Effects of whole wheat feeding on the development of coccidial infection in broiler chickens

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Effects of Whole Wheat Feeding on the Development of Coccidial Infection in Broiler Chickens

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ABSTRACT A complete ground and pelleted feed was compared to free choice feeding of whole wheat and a pelleted protein concentrate during three experimental infections with coccidia in broiler chickens. At 22 d of age birds were inoculated with different doses of sporulated oocysts of a cecal species (*Eimeria tenella*) in experiment 1 or intestinal species *E. maxima* or *E. acervulina* in experiments 2 and 3, respectively. The effects of diets were assessed on weight gain, hematocrit (during cecal coccidiosis), serum coloration (during intestinal coccidiosis), oocyst excretion, and lesion score until 7 d post-inoculation. In experiment 1 before inoculation, the birds fed whole grain had more beneficial microflora with lower counts of coliform bacteria. As shown by oocyst output and lesion score, whole wheat feeding increased parasite development during infection with the cecal parasite *E. tenella*. This led to significantly lower weight gain with whole wheat than with ground wheat from 5 to 6 d post-inoculation and to lower hematocrit at the highest infective dose. Parasite development in experiments 2 and 3 was similar among diets, during intestinal infection with *E. maxima* and *E. acervulina*, respectively, with no significant differences in lesion score. During the acute phase (4 to 7 and 3 to 5 d post-inoculation), when a difference appeared between diets, whole wheat fed-birds were always more affected than ground diet-fed birds in terms of serum coloration and weight gain. These results might be explained by modifications of digestive physiology and intestinal microflora by whole wheat.

(*Key words*: broiler chicken, coccidiosis, whole grain, wheat)

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INTRODUCTION

Coccidiosis is an important disease in intensive poultry production, leading to significant economic losses of up to $1 billon annually (Augustine, 2000). It is controlled by chemotherapy using anticoccidial drugs (synthetic products or antibiotic ionophores) in feed. The appearance of resistance to coccidiostats, consumer demand for fewer feed additives, and European Union regulations (withdrawal of antibiotic feed additives as a precautionary measure) might restrict the use of coccidiostats in the future. If this happens, methods of production, management, and hygiene will be changed. Therefore in the absence of anticoccidial vaccines that are not yet generally available in broiler production or to complement their action, feed composition, or presentation may be used as an alternative to help control coccidiosis. Several dietary supplements such as vitamins, n-3 fatty acids, and plant extracts have been reported to have beneficial effects (Allen et al., 1998; Banfield and Forbes, 1999; Crévieu-Gabriel and Naciri, 2001).

Feeding whole grain to poultry has become a more common practice in Europe for economic reasons and to meet consumer demands for more "natural" feeding systems (Cumming, 1992a; Noirot et al., 1998). However little is known about the effects of such a practice on animal health. Cumming (1987) reported that whole grain feeding led to decreased oocyst output and lower mortality when chickens were infected with a single mixed dose of *Eimeria tenella* (70%), *E. acervulina* (20%), and *E. maxima* (10%). These findings were confirmed by floor pen studies with natural coccidiosis challenge in the litter (Cumming, 1989). In the past 5 yr, European teams have studied the effects of whole wheat feeding on coccidiosis. However, they reported no effects of whole grain feeding (Waldenstedt et al., 1998; Banfield and Forbes, 2001) or even detrimental effects (Banfield et al., 1999, 2002). Several parameters varied in these different studies, including parasite dose, species and strains of coccidia, ages and strains of birds, age of introduction of whole grain, quantity and type of whole grain fed, and age of inoculation. As pre-

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Abbreviation Key: CG = complete ground diet; PI = post-inoculation; WP = whole wheat plus protein concentrate diet.

TABLE 1. Composition of complete ground diet

Ingredients, g/kg	Complete ground diet
Wheat	400.0
Maize	147.6
Soybean meal	360.0
Rapeseed oil	50.0
DL-Methionine	1.4
$CaCO_3$	14.0
Ca_2PO_4	17.0
NaCl	4.0
Mineral mixture[1]	1.0
Vitamin mixture[2]	5.0
Calculated analysis	
AME, kcal/kg	3,090
Crude protein	228
Methionine + cystine	9.0
Lysine	12.9
Calcium	10.6
Available phosphorus	4.2

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

[2]The vitamin mixture supplied the following vitamins (per kg of diet): 10,000 IU vitamin A (all-*trans*-retinol), 1,500 IU vitamin D_3 (cholecalciferol), 15 IU vitamin E (DL-α-tocopheryl acetate, 125 mg butylated hydroxy toluene, 1.25 mg vitamin K_3 (menadione), 0.5 mg thiamin, 3.2 mg riboflavin, 3.6 mg calcium pantothenate, 25 mg niacin, 1 mg pyridoxine, 8 μg cobalamin (vitamin B_{12}), 1.5 mg folic acid, 0.2 mg biotin, 0.75 mg choline chloride. These vitamins were mixed with oats (65% of vitamin mixture) before mixing the vitamin mixture with other ingredients.

viously observed for other compounds such as n-3 fatty acids, whole grain could have different effects depending on *Eimeria* species (Allen et al., 1997a), and diet effects may appear only with certain doses of coccidia.

The following experiments were undertaken to assess the effects of whole grain during coccidiosis. Studies were performed on broilers with three of the most common species of coccidia (*E. tenella*, *E. maxima*, and *E. acervulina*) infecting different parts of the digestive tract by using a wide range of parasite doses.

MATERIALS AND METHODS

Experimental Diets

Two diets were used for these experiments: a complete ground and pelleted diet (CG) and whole grain wheat with pelleted protein concentrate (WP). The CG diet was composed primarily of wheat (40%), soybean meal, maize, and a vitamin mineral mixture with no coccidiostat included in the diet (Table 1). The same batch of wheat was used for CG diet and whole grain wheat. The wheat used (88% of dry matter) was a mixture of two varieties (Altria and Tremie) frequently used in poultry nutrition. Both have moderate viscosity with Altria a soft variety and Tremie a medium to hard variety (ITCF, 2001). The protein concentrate comprised the same ingredients as CG diet except that wheat was removed from the formulation. When added to whole grain wheat at the ratio 60:40 this protein concentrate provided the same nutrients as CG diet. The protein concentrate and diet CG were pelleted (2.5 mm diameter) without steam (temperature between 45 and 50°C). Pelleting at low temperatures limits biochemical changes that may affect nutrient availability (Pettersson et al., 1991). Thus, the difference between the two dietary treatments was mainly the structure of the wheat (ground or whole grains). The protein concentrate and whole wheat were offered in two separate feeders. To accustom the chickens to whole wheat, grains were coarsely ground for the first 2 d of feeding. No grit was provided.

Birds and Housing

For each experiment, 100 male Ross broiler chickens (1 d of age) vaccinated against infectious bronchitis were obtained from a commercial hatchery.[2] They were raised in wire-floored cages in thermostatically controlled heated battery in overpressure in order to avoid any contamination. Therefore chickens had same initial immune status. Lighting and temperature conditions were as described by Gabriel et al. (2003). From 0 to 6 d of age, all chickens were fed the CG diet and received water ad libitum. At 7 d of age, birds of extreme weights were discarded, and 72 birds were selected and separated into two dietary treatment groups of similar body weights. Birds were housed three per cage, with 12 cages per group; one group was fed the CG diet, and the other received whole grain and protein concentrate. The concentrate was restricted to encourage birds to eat whole wheat. Water was available ad libitum for all birds. On d 14, all WP-fed chickens were individually housed (36 cages) to measure individual feed intake. Birds received whole grain and protein concentrate by free choice feeding. CG-fed chickens of extreme weight were discarded and 24 birds were selected and housed two per cage in the 12 remaining cages of the battery. Cage size and access to feeders allowed the two birds to eat together without feed restriction. On d 21, WP-fed birds of extreme weight or not eating enough whole wheat were discarded, and 24 chickens were selected. Each dietary group of chickens was then separated into four groups of similar weight (six chickens per group), and birds were individually housed until the end of the experiments. The birds in groups eating the WP diet consumed similar levels of wheat from 14 to 21 d. From 21 d to the end of the experiment, these birds received protein concentrate and whole wheat ad libitum.

Parasites

The strains of *E. tenella*, *E. maxima*, and *E. acervulina* used for the experiments were isolated from natural cases in commercial broiler houses in France. They were purified and maintained by serial passages through chickens in our facilities.

[2]Sicamen, Volnay, France.

Experimental Protocols

Experiments were conducted in accordance with the principles and specific guidelines presented in Decree No. 2001-646 (2001). Birds were infected at 22 d of age by crop intubation. They received 0.5 mL of an oocyst suspension containing different doses of coccidia. Uninfected control chickens received 0.5 mL of water.

Experiment 1. At 22 d of age, chickens were infected with 0, 5,000, 10,000, or 20,000 oocysts of *E. tenella*, a parasite of chicken ceca. Body weight was recorded prior to infection at 22 d (0 d post-inoculation; PI) and from 26 d (4 d PI) to 29 d (7 d PI). Blood was collected from the main wing vein at 5 d PI for hematocrit determination. Feces were collected from 6 to 7 d PI for oocyst count. Samples of feces without cecal content were collected before inoculation (21 d) and at 5 d PI (27 d) for microflora counts. At 7 d PI, chickens were weighed and killed by intracardiac injection of pentobarbital, and ceca were scored for lesions.

Experiment 2. The infective doses used were 0, 500, 5,000, or 50,000 oocysts of *E. maxima*, a parasite of the median and distal portions of the small intestine. Chickens were weighed as in the first experiment, and blood was collected at 6 d PI for determination of serum coloration. Feces were collected from 6 to 7 d PI for oocyst counts. At 7 d PI, chickens were killed, and the jejunum and ileum were scored for lesions.

Experiment 3. The infective doses used were 20,000, 60,000, 180,000, and 540,000 oocysts of *E. acervulina*, a parasite of the duodenum. In order to test four infective doses for this species, no uninfected group was included in this experiment. Body weight was recorded prior to infection at 22 d (0 d PI), and from 25 d (3 d PI) to 29 d (7 d PI). Blood was collected at 4 d PI to determine serum coloration. Feces were collected from 3 to 7 d PI for oocyst counts. As in previous experiments, chickens were killed at 7 d PI, and the lesions in the duodenum were scored.

Analyses

To compare the two dietary treatments on development of coccidial infection, we have used the main criteria laid down in the guidelines of Commission Directive 2001/79/EC (2001).

Serum Coloration. Serum carotenoid levels were estimated by direct colorimetry as described by Yvoré et al. (1993). Analyses were carried out in 96-well microtiter plates. Absorbance was read with a multiscan spectrophotometer at 490 nm (LP 500 microplate reader).[3]

Hematocrit. A criterion to assess *E. tenella* infection is packed blood cell volumes (Visco, 1975; Ryley and Hardman, 1978; Yvoré et al., 1980). In this species, second-generation meronts, which develop deeply in the cecal mucosa, cause hemorrhages when they burst out 5 d PI. Packed blood cell volumes were determined by a microhematocrit method (Johnson, 1955). Blood samples were collected in heparinized capillary tubes (75 mm, diameter 1.3 to 1.4 mm).[4] The tubes were sealed and centrifuged for 10 min at 10,000 rpm (MIKRO 20).[5] The packed red corpuscle volume was read directly as a percentage on a graphic reader.

Oocyst Excretion. Oocyst numbers were determined using a McMaster counting chamber (double standard)[6] (Raynaud, 1970) and expressed as log of total number of oocysts shed per chicken.

Lesion Score. The lesions were scored macroscopically from 0 (no lesions) to 4 (severe lesions) as described by Johnson and Reid (1970). This criterion is crucial to assess *Eimeria* pathogenicity (Yvoré et al., 1980; Allen et al., 2000).

Microflora Counts. Viable bacteria were counted in feces after successive 1/10 dilution in 0.9% NaCl. Lactobacilli and coliform bacteria were counted using Difco Lactobacilli MRS broth[7] (Man, Rogosa, Sharpe) and Drigalski lactose[8] growth culture media, respectively. Media were incubated aerobically at 37°C for 1 d (Drigalski agar) or 2 d (MRS agar). Results were expressed as the log of colony-forming units per gram of feces.

Statistical Analysis

Data were computed using Statview 5 software.[9] Significant differences between two treatment group means (dietary treatments) were determined by Student's *t*-test ($P \leq 0.05$). Significant differences between four treatment group means (parasite doses) were determined by ANOVA. When appropriate, means were separated using Student-Newman-Keuls' test ($P \leq 0.05$).

RESULTS

Experiment 1. E. tenella

One week before inoculation (14 to 21 d of age), the mean level of wheat consumption was 40 ± 2% (mean ± SEM). After inoculation (22 to 29 d of age), level of wheat consumption was independent of the infective dose (43 ± 3%).

From 22 to 29 d, no effect of dietary treatment was observed on weight gain of uninfected birds (Table 2). No significant differences in weight gain were observed between groups for infected birds from 0 to 4 d PI. Weight gains were not significantly different between infected birds and uninfected chickens for CG-fed birds during the period studied. Weight gains of WP-fed chickens infected with the highest dose were lower than uninfected birds from 4 to 5 d PI. From 5 to 6 d PI, weight gains of all infected WP-fed birds were lower than those of their

[3]Bio-Rad, Marnes la coquette, France.
[4]Merck Eurolab Polylabo, Strasbourg, France.
[5]Hettich Zentrifugen, Tuttlingen, Germany.
[6]Merck Eurolab Polylabo, Strasbourg, France.
[7]Reference 288130, Becton, Dickinson and Company, Le pont de claix, France.
[8]Reference 64664, Bio-Rad, Marnes la coquette, France.
[9]Abacus Concepts, Berkeley, California.

TABLE 2. Effects of whole wheat feeding on various parameters during *Eimeria tenella* infection in broiler chickens[1] until 7 d post-inoculation

Parameter	Age (d)	Infective period (d PI)[2]	Diet[3]	Parasite dose (oocysts/chick) 0	5,000	10,000	20,000
Weight gain, g/d	22–26	0–4	CG	69 ± 3	69 ± 5	70 ± 5	68 ± 4
			WP	73 ± 7	69 ± 6	74 ± 9	72 ± 4
	26–27	4–5	CG	69 ± 1	65 ± 7	64 ± 20	64 ± 6
			WP	74 ± 9^{a}	50 ± 8^{ab}	49 ± 8^{ab}	33 ± 13^{b}
	27–28	5–6	CG	69 ± 1	58 ± 9^{y}	63 ± 9^{y}	52 ± 8^{y}
			WP	76 ± 10^{a}	18 ± 15^{b,z}	10 ± 22^{b,z}	11 ± 16^{b,z}
	28–29	6–7	CG	70 ± 1	71 ± 8^{z}	65 ± 5	75 ± 4^{z}
			WP	73 ± 10	114 ± 7^{y}	85 ± 25	94 ± 6^{y}
	22–29	0–7	CG	69 ± 2	67 ± 4	67 ± 4	66 ± 3
			WP	74 ± 7	65 ± 4	63 ± 9	61 ± 4
Hematocrit, %	27	5	CG	31.0 ± 1.5	32.2 ± 1.2	31.6 ± 1.6	31.3 ± 1.0^{y}
			WP	31.8 ± 1.8	31.5 ± 0.8	31.0 ± 1.9	27.8 ± 1.2^{z}
Oocyst output, Log	28–29	6–7	CG	0	7.3 ± 0.3^{z}	7.3 ± 0.3	7.4 ± 0.2^{z}
			WP	0	8.1 ± 0.2^{y}	7.7 ± 0.3	8.0 ± 0.1^{y}
Lesion score[4]	29	7	CG	0	1.8 ± 0.7^{z}	0.9 ± 0.5^{z}	2.5 ± 0.5^{z}
			WP	0	3.9 ± 0.1^{y}	4.0 ± 0.0^{y}	4.0 ± 0.0^{y}
Escherichia coli, log cfu	27	5	CG	4.7 ± 0.6	4.8 ± 0.8	3.7 ± 0.8	4.3 ± 0.4
			WP	3.9 ± 0.5	3.6 ± 0.6	4.6 ± 0.8	4.4 ± 0.8
Lactobacillus, log cfu	27	5	CG	6.8 ± 0.3	7.2 ± 0.5	7.4 ± 0.3	6.9 ± 0.3
			WP	6.5 ± 0.3	6.8 ± 0.2	6.6 ± 0.4	7.4 ± 0.5

^{a,b}Means ± SEM. Within variable rows, means without common superscript letter differ significantly ($P \leq 0.05$).

^{y,z}Means ± SEM. Within variable columns, means without common superscript letter differ significantly ($P \leq 0.05$).

[1]The number of birds per group was six.

[2]d PI = days post-inoculation.

[3]CG = complete ground diet; WP = whole wheat and protein concentrate diet.

[4]Scores ranged from 0 to 4, indicating no lesion to severe lesions, respectively.

uninfected counterparts. No further differences were observed between groups from 6 to 7 d PI. When comparing WP-fed birds to CG-fed birds weight gains from 4 to 5 d PI tended to be lower for WP-fed birds ($P = 0.06$) with the highest infective dose and was significant for all infective doses from 5 to 6 d PI. On the other hand, from 6 to 7 d PI, weight gains were higher for chickens fed the WP diet for the lowest and highest infective doses. The lack of significant difference with the intermediate dose was due to wide variability in the whole grain eating group. No differences in weight gain were observed between diets over the whole study period (from 0 to 7 d PI), and no mortality due to coccidiosis occurred.

A lower hematocrit was observed at 5 d PI with the highest infective dose in WP-fed birds compared to CG-fed birds.

No oocysts were detected in the feces of uninfected birds from 6 to 7 d PI. Oocyst outputs were significantly higher for infected birds in WP-fed chickens compared to CG-fed chickens for the lowest and highest infective doses. Lesion scores in ceca at 7 d PI showed no contamination of uninfected birds. No dose effect was observed for infected birds, whatever the dietary treatment. Lesion scores for each dose were higher for WP-fed chickens than for CG-fed chickens. All chickens fed the WP diet reached the maximum score of 4 except for one chick inoculated with the lowest dose.

The CG-fed chickens excreted more *Escherichia coli* per gram of feces than WP-fed chickens before inoculation (3.9 ± 0.3 and 3.1 ± 0.2 log cfu, respectively, data not shown in Table 2). No differences were observed between CG and WP diets for *Lactobacillus* (6.5 ± 0.2 and 6.4 ± 0.2 log cfu, respectively). No significant differences were observed between dietary treatments or infective doses of *E. tenella* at 5 d PI whatever the bacteria species (Table 2).

Experiment 2. E. maxima

One week before inoculation (14 to 21 d of age), level of wheat consumption was 44 ± 1%. As in the first experiment after inoculation, level of wheat consumption was independent of the infective dose (44 ± 2%).

Weight gain was greater in uninfected WP-fed birds compared to CG-fed birds from 22 to 29 d of age (Table 3). Weight gains were not significantly different between groups for infected birds from 0 to 4 d PI. From 5 d PI, CG-fed birds infected with the highest infective dose had lower weight gain than their uninfected counterparts. Weight gain was lower in infected WP-fed birds than their uninfected counterparts from 4 d PI with the intermediate and highest infective doses, and from 5 d PI they had lower weight gain with all infective doses. Comparison of WP-fed birds and CG-fed birds from 4 to 5 d PI showed lower weight gains for WP-fed birds with the intermedi-

TABLE 3. Effects of whole wheat feeding on various parameters during *Eimeria maxima* infection in broiler chickens[1] until 7 d post-inoculation

Parameter	Age (d)	Infective period (d PI)[2]	Diet[3]	Parasite dose (oocysts/chick) 0	500	5,000	50,000
Weight gain, g/d	22–26	0–4	CG	79 ± 1	74 ± 3	79 ± 4	79 ± 9
			WP	86 ± 3	76 ± 3	80 ± 4	80 ± 5
	26–27	4–5	CG	77 ± 3^{z}	79 ± 8	71 ± 6^{y}	49 ± 16^{y}
			WP	92 ± 5^{ay}	85 ± 6^{a}	5 ± 13^{bz}	11 ± 5^{bz}
	27–28	5–6	CG	77 ± 4^{az}	68 ± 7^{ay}	54 ± 10^{ay}	–14 ± 30^{b}
			WP	90 ± 4^{ay}	18 ± 17^{bz}	–35 ± 14^{bz}	–41 ± 34^{b}
	28–29	6–7	CG	81 ± 3^{a}	77 ± 4^{ay}	41 ± 14^{ay}	–34 ± 41^{b}
			WP	87 ± 4^{a}	–26 ± 22^{bz}	–49 ± 20^{bz}	–21 ± 29^{b}
	22–29	0–7	CG	79 ± 1^{az}	74 ± 3^{ay}	69 ± 4^{ay}	45 ± 11^{b}
			WP	87 ± 3^{ay}	55 ± 5^{bz}	35 ± 4^{cz}	38 ± 10^{bc}
Serum coloration, optical density at 490 nm	28	6	CG	0.32 ± 0.02^{az}	0.24 ± 0.03^{ab}	0.17 ± 0.02^{b}	0.16 ± 0.04^{b}
			WP	0.45 ± 0.01^{ay}	0.16 ± 0.02^{b}	0.13 ± 0.01^{b}	0.14 ± 0.02^{b}
Oocyst output, log	28–29	6–7	CG	0	6.9 ± 0.2^{z}	7.1 ± 0.2	7.1 ± 0.2
			WP	0	7.6 ± 0.1^{y}	7.5 ± 0.1	7.4 ± 0.2
Lesion score[4]	29	7	CG	0	1.0 ± 0.0^{b}	1.5 ± 0.2^{b}	2.5 ± 0.5^{a}
			WP	0	1.7 ± 0.3	2.2 ± 0.3	2.2 ± 0.5

$^{a–c}$Means ± SEM. Within variable rows, means without common superscript letter differ significantly ($P \leq 0.05$).

^{y,z}Means ± SEM. Within variable columns, means without common superscript letter differ significantly ($P \leq 0.05$).

[1]The number of birds per group was six.

[2]d PI = days post-inoculation.

[3]CG = complete ground diet; WP = whole wheat and protein concentrate diet.

[4]Scores ranged from 0 to 4, indicating no lesion to severe lesions, respectively.

ate and highest infective doses, and from 5 to 7 d PI WP-fed chickens gained significantly less than CG-fed chickens with the lowest and intermediate infective doses, but the difference disappeared with the highest dose. During the whole observation period (from 0 to 7 d PI), WP-fed birds were affected with the lowest infective dose, whereas CG-fed birds were affected only with the highest infective dose. WP-fed birds had lower weight gain than CG-fed birds with the lowest and intermediate infective doses. No mortality occurred during infection.

The intermediate and highest infective doses led to lower serum coloration for CG-fed chickens compared to their uninfected control group. All infected WP-fed chickens had lower serum coloration than their controls. No significant differences were observed between diets for the infected groups.

No oocysts were counted in the feces of uninfected birds from 6 to 7 d PI. Significantly higher excretion was observed with the lowest dose in infected WP-fed birds compared to infected CG-fed birds. No lesions were observed in the small intestine of uninfected birds at 7 d PI. A higher score was observed with the highest dose with the CG diet, whereas no dose effect was noted with the WP diet. No differences were observed between diets for each infective dose.

Experiment 3. E. acervulina

One week before inoculation (14 to 21 d of age), wheat consumption level was 40 ± 2%. As with the other experiments, wheat consumption level after inoculation was independent of the infective dose. However, the mean level was slightly lower than in the previous experiments (36 ± 2%).

Weight gains from 0 to 3 d PI were not affected by diet or parasite dose (Table 4). For CG-fed chickens the highest infective dose led to lower weight gain than other infective doses from 4 to 6 d PI. During the whole study (from 0 to 7 d PI) only the highest infective dose led to lower weight gain than the lowest for this diet. For WP-fed chickens, the highest infective dose led to lower weight gain than the other infective doses from 3 to 4 d PI. No differences in weight gain were observed from 4 to 5 d PI. Weight gains were significantly higher with 60,000 oocysts than with 540,000 oocysts from 5 to 6 d PI. During the whole study (from 0 to 7 d PI) the highest infective dose led to lower weight gains than the two lowest infective doses. Significant differences were observed when the two diets were compared. From 3 to 4 d PI, WP-fed chickens were significantly more affected than CG-fed chickens whatever the infective dose. The same pattern was observed from 4 to 5 d PI, but it was not significant for 180,000 oocysts. Conversely, weight gains in WP-fed chickens were higher than in CG-fed chickens from 5 to 6 d PI with 60,000 oocysts. No differences in weight gain were observed between diets during the whole study (from 0 to 7 d PI).

Serum coloration was significantly affected by diet and by infective dose at 4 d PI. For CG-fed chickens serum coloration was lower with the highest infective dose but no dose effect was observed for WP-fed chickens. Serum carotenoid levels were lower in WP-fed birds than in CG-fed birds for all infective doses except the highest.

TABLE 4. Effects of whole wheat feeding on various parameters during *Eimeria acervulina* infection in broiler chickens[1] until 7 d post-inoculation

Parameter	Age (d)	Infective period (d PI)[3]	Diet[3]	Parasite dose (oocysts/chick) 20,000	60,000	180,000	540,000
Weight gain, g/d	22–25	0–3	CG	67 ± 9	65 ± 6	56 ± 3	68 ± 3
			WP	77 ± 6	70 ± 5	68 ± 5	70 ± 4
	25–26	3–4	CG	91 ± 5^{y}	77 ± 6^{y}	84 ± 8^{y}	74 ± 8^{y}
			WP	56 ± 9^{az}	26 ± 10^{az}	30 ± 14^{az}	–17 ± 15^{bz}
	26–27	4–5	CG	59 ± 8^{ay}	57 ± 10^{ay}	54 ± 13^{a}	–1 ± 12^{by}
			WP	25 ± 6^{z}	27 ± 9^{z}	18 ± 21	–19 ± 15^{z}
	27–28	5–6	CG	84 ± 10^{a}	68 ± 10^{az}	80 ± 10^{a}	37 ± 10^{b}
			WP	89 ± 10^{ab}	111 ± 7^{ay}	66 ± 13^{ab}	60 ± 17^{b}
	28–29	6–7	CG	85 ± 9	82 ± 10	67 ± 7	90 ± 11
			WP	87 ± 8	85 ± 5	78 ± 9	91 ± 12
	22–29	0–7	CG	74 ± 4^{a}	68 ± 4^{ab}	63 ± 3^{ab}	58 ± 3^{b}
			WP	70 ± 4^{a}	65 ± 3^{a}	56 ± 3^{ab}	46 ± 6^{b}
Serum coloration, optical density at 490 nm	26	4	CG	0.30 ± 0.04^{ay}	0.26 ± 0.04^{ay}	0.25 ± 0.03^{ay}	0.14 ± 0.02^{b}
			WP	0.19 ± 0.01^{z}	0.15 ± 0.01^{z}	0.15 ± 0.03^{z}	0.11 ± 0.02
Oocyst output, log	25–29	3–7	CG	8.4 ± 0.1^{b}	9.0 ± 0.2^{a}	8.6 ± 0.2^{ab}	8.9 ± 0.1^{aby}
			WP	8.8 ± 0.2	8.6 ± 0.1	8.6 ± 0.1	8.4 ± 0.2^{z}
Lesion score[4]	29	7	CG	0.8 ± 0.2	1.5 ± 0.2	1.3 ± 0.3	1.3 ± 0.2
			WP	1.2 ± 0.2	1.0 ± 0.3	0.8 ± 0.2	0.8 ± 0.2

[a,b]Means ± SEM. Within variable rows, means without common superscript letter differ significantly ($P \leq 0.05$).

[y,z]Means ± SEM. Within variable columns, means without common superscript letter differ significantly ($P \leq 0.05$).

[1]The number of birds per group was six.

[2]d PI = days post-inoculation.

[3]CG = complete ground diet; WP = whole wheat and protein concentrate diet.

[4]Scores ranged from 0 to 4, indicating no lesion to severe lesions, respectively.

Oocyst excretion from 3 to 7 d PI was higher in CG-fed birds than WP-fed birds only with the highest infective dose. Lesion scores at 7 d PI in the duodenum showed no diet or dose effects.

DISCUSSION

As the structure of diet can affect the parts of the digestive tract differently (Nir et al., 1994; Gabriel et al., 2002), the incidence of coccidiosis may vary according to the location of parasite development. For this reason we compared WP and CG diets by using birds infected with the three main chicken *Eimeria* species, *E. tenella*, *E. maxima*, and *E. acervulina*, which are specific to different intestinal locations; the ceca, jejunum and ileum, or duodenum, respectively.

Chickens consuming whole wheat were more affected than CG-fed chickens in all of the coccidia species studied. However, the incidence of the diet on parasite development differed between cecal and intestinal species.

Effects of Whole Wheat on Cecal Infection (E. tenella)

The infective doses of *E. tenella* used in the experiment were chosen to be low to moderate in order to emphasize the dietary effects on *E. tenella* infection. Low and medium lesion scores were therefore observed in ceca with our control diet CG. As this species is localized in the ceca, weight gain can be unaffected as observed for CG diet. The two phases observed are in concordance with the *E. tenella* life-cycle: an acute phase from 4 to 6 d PI, with hemorrhage due to the bursting of second generation meronts, followed by a recovery phase.

The pathogenicity of *E. tenella* was increased in WP-fed birds during the acute phase compared to CG-fed birds, as shown mainly by increased cecal lesion scores (maximum scores with WP diet) and also by decreased hematocrits or increased oocyst excretion with some parasite doses. As a consequence WP-fed birds were always affected, whereas weight gain in CG-fed birds was not affected at any infective dose. The weight gains thus decreased with the highest infective dose from 4 d PI and from 5 d PI with all the infective doses. These results are in contradiction with those of Cumming (1987) who observed lower oocyst excretion with whole grain and protein concentrate compared to the same diet finely ground and pelleted with a mixed inoculation containing 70% of *E. tenella* oocysts. This apparent contradiction could be due to differences in several parameters. The rate of whole grain incorporation was 60 to 70% in the experiment by Cumming instead of 40% in our study, and the energy of those diets (2,800 to 2,900 kcal/kg) was lower than that of our diets (3,090 kcal/kg). Birds were infected later than in our study, i.e., at 28 d of age instead of 22 d, an age when birds are more susceptible to coccidiosis (Gerriets, 1961). The time of whole grain feeding before inoculation may also have an effect on infection, as observed with other compounds in the diet such as artemisin from *Artemisia annua* (Allen et al., 1997b). In

Cumming's study, birds ate whole wheat for 21 d before inoculation instead of the 15 d in our experiment.

As shown by lesion score and oocyst excretion, whole wheat increased parasite development. This higher rate of development may be explained by changes in digestive tract physiology. It has been observed that feeding whole grain or large particle size in the diet led to higher gizzard weight and lower pH of its content (Cumming, 1992b; Nir et al., 1994; Forbes and Covasa, 1995; Gabriel et al., 2003). As coccidian sporocysts are believed to be mechanically liberated from the oocysts in the gizzard (Farr and Doran, 1962; Fernando, 1990), a higher level of development of this organ may help break the oocyst wall and thus increase parasite infection. However, this stage seems not to be essential for oocyst excystation (Ikeda, 1956). Other changes in the digestive tract may be involved. Banfield et al. (2002) showed that whole wheat leads to higher pancreas weights which may, in turn, lead to higher pancreatic protease secretion which is known to contribute to excystation (Ikeda, 1955; Farr and Doran, 1962), and may increase the severity of disease (Guyonnet et al., 1989). Moreover, microflora contribute to expression of *E. tenella* pathogenicity (Bradley and Radhakrishnan, 1973; Lafont et al., 1975; Visco, 1975). We observed that, as shown by the lower *E. coli* population with the whole wheat diet in healthy birds, the size of diet particles affects microflora. This was also suggested by the changes in fermentation characteristics of the cecal contents between coarse and fine particle diets (Williams et al., 1997). Modification of the bacterial population by the physical form of the diet may explain the different level of multiplication of *E. tenella* according to wheat structure in the diet and may be due to the higher level of gizzard development. First, lower pH in the gizzard (Gabriel et al., 2003) may increase its bactericide action. Second, a more functional gizzard leads to a higher level of digestion generating different substrates for microflora. In contrast to the ground diet, which appeared in the duodenal content as a suspension of relatively unchanged particles, whole grains appeared in the duodenum after mechanical and enzymatic action which may lead to finer particles (Hill, 1971; Svihus et al., 1997). These differently digested feed particles in the upper small intestine may result in a shift in the microbial population. Moreover, the pH of the duodenum is increased by feeding whole wheat or large particles of diet (Nir et al., 1994; Gabriel et al., 2003), which may also contribute to differences in microflora. These modifications of microflora may be responsible for the changes in coccidia development in whole wheat fed-chickens.

The WP-fed chickens had greater mean weight gains during the recovery phase, which was probably compensatory growth for the lower weight gain of WP-fed chickens during the acute phase. As observed by Waldenstedt et al. (1998) with low infective dose of *E. tenella*, the succession of the two phases led to similar weight gains between 0 and 7 d PI. In fact, whole wheat feeding led to increased sensitivity to *E. tenella*, with lower hematocrits, higher lesion scores, and higher oocyst excretion, which although transient, led to significantly lower weight gains.

Effects of Whole Wheat on Intestinal Infection (*E. maxima*, *E. acervulina*)

The doses of *E. maxima* parasites inoculated led to moderate levels of parasite development, as indicated by low intestinal lesion scores. As expected for this *Eimeria* species localized in the jejunum and ileum, considerable digestive disturbances occurred resulting in lower weight gains. Thus, a decrease in nutrient absorption was observed, demonstrated by decreased serum coloration. This criterion has been proved to be very sensitive for the evaluation of intestinal coccidial infections (Yvoré et al., 1993).

A marked difference between diets was observed in our experiment. Whole grain feeding showed greater negative effects overall on absorption and weight gain. Weight gains decreased in whole wheat-fed birds sooner than in birds fed a ground diet (4 d PI instead of 5 d PI), and they were affected at lower infective doses (500 instead of 50,000 oocysts in this study). These effects are in contradiction with the study of Waldenstedt et al. (1998) which reported no significant effects of the same types of diet with *E. maxima*, or only a tendency for a slight negative effect on weight gain of standard feed compared to whole wheat. As previously explained for cecal coccidiosis, this discrepancy between studies may be the result of differences in experimental conditions. Waldenstedt et al. (1998) used a lower proportion of whole wheat, i.e., 10 to 30%, instead of more than 40% in our study. Relative gizzard weight was therefore only slightly higher with whole grain than with the standard diet (15 g/kg and 12 g/kg of bird weight, respectively), whereas under our conditions gizzard weight was twice as high in WP-fed than CG-fed chickens, and in a previous study gizzard weights were 17 g/kg and 8 g/kg for WP- and CG-fed birds, respectively (Gabriel et al., 2003). Moreover, Waldenstedt et al. (1998) used wheat in dilution instead of substitution, leading to a lower nutrient value of the whole wheat diet compared to the standard diet.

Relatively low lesion scores were observed for *E. acervulina*, even with the high inoculation doses. As in the case of *E. tenella*, acute and recovery phases were observed. However, as this species has a shorter cycle, these periods occurred early (3 to 5 d PI instead of 4 to 6 d PI with *E. tenella* for the acute phase).

With *E. acervulina* WP-fed birds were more affected than CG-fed birds during the acute phase. As for the other intestinal coccidia species, they were affected earlier than CG-fed birds (3 instead of 4 d PI) and with lower infective doses (20,000 oocysts instead of 540,000 oocysts in our study). These results confirmed the more detrimental effects of whole grain observed in other studies for a wide range of parasite doses. By using 20 or 40% whole wheat in substitution, Banfield et al. (1999, 2002) observed a decrease in weight gain during the acute phase when compared to ground wheat-fed birds. In a previous study

in our laboratory using sequential feeding, we also observed a decrease in weight gain and serum coloration (Crévieu-Gabriel et al., 2000). However, in another study no effect of whole wheat in substitution was observed during coccidiosis (Banfield and Forbes, 2001).

For the infective dose of 60,000 oocysts weight gain during the recovery phase was higher with the WP diet than with the CG diet. As for *E. tenella*, this may have been compensatory growth for the lower weight gain of WP-fed chickens during the acute phase. In addition, WP-fed birds infected with the highest infective dose excreted fewer oocysts than CG-fed birds, whereas Banfield et al. (1999) observed an increase in oocyst excretion with whole wheat. However this discrepancy between studies may be due to the high variability of this criterion to evaluate parasite development.

In the two types of intestinal coccidiosis studied here, i.e., *E. maxima* and *E. acervulina*, parasite development was similar or only slightly modified by diet structure compared to *E. tenella*. This absence of differences in parasite development between diets may be due to various reasons. First, the modifications of the digestive tract due to the diet structure may have a different impact according to the species of parasite. Intestinal and cecal coccidia species have different sensitivity of the oocyst wall to mechanical breakdown and of the sporocyst Stieda body to enzymatic hydrolysis (Farr and Doran, 1962). Second, as intestinal coccidia species are not known to be influenced by microflora (Lafont et al., 1975), a shift in microbial population induced by diet structure might be without consequence on these parasites.

A strongly negative effect of whole wheat was observed on absorption during intestinal coccidiosis, as shown by the decrease in serum coloration. This effect may be explained by the lower digestive capacity of the intestine of uninfected birds fed whole wheat as demonstrated by lower enzyme activity (Gabriel et al., 2003). This lower intestinal digestive capacity seems to have no detrimental consequences on healthy birds, but when the mucosa is degraded by parasite development, intestine capacity may become a limiting factor for nutrient digestion and as a consequence may increase the deleterious effects of a parasite.

In conclusion, free choice feeding of whole wheat with protein concentrate to broiler chickens under our experimental conditions (battery, 40% whole wheat from 7 d of age, free choice feeding) led to worse effects than a ground pelleted diet after experimental coccidia inoculation at 22 d of age. The mechanisms involved may be different between cecal and intestinal coccidiosis, as these parasites differ in their development pattern. In the case of cecal coccidiosis, modification of microflora may lead to a higher level of development of the parasite in the case of whole wheat feeding. These dietary effects on microflora need further investigation because of the increasing tendency for antibiotic suppression in poultry feed. Modification of excystation conditions due to change in the digestive tract (greater gizzard development and possibly increased pancreatic function) may contribute to higher levels of parasite development in birds fed whole wheat. This increased level of development of coccidia may be responsible for lower weight gains with whole wheat feeding. Parasite development of intestinal coccidiosis was similar between the two dietary treatments. However, the lower intestinal digestive capacity of whole grain-fed chickens may accentuate the deleterious effects of coccidia on nutrient digestion, which leads to lower serum coloration and weight gain. These hypotheses will require further study to establish conditions in which whole grain may be beneficial or detrimental during coccidia infections, and to understand the mechanisms involved.

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