

Effects of diet's physical form on the outcome of an artificial salmonella infection in broiler chickens

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Background

Zoonotic agents communicable by food of animal origin (e.g. *Salmonella* spp., *Campylobacter* spp.), are of great interest regarding food safety. The study presented is focussed on effects of diet's physical form on the outcome of an artificial infection with *Salmonella* (S.) Enteritidis in broiler chickens. The hypothesis was that coarsely ground diets might reduce the colonisation of ceca and the translocation of salmonella into the liver tissue (salmonella in the liver: indicates the transition into the food chain) as already proven in pigs (VISSCHER 2006).

Material and methods

Animals and housing:

- 312 male broilers (Ross 708)
- 3 trials with 4 groups à 26 broilers in each

Diets / Feeding:

- 1st – 6th day of life: Conventional starter diet including coccidiostats, fed ad libitum
- 7th – 36th day of life: Experimental diets (13 MJ ME, 234 g CP/kg DM), fed ad libitum

Tab. 1: Characterisation of experimental diets (based on wheat and soy bean meal) regarding grinding and further compaction

Parameter	Pellet fine	Pellet coarse	Pellet + w	Extrudate
Ground by	Hammer mill	Roller mill	Hammer mill	Roller mill
Structure	Fine	Coarse	Fine + wheat ¹	Coarse
Offered as	Pellet	Pellet	Pellet	Extrudate

¹ 22 % whole wheat added prior to pelleting

Artificial infection:

- 14th day of life: Instillation of ~10⁸ CFU *S. Enteritidis* (SE 147; METHNER et al. 1995) directly into the crop of two birds per experimental group in each trial
- immediately after instillation these birds ("artificially infected birds") were put back into their group (to the 24 "contact birds")



Fig. 1: Housing of broilers in groups



Fig. 2: Instillation of *Salmonella* Enteritidis (in 2 of 26 birds only)

Microbiological investigations:

- day 2, 4, 6 and 13 post infection sampling and qualitative analysis of cloacal swabs (of each individual)
- 21st/22nd day of life (9 contact birds and 1 artificially infected one of each group) and 35th/36th day of life (remaining birds) dissection and sampling of cecal content and liver tissue of each individual and qualitative analysis for salmonella

Qualitative analysis for salmonella:

- **Selective enrichment** of samples in Rappaport-Vassiliadis- and Tetrathionate-Brilliantgreen-Bile-Broth
- Plating on solid and selective culture media (Brilliance™ *Salmonella* and Brilliantgreen-Phenolered-Lactose-Sucrose Agar)
- **Further differentiation:** Subcultivation on Columbia Blood Agar including 6 % sheep blood and angular agar (Kligler Iron Agar); serological differentiation

Statistical analyses:

- SAS® version 3.9; proc. FREQ

Results and discussion

The experimental diets:

The diets were botanically identical but differed in the particle size distribution (Table 2).

Tab. 2: Particle size distribution of the 4 experimental diets (wet sieve analysis):

	Pellet fine	Pellet coarse	Pellet + w	Extrudate
> 1 mm (% of DM)	12.4	38.8	30.7	19.4
< 0.2 mm (% of DM)	42.9	32.4	37.3	58.9
GMD ¹ (µm)	300	467	422	250

¹ Geometric Mean Diameter (WOLF et al. 2012)

The diet "Extrudate" showed the highest percentage of particles smaller than 0.2 mm whereas the diet "Pellet coarse" the highest percentage of particles coarser than 1 mm. This diet was characterized also by the highest GMD. But regarding the particles remaining on the both sieves on top (3.15 mm and 2.0 mm) diet "Pellet + w" showed higher percentages than diet "Pellet coarse" (data not shown). Diets differed also in xylanase activity (NSP-degrading enzyme). Due to the higher process temperature at extrusion (diet "Extrudate") at least the xylanase got totally inactivated.

Performance data:

The performance of birds at the end of the trials despite of salmonella infection varied on a level comparable to conditions common in the field.

Tab. 3: Mean body weight (35th day of life) und feed conversion ratio (FCR among 4 weeks of feeding trial)

Experimental diet	Pellet fine	Pellet coarse	Pellet + w	Extrudate
Mean body weight (g)	2269 275	2254 259	2275 265	2101 266
FCR (kg/kg)	1.55	1.54	1.51	1.58

Discussion and conclusion

In this study offering the diet including 22 % whole wheat (added prior to pelleting) reduced the prevalence of salmonella positive samples in comparison to the other experimental diets. In poultry fed a coarsely ground diet less the inhibition of *Salmonella* invasion by higher SCFA concentration due to higher starch influx in the hind gut (mechanism in pigs; VISSCHER et al. 2006) than an improved barrier function of the stomach (acidification, passage duration) is discussed as basal mechanism. Further research which focus on possible mechanisms (potential effects of the "coarse diet" on stomach acidification/passage time/products of microbial fermentation) are necessary to understand the background of the effects described here. Furthermore the possible influences of the impaired activity of NSP-degrading enzymes in the diet on salmonella infections in broilers needs to be investigated.

Literature

METHNER, U., S. AL-SHABIBI and H. MEYER (1995): Experimental Oral Infection of Specific Pathogen-free Laying Hens and Cocks with *Salmonella* enteritidis Strains. *J. Vet. Med. B* 42, pp. 459-469; VISSCHER, C. F. (2006): Investigations (field study) on *Salmonella* prevalence of fattening pigs with regard to the influence of a low feed grinding intensity and feed additives (organic acids and potassium diformate respectively). Hannover, University of Veterinary Medicine, Foundation, Dissertation thesis; WOLF, P., M. ARLINGHAUS, J. KAMPHUES, N. SAUER and R. MOSENTHIN (2012): Impact of feed particle size on nutrient digestibility and performance in pigs. *Übers. Tierernähr.* 40, pp. 21-64

The project was supported by funds of the Federal Ministry of Food and Agriculture (BMELV) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme.

Feed technology" (diet's physical form) and outcome of artificial salmonella infection:

The results of the microbiological analyses showed marked effects of the different dietary treatments on the frequency of colonisation and translocation of *S. Enteritidis* (Figures 3 and 4).

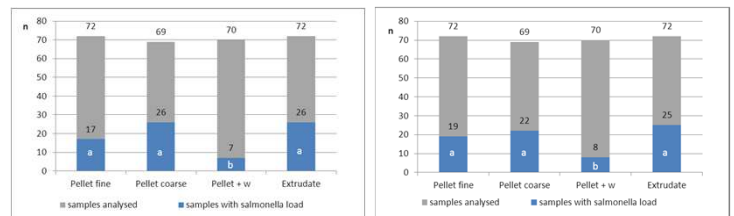


Fig. 3: Results of the qualitative analysis of contact bird's cecal content (at the left) and liver tissue (at the right) for salmonella; ^{a,b} denote significant differences (p < 0.05)

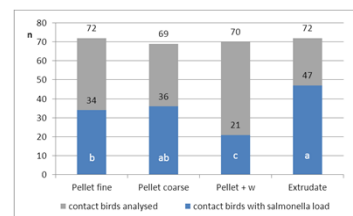


Fig. 4: Results of the qualitative analysis of contact bird's samples (cloacal swabs, cecal content and liver tissue) for salmonella; ^{a,b,c} denote significant differences (p < 0.05)

The rate/frequency of birds with salmonella in cecal content and in the liver tissue (translocation) was significantly reduced in group "Pellet + w" (Fig. 3).

Furthermore the number of birds with salmonella isolation in cloacal swab, cecal content and/or liver tissue was significantly decreased when pellets including 22 % whole wheat were fed (Fig. 4).

The "Extrudate", characterised by the lowest GMD and the lack of xylanase activity, showed the most unfavourable results in the microbiological examinations. Up to now the mechanisms behind these findings are still unclear.