved, however, the feed conversion was at the same level as with the growth promoter because this group showed the lowest feed consumption. All other ToyoCerin groups did better than the growth promoter groups.

Table 3: Influence of different ToyoCerin concentrations or of a growth promoter (zinc-bacitracin) on the performance of turkeys

	Control	ZBA	ToyoCerin 100*	ToyoCerin 50*	ToyoCerin 20*	ToyoCerin 100/50/20*
live-weight [kg]	19.92 ± 1.66	20.05 ± 1.88	20.37 ± 1.38	20.69 ± 1.96	20.30 ± 1.58	19.90 ± 1.69
feed-intake [kg]	56.43 ± 1.62	54.32 ± 1.41	55.29 ± 1.53	54.32 ± 1.79	54.02 ± 1.80	53.87 ± 1.19
feed-conversion ratio [kg/kg]	2.84 ± 0.12	2.72 ± 0.11	2.72 ± 0.10	2.63 ± 0.09	2.67 ± 0.08	2.72 ± 0.07

JEROCH, STROBEL and MATZKE, 1998 -not published-

* mg ToyoCerin 10^{10}/kg feed

The mortality was only 0.9 % over the whole trial period and losses only occured in phases 1 and 2. Female birds as well as turkeys with wing fractures or crop deformation were selected. The losses were not influenced by the

treatments. The results show no significant influence of the ToyoCerin dosage.

Furthermore the analysis of the single fattening periods did not indicate that higher ToyoCerin concentrations led to additional improvements. A reason may be the fact that no particular digestion problems occured during the fattening period which could be seen from the very low mortality rate. Also, during the sensitive growing phase (phase 4), the ToyoCerin 100 group was not superior. It was on a similar level to the control group whereas all other additive groups showed an improved feed conversion rate by 3.3 % to 6.2 %.

Summary

In basic poultry trials it could be demonstrated that B. toyoi spores quickly germinate in the intestinal tract of broilers which is an essential precondition for the efficiency of ToyoCerin. Furthermore, sporulated cells were found in the intestinal tract after one single administration of pre-germinated (metabolically active) B. toyoi cells. From this, initial evidence is provided that, after germination, B. toyoi forms endospores in the intestinal tract which in turn germinate again. This could be interesting for the stabilization of the steady rate of the microflora in the entire intestinal tract.

The fattening trials demonstrate that the performance of broilers and fattening turkeys can be improved by the use of the probiotic ToyoCerin. The performances in the turkey trial were at least on the same level with the used growth promoter and partly higher effects could be achieved compared with the conventional growth promoter. The trials show that even under good hygienic conditions, also the supplementation with ToyoCerin had a positive influence on performance. A clear recommendation for the dosage could not be deduced.

However, under practical conditions the ToyoCerin dosage should be $1x10^9$ cfu/kg (broiler starter resp. turkey phase P1 - P2) at the beginning of the fattening because of lower feed intake and the microflora which is not yet in the steady state. From current experience a dosage of $0.2x10^9$ cfu/kg is sufficient for the fattening periods which follow.

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