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## Effects of whole wheat feeding on the development of coccidial infection in slow-growing broiler chickens

Einfluss der Fütterung von ganzem Weizen auf die Entwicklung von Kokzidiosen bei langsam wachsenden Masthühnern

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### Introduction

Coccidiosis caused by *Eimeria* species is an important disease in poultry production. Depending on *Eimeria* species, it leads to a reduced growth and sometimes to death such as with *E. tenella* in chickens. Moreover, this disease predisposes the chickens to intestinal bacterial infections (WILSON et al., 2005). Therefore, in order to prevent and limit this disease, anticoccidial drugs, synthetic products or antibiotic ionophores, are routinely used as feed additives. However, due to the appearance of resistance to coccidiostats, the consumer demand for less feed additives, and the expected ban of coccidiostats in 2012 in Europe, alternatives have been proposed as vaccines (CROUCH et al., 2003) or by dietary means (ALLEN et al., 1998; CRÉVIEU-GABRIEL and NACIRI, 2001). Among these latest alternatives, feeding whole grain was reported to have a beneficial effect during coccidiosis (CUMMING, 1989). As this mode of feeding has several advantages including reducing feed cost and meeting consumer demands for more 'natural' feeding systems, its use has been developed in northern Europe (NOIROT et al., 1998). This led to further research in Europe on the effect of whole grain during coccidiosis. Several authors reported no beneficial effects of this mode of feeding (WALDENSTEDT et al., 1998; BANFIELD and FORBES, 2001) or even detrimental effects (BANFIELD et al., 2002; GABRIEL et al., 2006a). All these studies were performed with fast-growing standard lines of chickens. To meet a specific consumer demand, particularly in France, rearing systems for slow-growing type lines have been developed and some use whole grain in the diet without knowing the incidence of this mode of feeding on coccidiosis. However, selection for growth rate has an effect on disease development such as coccidiosis (MAATMAN et al., 1993; ZULKIFLI et al., 1993; SAIF and NESTOR, 2002). Consequently, the effects of whole wheat feeding during coccidiosis may be different between fast-growing and slow-growing lines of chickens.

In order to assess the effects of this mode of feeding on coccidial development in a slow-growing line, the following experiment was undertaken with the three most com-

mon species of coccidia present in chicken production, and infecting different parts of the digestive tract: *E. acervulina* (duodenum), *E. maxima* (jejunum and ileum) and *E. tenella* (caeca).

### Materials and methods

#### Experimental diets

Two types of diets, differing only in the structure of the wheat (ground or whole wheat), were used: complete ground and pelleted diets (C) and whole grain wheat with pelleted protein concentrates (W). The C diets were mainly composed of wheat, soyabean meal, maize and a vitamin-mineral mixture (Table 1). The starter (0-27 d of age) and grower C diets (28 d to the end of the experiment, i.e. 43 d of age) were adapted to the needs of slow-growing lines. Moreover, in accordance with this production system, a minimum of cereal inclusion rate was used for starter (500 g/kg) and grower complete diets (750 g/kg). Protein concentrates contained the same ingredients as the corresponding C diets, except that all the wheat or a part of the wheat (400 g/kg) was removed from the formulation of starter and grower protein concentrates, respectively. Thus, when whole grain wheat was added to the protein concentrates at the ratio of 0.4:0.6, W diets provided the same nutrients as the corresponding C diets. The same batch of wheat (855 g DM/kg) from the variety Sideral, a soft variety with moderate viscosity (ITCF, 2001), was used for the C diets, the grower protein concentrate and whole grain wheat. Neither coccidiostats nor growth enhancers were included. Protein concentrates and C diets were pelleted (2.5 mm diameter) without steam (temperature between 45°C and 50°C) to limit biochemical changes which may affect nutrient availability (PETTERSON et al., 1991). Protein concentrates and whole wheat were given in two separate feeders. In order to accustom the chickens to whole wheat, grains were coarsely ground for the first two days of feeding. No grit was provided.

#### Animals and housing

Male slow-growing French 'label' chickens (Hubbard I 657, 1 d of age), vaccinated the day of hatching against infectious bronchitis (spraying) and Marek disease (intramuscular), were obtained from a commercial hatchery (Sicamen, Volnay, France). They were raised in two thermostatically controlled heated batteries. From 0 to 6 d of age, the lighting program was continuous, and from 7 d to the end

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Table 1. Composition of complete diets  
Zusammensetzung der Versuchsrationen

	Complete diets	
	Starter 0-27 d	Grower 28-43 d
Ingredients (g/kg)		
Wheat	400.0	600.0
Maize	228.7	79.0
Soyabean meal	315.5	135.7
Maize germ	-	59.0
Rapeseed oil	15.0	15.0
Wheat bran		70.8
DL methionine	1.1	0.4
HCl lysine	-	2.3
CaCO <sub>3</sub>	13.3	15.2
Ca <sub>2</sub> PO <sub>4</sub>	16.4	12.6
NaCl	4.0	4.0
Mineral mixture <sup>1</sup>	1.0	1.0
Vitamin mixture <sup>2</sup>	5.0	5.0
Calculated analysis (g/kg)		
ME, MJ/kg	12.0	12.1
Crude protein	208.6	180.1
Methionine + cystine	8.0	7.2
Lysine	11.0	8.7
Calcium	10.0	9.5
Available phosphorus	4.0	3.8
Analyzed composition (g/kg) <sup>3</sup>		
ME, MJ/kg <sup>4</sup>	12.0	12.0
Crude protein	204.2	178.9
Crude fat	36.8	34.1

ME = metabolisable energy

<sup>1</sup> The mineral mixture supplied (mg per kg diet): Co, 0.36; Cu, 8.7; I, 1.2; Se, 0.24; Zn, 84, Fe, 44; Mn, 106, and Ca, 210, as support.

<sup>2</sup> The vitamin mixture supplied the following vitamins (per kg diet): vitamin A (all-*trans*-retinol) 20 mg, cholecalciferol 3 mg, vitamin E (*d*/alpha-tocopheryl acetate) 15 mg, butylated hydroxy toluene 125 mg, menadione 1.25 mg, thiamin 0.5 mg; riboflavin 3.2 mg, calcium pantothenate 3.6 mg, niacin 25 mg, pyridoxine 1 mg, vitamin B12 8 µg, folic acid 1.5 mg, biotin 0.2 mg, choline chloride 750 mg. These vitamins were mixed with oats (65 % of vitamin mixture) before mixing vitamin mixture with other ingredients.

<sup>3</sup> For starter and grower W diets when whole wheat was added to the protein concentrates at the ratio 0.4:0.6, ME, crude protein and crude fat were 11.9 and 12.0 MJ/kg, 199.7 and 173.8 g/kg, and 36.6 and 35.5 g/kg, respectively.

<sup>4</sup> Calculated from fat, mineral and water-insoluble cell walls contents and predictive equation from CARRE and BRILLOUET (1989)

of the experiment it consisted of 23 h light and 1 h dark (0h-1 h). The temperature was gradually decreased from 33°C (0 d) to 22°C (32 d). From 0 to 6 d of age, all chickens were fed the starter C diet and received water *ad libitum*. From 7 d of age, 288 birds were separated into two groups of similar body weight. One group continued to be fed with the starter C diet, and the other received the starter W diet. During the first week of the whole wheat introduction, the protein concentrate was limited to avoid its excess consumption at the expense of whole wheat. At 21 d of age, chickens of extreme weight in each dietary group

were discarded and 48 chickens were separated into four groups (Control, *E. acervulina*, *E. maxima* and *E. tenella*) of similar weight (12 chickens / group), which were individually housed in wire-floored cages (30 cm x 30 cm x 36 cm) at random in the two batteries (2 x 48 cages).

#### Experimental protocol

In each dietary group, C and W, birds of three of the four groups were inoculated at 22 d of age by crop intubation with 0.5 ml of water containing in suspension either 250,000 oocysts of *E. acervulina*, 5,000 oocysts of *E. maxima* or 20,000 oocysts of *E. tenella*. These coccidia originated from natural coccidiosis cases in commercial broiler houses in France. They had been purified and maintained regularly and separately in specific pathogen free chickens. The fourth group in each dietary group was used as uninfected control, and birds in this group received 0.5 ml of water. In order to compare the effect of the two dietary treatments on the development of the coccidial infection, the following parameters were measured: growth performance (feed intake, body weight), serum IgG against each *Eimeria* species, oocyst output kinetics and bacterial counts. Times of measurement after infection were chosen according to life cycle of each *Eimeria* species. Blood for serum IgG determination was collected at 10, 13, 17 and 21 d post-inoculation (d PI). In order to assess the kinetics of the oocyst excretion by the birds infected with *E. acervulina*, *E. maxima* and *E. tenella* excreta were collected each day and pooled per group from 2 to 11, 4 to 12 and 4 to 13 d PI, respectively. For uninfected birds, excreta were collected from 2 to 13 d PI. For bacterial counts, excreta were individually collected before inoculation (22 d) and at 14 d PI (36 d).

#### Analyses

Blood, collected from the main vein of the wing, was allowed to clot for 4 h and centrifuged for 20 min at 2,500 rpm. The sera were aliquoted in 0.5-ml vials and kept at -20°C for further serum antibody determination. Serum IgG were assayed by the ELISA method described by GIRARD et al. (1997) with some modifications. Briefly, microtitre plates (Nunc) were coated overnight at 37°C with 10 µg ml<sup>-1</sup> soluble purified *E. acervulina*, *E. maxima* or *E. tenella* antigen. After subsequent washing with tap water, plates were coated with bovine serum albumin and incubated for 1 h at 37°C. After three washing with 0.05% tween in phosphate buffer saline, plates were incubated for 1 h at 37°C with serum samples in an appropriate dilution (1/10 for *E. acervulina*, 1/40 for *E. maxima* and 1/200 for *E. tenella*). Each plate contained positive and negative reference samples. After three washing with 0.05% tween in phosphate buffer saline, 100 µl of a 1:10,000 dilution of alkaline phosphatase-conjugated rabbit anti-chicken IgG was added and incubated for 1 h at 37°C. The wells were washed with 0.05% tween in phosphate buffer saline. Finally, the colour was developed by adding 100 µl per well of a 1 mg/ml solution of p-nitrophenylphosphate and incubating the plate for 1 h at 37°C. Serum IgG was quantified by the absorbance at 405 nm read with a multiscan spectrophotometer (Argus 300 Microplate reader, Bio-tek instruments, Winooski, Vermont, USA). For each diet, the mean value obtained for uninfected birds was subtracted from the individual value of each infected bird. Antibody titres were expressed as the log<sub>10</sub> of absorbance (x 1000) to approach a normal distribution of the values.

Bird droppings were collected each day per group during oocyst output. Excreted oocysts were counted using

Table 2. Effect of whole wheat feeding on daily feed intake and weight gain (g/d) during coccidiosis in slow-growing chickens until 20 d PI (n=12 / treatment)  
 Einfluss der Fütterung von ganzem Weizen auf die tägliche Futteraufnahme und den täglichen Zuwachs (g/d) während einer Kokzidiose bei langsam wachsenden Masthühnern bis zum 20. Tag nach der Inokulation (n=12/ Behandlung)

Age (d)	Postinoculation period (d PI)	Treatment												SEM	P value
		Uninfected		<i>E. acervulina</i>		<i>E. maxima</i>		<i>E. tenella</i>		Diet	Parasite	Diet x Parasite			
		C	W	C	W	C	W	C	W						
<b>Daily feed intake</b>															
21 - 26	(-1) - 4	60 b	64 ab	60 b	60 ab	65 ab	63 ab	66 a	61 ab	1.4	0.39	*	*		
26 - 27	4 - 5	65 a	64 a	47 b	29 c	68 a	56 b	66 a	53 b	2.5	**	**	**		
27 - 28	5 - 6	65 a	65 a	69 a	47 b	62 a	45 b	62 a	49 b	2.9	**	**	**		
28 - 29	6 - 7	71 ab	75 ab	84 a	85 a	64 b	45 c	77 ab	69 b	3.4	*	**	**		
29 - 42	7 - 20	85	85	90	89	90	88	91	87	1.6	0.16	*	0.47		
<b>Daily weight gain</b>															
21 - 26	(-1) - 4	28 ab	30 ab	29 ab	26 b	32 a	31 ab	33 a	26 b	1.3	*	0.07	*		
26 - 27	4 - 5	33 a	35 a	11 b	-4 c	37 a	28 a	28 a	10 b	3.1	**	**	**		
27 - 28	5 - 6	32 a	33 a	38 a	32 a	31 a	15 b	20 b	11 b	2.8	**	**	*		
28 - 29	6 - 7	32 abc	28 bc	31 abc	44 a	21 c	3 d	41 ab	38 ab	3.4	0.16	**	**		
29 - 42	7 - 20	38	37	40	40	40	38	40	39	1.0	0.20	0.09	0.81		
<b>Final weight</b>															
42	20	1166 ab	1147 ab	1175 ab	1147 ab	1200 a	1121 b	1205 a	1116 b	18.6	**	0.99	0.17		

\*: p < 0.05; \*\*: p < 0.01  
 d PI = day post-inoculation, C = complete diet, W = whole wheat / protein concentrate  
 SEM = Standard error of the mean  
 (a,c) Means in the same line with different letters differ significantly (p ≤ 0.05).

McMaster technique modified by RAYNAUD, (1970) and their numbers were expressed per bird and per day.

Viable bacteria were counted after successive 1/10 dilutions in 0.9% NaCl. Lactobacilli, coliform and *Enterococcus* were counted using Difco™ Lactobacilli MRS Agar (Man, Rogosa, Sharpe) (Becton 288130, Dickinson and Company, Le Pont de Claix, France), Drigalski lactose Agar (Bio-Rad 64664, Marnes-la-Coquette, France) and mEnterococcus Agar (Difco 274620, Dickinson and Company, Le Pont de Claix, France) growth culture media, respectively. Media were incubated aerobically at 37°C for 1 day (Drigalski lactose Agar) or 2 days (MRS Agar and mEnterococcus Agar). Results were expressed as the log<sub>10</sub> of Colony Forming Units (CFU) per g of excreta.

### Statistical analysis

Data were computed using the Statview® software programme version 5 (Abacus Concepts, Berkeley, CA, USA).

The ANOVA model used for weight of uninfected chicken (21 d, 25 d), microflora counts before infection (22 d), and for antibody response during coccidiosis was:

$$Y_{ij} = \alpha_i + \varepsilon_{ij}$$

where  $Y_{ij}$  was chicken weight, microflora counts or antibody response,  $\alpha_i$  was diet effect (C or W) and  $\varepsilon_{ij}$  was residual error. Weights of uninfected chicken (21 d, 25 d) are presented in the text as mean  $\pm$  SE.

The ANOVA model used during coccidiosis for performance and microflora counts was:

$$Y_{ijk} = \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$$

where  $Y_{ijk}$  was chicken performance and microflora counts during coccidiosis,  $\alpha_i$  was diet effect (C or W),  $\beta_j$  was parasite effect (uninfected, *E. acervulina*, *E. maxima* or *E. tenella*),  $\alpha\beta_{ij}$  was interaction between diet and parasite, and  $\varepsilon_{ijk}$  was residual error.

When appropriate ( $P \leq 0.05$ ), the group means were compared using the Student-Newman-Keuls test with level of significance of  $P \leq 0.05$ .

The amount of whole wheat and protein intake of W-fed birds were compared to the expected value consumed by C-fed birds by unilateral T-test ( $P \leq 0.05$ ). These results are presented in the text as mean  $\pm$  SE.

### Results

At 21 d of age, live weight of W-fed birds was slightly lower than that of C-fed birds, 422  $\pm$  1 g and 432  $\pm$  1 g, respectively ( $p < 0.01$ ), but as early as 25 d, for uninfected birds this difference was no longer observed (547  $\pm$  3 g). Thus, from 21 to 42 d, for uninfected birds, no significant differences were found in feed intake, weight gain and body weight at 42 d (Table 2). The amount of whole wheat intake by W-fed birds ( $n=12$ ) was not significantly different from the expected value, 287  $\pm$  75 g/kg and 400 g/kg diet consumed, respectively.

For infected birds, no death due to coccidiosis was observed. The feed intake and weight gain were differently affected depending on the diet (Table 2). Whatever the *Eimeria* species, a lower feed intake was observed for W-fed birds compared to C-fed birds from 4 to 6 d PI, 4 to 7 d PI and 4 to 6 d PI with *E. acervulina*, *E. maxima* and *E. tenella*, respectively. W diet led to significant lower weight gains than C diet: from 4 to 5 d PI, 5 to 7 d PI and (-1) to 5 d PI with *E. acervulina*, *E. maxima* and *E. tenella*, respectively. From 6 to 7 d PI, *E. acervulina*-infected birds receiving W diet showed a higher weight gain compared to their uninfected counterparts. At 20 d PI (42 d), the weight of W-fed birds was lower than that of C-fed birds,

for *E. maxima* and *E. tenella*-infected birds, but similar for *E. acervulina*-infected birds. The amounts of whole wheat intake by W-fed birds were similar to the expected value (400 g/kg diet consumed), the first week after infection. The two following weeks, the amounts of whole wheat consumed were lower than expected with *E. acervulina*, 139  $\pm$  37 g/kg ( $p < 0.01$ ) and *E. tenella*, 246  $\pm$  56 g/kg ( $p = 0.02$ ), but similar with *E. maxima*, 294  $\pm$  62 g/kg. This led to higher protein intake by W-fed birds compared to C-fed birds (180 g/kg diet consumed), with *E. acervulina*, 215  $\pm$  5 g/kg ( $p < 0.01$ ) and *E. tenella*, 201  $\pm$  7 g/kg ( $p = 0.02$ ), and similar protein intake with *E. maxima*, 194  $\pm$  8 g/kg.

The specific IgG antibody response was higher in W- than C-fed birds from 13 to 21 d PI with *E. acervulina* (although not significantly at 17 d PI due to high variability), at 17 d PI with *E. maxima*, and tended to be higher at 10 d PI with *E. tenella* (Table 3).

No oocysts were detected in the excreta of uninfected birds from 2 to 13 d PI. With the intestinal species, *E. acervulina* and *E. maxima*, the profiles and total oocyst excretion were similar between the two diets (Figure 1). In *E. acervulina*-infected birds, the maximum excretion occurred at 5 d PI, and total oocyst excretions from 3 to 11 d PI were 470  $\times 10^6$  and 452  $\times 10^6$  for W- and C-fed birds, respectively. In *E. maxima*-infected birds, the maximum excretion occurred at 7 d PI and total oocyst excretions from 6 to 12 d PI were 40  $\times 10^6$  and 35  $\times 10^6$  for W- and C-fed birds, respectively. In *E. tenella*-infected birds, the profiles of excretion were different between the two diets (Figure 1). The peak of excretion of W-fed birds was of two days (6 and 7 d PI) compared to only one day (6 d PI) for C-fed ones. Thus, total oocyst output from 5 to 12 d PI was 1.3 times higher in W- than in C-fed birds, 203  $\times 10^6$  versus 153  $\times 10^6$ .

*Lactobacillus*, *Enterococcus* and *E. coli* counts were similar for uninfected birds between C and W diets (Table 4). At 14 d PI, the coccidial infections had no effect on the microflora in C-fed chickens, while in W-fed chickens *E. coli* counts were higher in *E. tenella*-infected birds compared to their uninfected counterparts.

### Discussion

Whole grains of wheat are used in some rearing systems of slow-growing lines of chickens and although this mode of feeding has no negative effect in healthy fast-growing broilers, detrimental effects were shown during coccidiosis (BANFIELD et al., 2002; GABRIEL et al., 2006a). The present experiment was thus performed with 'label' chickens, a slow-growing line, infected with the three most common species of coccidia present in chicken production, to assess the effect of whole wheat feeding during coccidiosis in this type of chickens.

The introduction of whole wheat at 7 d of age, led to a slightly lower weight at 21 d for W-fed birds (0.98 of C-fed birds) as previously observed for fast-growing broiler chickens (GABRIEL et al., 2006a). This difference between both diets did not persist afterwards in uninfected birds. Thus in healthy birds, without coccidial challenge, these results show that in slow-growing chicken, whole wheat feeding does not have any detrimental effect on growth performance, as observed in fast-growing broiler chickens (CUMMING, 1994; PRESTON et al., 2000; GABRIEL et al., 2003; 2006a).

The effect of diet observed on parasite development was different according to *Eimeria* species. Whereas total oocyst excretion was similar between diets with intestinal

Table 3. Effect of whole wheat feeding on seric IgG antibody response (Log absorbance at 405 nm x 1000) during coccidiosis in slow-growing chickens until 21 d PI (n=12 / treatment)

Einfluss der Fütterung von ganzem Weizen auf die IgG Antikörper-Titer im Serum (Log Absorption bei 400 nm x 1000) während einer Kokzidiose bei langsam wachsenden Masthühnern (n=12/ Behandlung)

Eimeria species	Age (d)	Post-inoculation period (d PI)	Treatment		SEM	P value Diet
			C E	W E		
<i>E. acervulina</i>	32	10	2.99	3.00	0.061	0.92
	35	13	2.78 b	2.99 a	0.056	*
	39	17	2.74	2.94	0.079	0.08
	43	21	2.68 b	2.92 a	0.080	*
<i>E. maxima</i>	32	10	2.66	2.63	0.099	0.81
	35	13	2.47	2.62	0.088	0.27
	39	17	2.29 b	2.57 a	0.062	**
	43	21	2.25	2.24	0.072	0.95
<i>E. tenella</i>	32	10	3.06	3.25	0.070	0.07
	35	13	3.14	3.20	0.063	0.52
	39	17	3.13	3.19	0.060	0.48
	43	21	2.98	3.05	0.068	0.48

\*: p &lt; 0.05; \*\*: p &lt; 0.01

d PI = day post-inoculation, C = complete diet, W = whole wheat / protein concentrate

E = *Eimeria*

SEM = Standard error of the mean

Table 4. Effect of whole wheat feeding on intestinal microflora (log<sub>10</sub> of Colony forming Units/ g of excreta) before (0 d PI) and 14 d after coccidial infection in slow-growing chickens (n=12 / treatment)Einfluss der Fütterung von ganzem Weizen auf die Mikroflora im Darm (log<sub>10</sub> Kolonie bildende Einheiten/ g Exkrement) vor (0 Tage vor Inokulation) und 14 Tage nach einer Kokzidieninfektion bei langsam wachsenden Masthühnern (n=12/ Behandlung)

Microflora	Age (d)	Post-inoculation period (d PI)	Treatment								SEM	P value		
			Uninfected		<i>E. acervulina</i>		<i>E. maxima</i>		<i>E. tenella</i>			Diet	Para-site	Diet x Parasite
			C	W	C	W	C	W	C	W				
<i>Lactobacillus</i>	22	0	8.0	7.8							0.23	0.65		
	36	14	7.6	7.6	7.7	7.3	7.3	7.9	7.9	7.4	0.23	0.49	0.90	0.12
<i>Enterococcus</i>	22	0	5.5	5.7							0.16	0.47		
	36	14	5.6	5.6	5.3	5.1	5.2	5.6	5.5	5.9	0.27	0.57	0.30	0.54
<i>E. coli</i>	22	0	5.5	5.1							0.19	0.28		
	36	14	5.1 b	5.1 b	5.4ab	5.0 b	5.1 b	5.1 b	5.6ab	6.3 a	0.28	0.73	**	0.28

\*: p &lt; 0.05; \*\*: p &lt; 0.01

d PI = day post-inoculation, C = complete diet, W = whole wheat / protein concentrate

SEM = Standard error of the mean

(a,b) Means, in the same line with different letters differ significantly (p ≤ 0.05).

species (*E. acervulina* and *E. maxima*), a higher oocyst excretion was observed with the caecal species (*E. tenella*) in W-fed birds compared to C-fed ones. This difference between diets might be explained by the modifications of the

digestive tract due to whole wheat feeding such as a higher development of the gizzard (FORBES and COVASA, 1995; GABRIEL et al., 2003) and of the pancreas (BANFIELD et al., 2002; ENGBERG et al., 2004). The higher mechanical

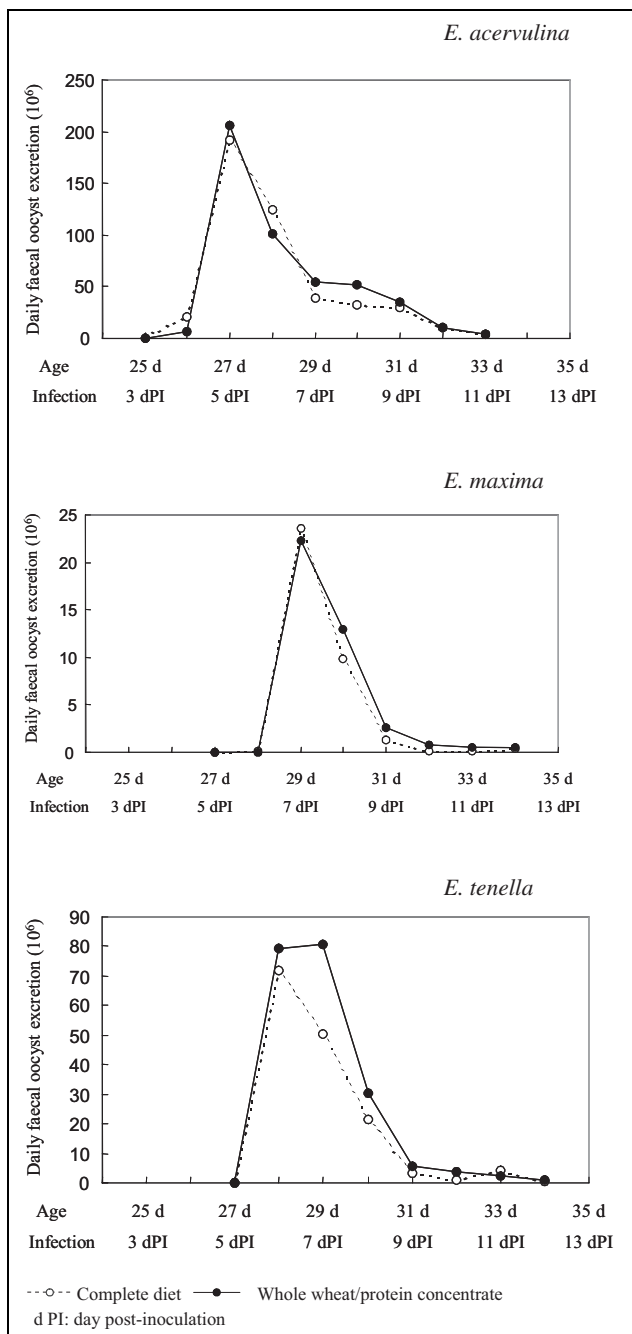


Figure 1. Effect of whole wheat feeding on daily oocyst excretion (per bird) during coccidiosis in chickens (one value per dietary group)  
*Einfluss der Fütterung von ganzem Weizen auf die tägliche Oozysten-Ausscheidung während einer Kokzidiose bei Masthühnern (ein Wert je Fütterungsgruppe)*

strength of the gizzard may help to break the oocyst wall, and thus increase parasite development. The higher development of the pancreas might also lead to higher pancreatic protease secretion, particularly chymotrypsin. These enzymes are implied in excystation of the sporocyst for the hydrolysis of the Stieda body (WANG, 1982). The susceptibility of the oocyst wall to mechanical breakdown and of the Stieda body of the sporocyst to enzymatic hydrolysis depend on the coccidial species and on its site of development (FARR and DORAN, 1962). Consequently, the modifications of digestive tract by whole wheat may have no effect on intestinal coccidial species. However the increase in *E.*

*tenella* development in 'label' chicken (x 1.3) was not as high as previously reported in fast-growing broiler chicken (x 10) (GABRIEL et al., 2006a). This lower effect of the diet on parasite development according to the line of chickens, might be due to differences in the levels of physiological modifications of the digestive tract due to whole wheat. For example, gizzard weight might be less modified by whole grains in slow-growing lines than in fast-growing lines, as the former have a more developed gizzard (BOA-AMPONSEM et al., 1991).

Although the effects of whole wheat feeding on parasite development varied according to *Eimeria* species, this mode of feeding, whatever the *Eimeria* species, led to more deleterious effects on the animal the first week post-inoculation compared to the control diet as observed in fast-growing broiler chickens (BANFIELD et al., 2002; GABRIEL et al., 2006a). The lower feed intake of W-fed birds compared to C-fed birds, may be related to a higher inflammatory response (KLASING et al., 1987) that might be due to a change in immune response of the digestive tract to the pathogen depending on the diet. This lower feed intake of W-fed birds contributed towards lower weight gain during the first week post-inoculation of coccidia compared to the control diet. From 6 to 7 d PI, the presence of compensatory growth of W-fed birds infected with *E. acervulina*, probably due to their higher protein intake compared to C-fed birds, led to no difference in weight between the two diets at the end of the period studied (42 d). For W-fed birds infected with *E. tenella*, the higher protein intake compared to C-fed birds, was probably insufficient to lead to compensatory weight gain. Thus, in the case of *E. tenella* and *E. maxima*, as no compensatory weight gain was observed, this led to a negative effect on weight at 42 d. Nevertheless, as the rearing period of slow-growing lines of chickens is about twelve weeks, they may reach their final weight later during the following weeks of rearing.

In addition to the effect of whole wheat on chicken performance during coccidiosis, this mode of feeding had consequences on the digestive microflora and immunity, which may have an impact on further infections which occur under field conditions. After *E. tenella* infection, an alteration of the microflora was observed, consisting in an increase in the *E. coli* population in W-fed birds but not in C-fed birds. Changes in the microflora following *Eimeria* infection (TURK and LITTLEJOHN, 1987), may be due to changes in the physiology of the digestive tract, such as a modification of intestinal cells with different receptors for bacteria (BABA et al., 1993), more substrate for bacteria (cells due to the epithelium degradation), a modification of intestinal contractility along the whole tract (OIKAWA and KAWAGUCHI, 1974, 1975) or an increased transit time (AYLOTT et al., 1968). These changes in the physiology of the digestive tract may depend on the diet. No microflora changes were observed with *E. acervulina* and *E. maxima* infection unlike in fast-growing broiler chickens (GABRIEL et al., 2006a). This might be due to the fact that the microflora may be different between fast and slow-growing lines, as it seems to depend on genetic factors (GABRIEL et al., 2006b) and may not be modifiable in a similar manner. As *E. tenella* develops in the caeca, where the microflora is the most abundant (GABRIEL et al., 2006b), this *Eimeria* species may have stronger effect.

After primary infection, birds are specifically immunized and antibodies can be detected in the sera. These antibodies are markers of the infection, but their role in the recovery or the protection of the chickens is not clearly established unlike cellular immunity (LILLEHOJ and LILLEHOJ, 2000). However, after a primary infection with *E. acervulina*, a secondary infection with the same coccidial species

was less pronounced in lines of birds which presented significantly higher total serum antibody titres (PARMENTIER et al., 2001). In our study, slow-growing lines of chickens fed W diet exhibited a more intense antibody response than C-fed birds against two of the three coccidial species, *E. acervulina* and *E. maxima*, and antibody response also tended to be higher with *E. tenella*. This difference in antibody response between diets may be explained by a change of the immune response of the digestive tract to *Eimeria* depending on the diet, as proposed with the inflammatory response leading to a lower feed intake in whole wheat-fed birds than in control-fed birds. Moreover the free choice feeding of whole wheat and protein concentrate, allowed birds to increased protein intake particularly in the case of birds infected with *E. acervulina*. These proteins may be used partly to maximise the immune response which requires large amounts of amino acids (TSIAGBE et al., 1987). For *E. tenella*-infected birds, changes in the microflora may also be involved in the increased antibody response as the microflora has an effect on immunity (GABRIEL et al., 2006b). Likewise, W-fed birds which were more affected during the first infection and produced more immunoglobulins may be more protected during further infections that occur under field conditions.

In conclusion, after an experimental infection with the three most important species of chicken coccidia (*E. acervulina*, *E. maxima* and *E. tenella*) at 22 d of age (age of contamination in field condition), free choice feeding of whole wheat and a protein concentrate to slow-growing chickens under our experimental conditions severely affected bird performance compared to a ground pelleted diet, as it was observed previously in fast-growing broiler chickens. However, a compensatory growth was observed with one of the three species (*E. acervulina*) resulting for this species in no difference in weight between diets after the three weeks following inoculation. With the two other coccidial species, although no compensatory growth was observed during the period studied (until 20 d PI), chicken may reach their final weight later during the following weeks of rearing. Moreover, the higher antibody production observed in birds fed whole wheat might be a protection against further infections. Contrarily to fast-growing broiler chickens, only a slightly higher oocyst excretion of *E. tenella* was observed with whole wheat, thus the W diet did not lead to a higher potential contamination under field conditions. Thus, in birds experimentally infected with *Eimeria* whole wheat had a more detrimental effect than ground wheat during the first week post-inoculation. However, under field conditions, the effect at the end of the rearing period may be not detrimental for birds, which need to be confirmed by studies carried out under field conditions.

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### Summary

Whole wheat feeding has been shown to have an effect on coccidial development in fast-growing standard lines of chickens. As whole wheat is used for rearing slow-growing chickens, the effect of this mode of feeding was studied in this type of chickens during an experimental coccidiosis. After two weeks of consumption of whole wheat and a pelleted protein concentrate (W) or a complete ground and

pelleted diet (C), birds of each dietary treatment were either used as uninfected control, or inoculated (22 d of age) with either 250,000 oocysts of *Eimeria acervulina*, 5,000 oocysts of *E. maxima*, or 20,000 oocysts of *E. tenella*, infecting duodenum, jejunum/ileum or caeca, respectively. Whatever the *Eimeria* species, whole wheat led to a lower weight gain than the C diet: from 4 to 5 day post-inoculation (d PI) with *E. acervulina*, from 5 to 7 d PI with *E. maxima* and from (-1) to 5 d PI with *E. tenella*. From 6 to 7 d PI, *E. acervulina*-infected birds fed with W diet showed a higher weight gain than their uninfected counterparts. The specific serum IgG antibody response to *E. acervulina* and *E. maxima* was higher with the W than the C diet, and tended to be higher with *E. tenella*. Total oocyst excretion was similar between the two diets for *E. acervulina* and *E. maxima*, and slightly increased in *E. tenella*-infected W-fed compared to C-fed chickens. After coccidial infection (14 d PI), no effect on the faecal microflora was observed in C-fed birds, while in *E. tenella*-infected W-fed chickens *E. coli* counts were increased. In conclusion, feeding wheat as whole grain compared to ground wheat to slow-growing chickens led to more detrimental effects during an experimental coccidial infection, but may have no impact on the weight at the end of the rearing period.

### Key words

Slow-growing broilers, nutrition, whole wheat, coccidiosis

### Zusammenfassung

#### Einfluss der Fütterung von ganzem Weizen auf die Entwicklung von Kokzidiosen bei langsam wachsenden Masthühnern

In früheren Untersuchungen wurde über einen positiven Einfluss von der Fütterung ganzer Weizenkörner auf die Entwicklung von Kokzidiosen bei schnell wachsenden Masthühnern berichtet. In der vorliegenden Untersuchung sollte daher geprüft werden, ob dieser Einfluss auch bei langsam wachsenden Masthühnern nach einer Kokzidieninfektion auftritt. Hierzu wurden langsam wachsende Broiler entweder mit einem Ergänzter und ganzem Weizen (W) oder einem pelletierten Alleinfutter (C) gefüttert. Am 22. Lebenstag wurden Tiere jeder Vorfütterung aufgeteilt in Kontrolltiere und in Behandlungstiere, die zur Infektion von Duodenum, Jejunum/Ileum bzw. Blinddarm jeweils entweder mit 250.000 Oozysten von *Eimeria acervulina*, 5.000 Oozysten von *E. maxima* oder 20.000 Oozysten von *E. tenella* inokuliert wurden.

Unabhängig vom Eimeriatyp führte die Fütterung von ganzem Weizen zu geringeren Zunahmen: 4-5 Tage nach der Inokulation (PI) mit *E. acervulina*, 5-7 Tage PI mit *E. maxima* und 0-5 Tage PI mit *E. tenella*. Zwischen dem 6. und 7. Tag PI zeigten die mit *E. acervulina* inokulierten Tiere bei Weizenfütterung höhere Zunahmen als die Tiere der nicht infizierten Gruppe. Die spezifischen IgG Antikörper-Titer gegen *E. acervulina* und *E. maxima* waren bei Weizenfütterung höher als in der Kontrollgruppe mit Alleinfutter und waren für *E. tenella* tendenziell höher. Die Oozysten-Ausscheidung war bei beiden Futtergruppen für *E. acervulina* und *E. maxima* ähnlich. Die Ausscheidung an *E. tenella* Oozysten war in der Behandlung W tendenziell höher als in der Kontrollgruppe. Nach der Kokzidien-Infektion (14 Tage PI) wurde bei den mit dem Alleinfutter gefütterten Tieren kein Einfluss auf die



Darmmikroflora festgestellt, während bei mit *E. tenella* infizierten Tieren der Weizenfütterung erhöhte *E. coli*-Zahlen registriert wurden. Es kann der Schluss gezogen werden, dass die Fütterung von ganzem Weizen bei langsam wachsenden Masthühnern zwar zu mehr schädlichen Effekten nach einer experimentellen Kokzidien-Infektion führt, aber keinen Einfluss auf das Lebendgewicht am Mastende hat.

## Stichworte

Broiler langsam wachsend, Fütterung, ganzer Weizen, Kokzidiose

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