

Effects of food deprivation and particle size of ground wheat on digestibility of food components in broilers fed on a pelleted diet

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- Abstract**
1. The first aim of the experiment was to study the effect of wheat (*Triticum aestivum*) particle size on the digestibility of starch in a pelleted diet given to broilers. The second aim was to study the consequences of food deprivation before the excreta collection period (from 21 to 24 d). Wheat from a strong hardness cultivar was incorporated at 546.1 g/kg in diets. The other main ingredients were soybean meal (353.5 g/kg) and rapeseed oil (55.0 g/kg). Diets were given as pellets. The experimental design was a 2 × 2 factorial design testing two particle sizes of wheat flour and two procedures of a balance experiment (with or without food deprivation).
 2. Birds given diet C (wheat coarse grinding before pelleting) had significantly greater gizzard weight than birds fed on diet F (wheat fine grinding before pelleting).
 3. Starch digestibility value was significantly increased when birds were fed on diet F. This effect was halved by food deprivation. No significant effect of grain particle size was observed for protein and lipid digestibility values. However, food deprivation decreased apparent protein digestibility, with an effect which was more pronounced for fine than for coarse grinding.
 4. AME_N of the diet was significantly improved by fine grinding of wheat and decreased by food deprivation. However, no significant differences in growth performance were induced by differences in wheat grinding.
 5. No significant effect of grinding was observed on the water excretion:feed intake ratio. No significant difference was observed for vent score between treatments.
 6. There was over-excretion of starch in the first hours of refeeding following food deprivation.

INTRODUCTION

Wheat is a major ingredient of broiler diets in many countries and, because of its high starch content, it is considered a suitable energy source for chickens. However, studies with wheat often revealed a low coefficient of starch digestibility (Mollah *et al.*, 1983; Rogel *et al.*, 1987; Svihus, 2001; Carré *et al.*, 2002). As reviewed by Carré (2003), starch granules from cereals are not really resistant compared to other sources, and no antinutritional factors can be expected to induce such low starch digestibility values. Thus, the hypothesis of an access problem in cereal particles has to be considered. Recently, negative correlations were observed between starch digestibility and hardness or particle size of wheats (Carré *et al.*, 2002, 2005), which is an argument in favour of the hypothesis of an access problem in coarse particles of wheats.

However, variations in hardness and particle size of wheat were linked (Carré *et al.*, 2002, 2005). Thus, different particle sizes came from different wheat samples. The aim of the current study was to examine the effect of particle size, per se, by comparing two flours with different particle size, coming from one wheat sample. A wheat cultivar of high hardness value was retained, and ground with two different mill screens in order to obtain a coarse wheat pelleted diet and a fine wheat pelleted diet.

Digestibilities of food components and morphology of the digestive tract were measured, as food particle size was also reported to affect the gut anatomy (Nir and Ptichi, 2001). Nir *et al.* (1994) reported that coarsely ground wheat increased gizzard weight significantly compared to fine grinding. Some similar effects were observed with whole grain compared to a pelleted ground diet (Forbes and Covasa, 1995;

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Noirot *et al.*, 1998) or with coarsely ground maize compared to finely ground maize (Dahlke *et al.*, 2003). This greater development is thought to be due to the increased frequency of gizzard contractions (Roche, 1981) needed to grind large particles. In the case of the small intestine, Nir *et al.* (1994) have shown that duodenum weight decreased significantly with increased particle size.

One of the difficulties in a comprehensive approach of cereal starch digestion is the great variability of response between birds (Choct *et al.*, 1999). As suggested by Svihus and Hetland (2001), who observed abnormal ileal starch concentrations with pelleted diets after 2 h of feed deprivation, the variability could be the consequence of an overload risk in the digestive tract of chickens. The pattern of feed intake may, therefore, be important for digestion processes. In the current experiment, digestibility values were measured by two distinct methods: the classical total collection method with a previous feed deprivation period, and a marker method with no feed deprivation.

MATERIALS AND METHODS

Animals

One hundred and fifty male broiler chickens (Ross), obtained from a commercial hatchery, were put in metal cages (44 cm length, 32 cm width, 36 cm height) with 4 birds per cage until d 7, then with one bird per cage. The cages were placed in ventilated rooms with controlled light (23 h light/d) and temperature (34°C d 1, 33°C until d 3, 31°C until d 8, 29°C until d 10, 28°C until d 15, 26°C until d 22, 24°C until d 24). At d 7, 96 birds were selected and distributed into two groups with 48 birds per group in order to give the same mean weight (144 g) and similar standard deviations for each group. At d 7, each group received one of the two experimental diets (Table 1). The selected birds were randomly distributed in 96 different cages, with one bird per cage and 48 cages per diet. Each cage was provided with a drinker, a feeder and a plastic tray for total collection of excreta.

At d 20, each group of 48 birds was divided into two subgroups for conducting balance experiments according to two different methods. The first subgroups (28 birds per diet) were assigned to a total collection method with starvation periods. The second subgroups (20 birds per diet) were assigned to a marker method without starvation periods.

For the subgroups of 28 birds, the total collection method was similar to that described by Bourdillon *et al.* (1990) with some modifications: at d 20, birds were fasted for 16 h, weighed,

Table 1. Composition of the experimental wheat pelleted diets

Ingredients	g/kg
Wheat (Baltimor cultivar) ¹	546.1
Rapeseed oil	55.0
Soyabean meal	353.5
Calcium carbonate	11.9
Dicalcium phosphate	15.0
Sodium chloride	3.5
Mineral ² and vitamin ³ mix	5.0
DL-methionine	1.5
Robenidine ⁴	0.5
Insoluble ash (Celite™)	8.0
Calculated values	
AME _N (MJ/kg)	12.23
Lysine	11.3
Methionine + cystine	8.6
Calcium	11.3
Available phosphorus	4.0
Measured values	
Starch	345.2
Crude protein	211.0

¹Wheat: hardness = 0.83; Real applied viscosity (RAV) = 1.65 ml/g.

²Supplies, as mg per kg of diet: Ca 1600, Co 0.6, Cu 25, Fe 50, I 1, Mn 85, Se 0.25, Zn 60.

³Supplies, per kg of diet: vitamin A (all-trans-retinol) 3.3 mg, cholecalciferol 0.05 mg, vitamin E (DL- α -tocopheryl acetate) 30 mg, thiamine 1.5 mg, riboflavin 4 mg, calcium pantothenate 10 mg, vitamin B₁₂ 0.015 mg, menadione 2 mg, pyridoxine hydrochloride 2.5 mg, folic acid 0.4 mg, biotin 0.2 mg, choline 500 mg, niacin 30 mg, butylated hydroxyl toluene 125 mg.

⁴Robenz®, American Cyanamid Co, Agricultural Division, Wayne, NJ, USA.

refed for 56 h, then fasted for 16 h and weighed. Individual food intakes were measured and excreta were individually collected daily. After each collection, droppings were weighed and immediately stored at -20°C. Subsequently, the droppings were freeze-dried, weighed, ground through a 0.5 mm screen and stored at 4°C until analysis. Water content was measured as the weight decrease following freeze-drying. On d 21, 8 birds from each subgroup of 28 birds were used to determine the kinetics of excretion: after refeeding following 16 h starvation, excreta were collected individually every hour, for approximately 6 h. Starch, arabinose + xylose and protein contents in excreta were then measured (Figure).

In the two subgroups of 20 birds, the balance experiment was performed in the same way except that there was no food deprivation at the beginning and end of the balance experiment. Excreta were also individually collected daily for 72 h, at the same times as in the other method, weighed and immediately stored at -20°C. AME_N and digestibilities were calculated using acid insoluble ash (AIA) as a marker, as previously described by Scott and Hall (1998). At the end of the balance experiment, for all birds, 4 people estimated the vent and assigned a score (0 to 5) increasing according to the increase in the dirtiness of appearance. The mean value

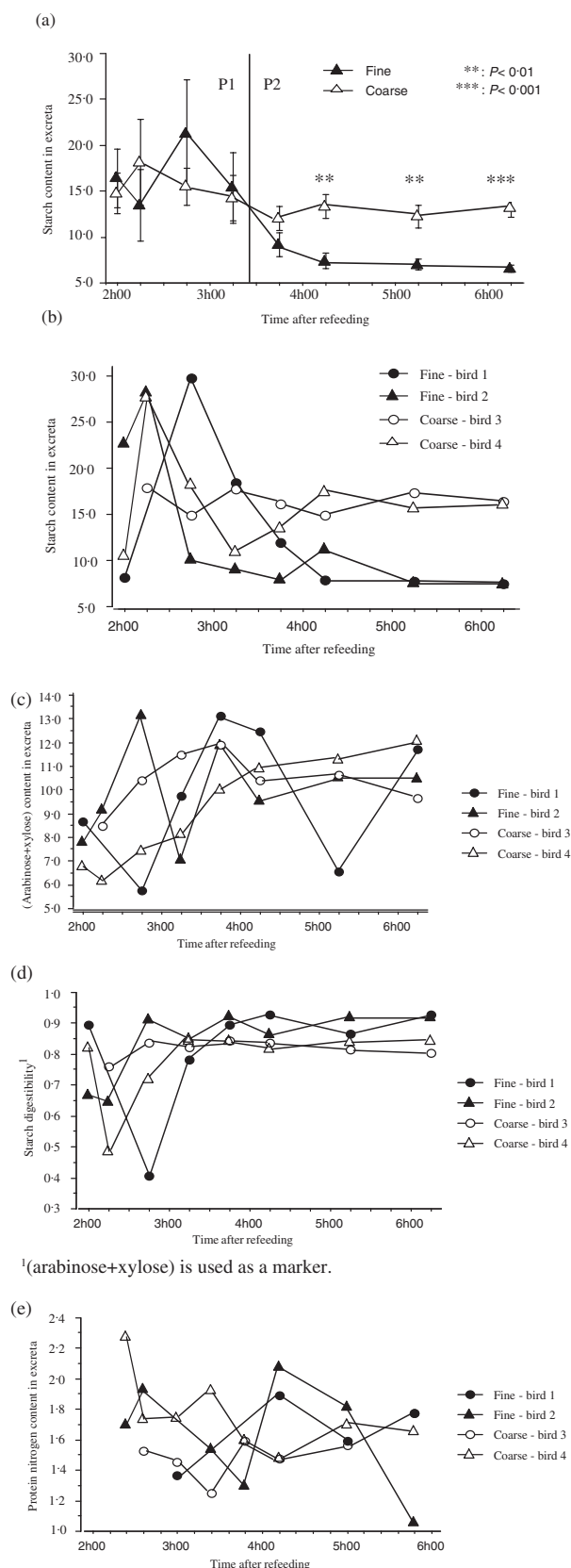


Figure. Kinetics of starch content (g/100g), (arabinose + xylose) content (g/100g), protein content (g/100g) and starch digestibility in dry excreta after a night food deprivation and refeeding. (a) Starch content, mean values \pm SEM, $n = 8$. (b) Starch content, 4 individual values. (c) Arabinose + xylose content, 4 individual values. (d) Starch digestibility, 4 individual values. (e) Protein nitrogen content, 4 individual values.

resulting from the scoring by the 4 people was attributed to each bird.

Growth performance

The chickens from each group were weighed at d 7 and d 15 after 17h food deprivation. Food intakes were also individually recorded between d 7 and d 15.

Experimental diets

The male broiler chickens were maintained on a standard starter diet (12.98 MJ/kg AME_N, 220 g crude protein per kg) containing maize, wheat and soybean meal until d 7. Then, each group was given one of the two experimental diets (Table 1) for 17 d. The only source of starch was wheat. The wheat cultivar Baltimor was chosen for its high hardness value. The two diets were the same except for the intensity of wheat grinding: in one diet, the wheat was finely ground (diet F), whereas, in the other, the wheat was coarsely ground (diet C). Grinding was with a hammer mill (speed, 3200 rpm) and two screen sizes, 2 mm for fine grinding and 6 mm for coarse grinding. In both experimental diets, 8 g/kg CeliteTM (a source of AIA) was added as an inert marker. Diets were given as pellets. Steam was used during pelleting and the diameter of the die was 2.5 mm.

Sampling procedures for gizzards and intestinal sections

At 24 d, after the total collection period, 10 birds from each diet group had continuous access to food and water until they were killed for sampling of the digestive organs. Birds were weighed and then killed by intracardiac injection of 1 ml of sodium pentobarbital (Sanofi, Marne la Coquette, France). Their digestive tract was then immediately excised. Gizzard, duodenum, jejunum and ileum were emptied and measured for weight and length (Table 4). Each dissection and tissue sampling lasted approximately 10 to 12 min per animal.

Analytical methods

Wheat samples were analysed for real applied viscosity (RAV) as described by Carré *et al.* (1994). Fine and coarse ground wheat samples were analysed for mean particle diameter by dry sieving. Two stacks of 12 sieves were used: one stack for coarse ground wheat (range: 2500 to 200 μ m), and the other stack for fine ground wheat (range: 1250 to 100 μ m). Geometric mean particle diameter (GMD) was calculated according to ASAE (1983). Fine and coarse ground

Table 2. Particle size (% retained on sieves) of ground wheat and flours from pelleted diets¹ by dry and/or wet sieving

Wheat (dry sieving)																	GMD ²
Sieve openings	(μm)	100	125	160	200	250	315	400	500	630	800	1000	1250	1600	2000	2500	(μm)
	Fine	4.3	8.9	4.7	6.4	8.2	7.5	8.3	11.4	12.7	12.4	7.7	2.6				380
	Coarse				2.4	3.1	3.6	3.9	5.4	6.8	9.9	12.6	14.6	14.8	8.9	5.1	955
Wheat (wet sieving)																	
Sieve openings	(μm)	<75	75	150	300	425	600	850	1180	1600	2000						
	Fine	37.9	5.5	4.8	5.8	7.5	14.5	12.5	8.2	2.6	0.7						
	Coarse	18.6	1.2	0.5	1.8	2.4	6.9	9.5	14.5	18.2	26.3						
Diets (wet sieving)																	
Sieve openings	(μm)	<75	75	150	300	425	600	850	1180	1600	2000						
	Fine	36.8	13.4	5.9	4.2	7.1	13.4	9.5	6.3	2.6	0.9						
	Coarse	30.5	10.0	4.8	3.5	5.7	9.7	10.1	10.0	8.7	7.1						

¹Durability of pellets was 25 for both diets C and F.

²Geometric mean particle diameter.

wheats were also analysed by wet sieving, using the same procedure as for pelleted diets (Table 2). A sample of ground wheats or pellets of each diet (9.1 g, dry matter basis) was stirred in 200 ml of water for 5 min. Then, the mix was put through a wet sieving analyser (Retsch WS-1, F. Kurt Retsch GmbH & Co. KG, Haan, Germany) with 9 sieves: 850, 2000, 1600, 1180, 600, 425, 300, 150 and 75 μm opening diameter. Water flowed through the sieves for 5 min. The sieves were then dried for 4 h at 103°C and weighed. Durability of pellets was measured as the percentage of materials remaining on a 2 mm screen after shaking in a Eurotest rotary mill (Sabe, Chauché, France) designed for durability measurement. Wheat hardness was estimated using near infrared reflectance (NIR) spectrometry according to AACC (1995), using whole-meal flour produced on a cyclotec mill fitted with a 0.8 mm sieve.

Diets and individual samples of excreta were analysed for starch, protein, lipid, gross energy and AIA. Starch content was determined using the amyloglucosidase-dimethylsulphoxide method (Boehringer Mannheim, 1980) as described by Carré *et al.* (1991). The AIA content was determined using the AFNOR V18-102 method (1977). Protein, lipid and gross energy contents of dry excreta collected for the balance experiments were measured using NIR spectroscopy as described by Mignon-Grasteau *et al.* (2004). The AIA content was used as an inert marker to calculate digestibilities.

For the kinetic experiment, water-insoluble arabinoxylan contents of diets (sample of 100 mg) and excreta (sample of 40 mg) were determined for 4 birds, as follows. Samples were boiled in acetate buffer (0.5 M, pH 5.6) with Termamyl[®] α -amylase for 10 min. The temperature was decreased to ambient and

amyloglucosidase was added (10 μg) and the suspensions were then stirred for 2 h. Then, the suspensions were centrifuged and the residues were rinsed with distilled water, ethanol and acetone. Residues were hydrolysed with sulphuric acid (2 N), and free neutral sugars were determined using gas-liquid chromatography as previously described (Blakeney *et al.*, 1983; Brillouet and Carré, 1983). Protein nitrogen in samples for the excretion kinetic was measured as the difference between total (Reardon *et al.*, 1966) and uric acid nitrogen (Marquardt, 1983).

Statistical analysis

Statistical analysis was performed using variance analyses (Statview Software program, version 5).

RESULTS

Wheat and diets

NIR analysis of the wheat sample confirmed its high hardness value (83). Particle size distributions are shown in Table 2. Geometric mean particle diameter of wheat flours were 380 μm for the fine grinding process and 955 μm for the coarse grinding process.

Wet sieving of ground wheat and pelleted diets showed that the difference in particle size distribution still existed between diets after pelleting, especially in the proportion of coarse particles. Diet C (coarse wheat diet) showed a high percentage of coarse particles (>1180 μm) compared to diet F (fine wheat diet) (25.8 vs 9.8%, see Table 2). Diet F also showed more small particles (Table 2). Durability of pellets was 25 for both diets (Table 2). Thus, diets were identical for wheat hardness value and pellet durability.

They only differed by the mean particle size of wheat flours introduced in diets before pelleting.

Growth performances

Results of growth performance and food intake are shown in Table 3. Weight gain (7 to 15 d) and gain:food ratio (7 to 15 d) tended to decrease with diet F, but the effect was not significant ($P > 0.05$). No significant differences were observed in growth performances concerning feed intake and body weight.

Digestive tract

The anatomical traits of the digestive tract did not differ between treatments except for gizzard, which was biggest in birds fed on diet C (Table 4).

Digestibility values and AME_N

In the balance experiment with food deprivation, the starch digestibility values calculated according to the marker method were very similar to those calculated according to the total collection method (Table 5).

Table 3. Effects of wheat grinding intensity on growth performances of male broiler chickens fed with wheat pelleted diets ($n = 48$) from 7 to 21 d (mean \pm SEM)

	Coarse grinding	Fine grinding
Body weight at 7 d (g)	144 \pm 1.2 ^a	144 \pm 1.2 ^a
Body weight at 15 d (g)	444 \pm 4.0 ^a	440 \pm 4.4 ^a
Body weight at 21 d (g)	723 \pm 8.6 ^a	723 \pm 11.2 ^a
Weight gain (7–15 d) (g)	299 \pm 3.5 ^a	296 \pm 4.2 ^a
Food intake ¹ (7–15 d) (g)	375 \pm 3.8 ^a	380 \pm 5.3 ^a
Gain:food ratio (7–15 d) (g:g)	0.80 \pm 0.006 ^a	0.78 \pm 0.007 ^a

¹Dry matter basis

^aMeans on the same row followed by the same letter are not significantly different at $P < 0.05$.

Table 4. Effects of wheat grinding intensity on digestive organ size (empty weight and length) in 3-week-old male broiler chickens fed with wheat pelleted diets (Mean \pm SEM, $n = 20$ chickens)

	Coarse grinding	Fine grinding
Gizzard		
Weight/body weight (g/g)	0.015 \pm 0.0008 ^a	0.013 \pm 0.0005 ^b
Duodenum		
Weight/body weight (g/g)	0.013 \pm 0.0005 ^a	0.012 \pm 0.0005 ^a
Length/body weight (cm/g)	0.033 \pm 0.0009 ^a	0.033 \pm 0.0008 ^a
Jejunum		
Weight/body weight (g/g)	0.020 \pm 0.0003 ^a	0.020 \pm 0.0007 ^a
Length/body weight (cm/g)	0.071 \pm 0.0014 ^a	0.075 \pm 0.0026 ^a
Ileum		
Weight/body weight (g/g)	0.015 \pm 0.0004 ^a	0.016 \pm 0.0009 ^a
Length/body weight (cm/g)	0.068 \pm 0.0019 ^a	0.070 \pm 0.0023 ^a

^{a,b}Means on the same row with different superscripts are significantly different ($P < 0.05$).

Dry matter and starch digestibilities were increased by fine grinding ($P < 0.01$) although the effect tended to be reduced by food deprivation ($P = 0.088$ and 0.051 , respectively; Table 5). Standard deviations of starch digestibilities were about 60% greater with food deprivation. The greatest individual variability in starch digestibility was observed with food deprivation and fine grinding. Food deprivation resulted in decreased dry matter and protein digestibilities ($P < 0.01$), especially with fine grinding ($P = 0.088$ and 0.041 , respectively; Table 5). Lipid digestibilities (Table 5) were not significantly different between treatments. Coarse grinding ($P < 0.001$) and food deprivation ($P = 0.02$) resulted in negative effects on AME_N, and increased water excretion (Table 5). AME_N was observed to be negatively correlated to the relative jejunum weight ($P = 0.0054$). No effects were observed on vent score.

Kinetics of starch excretion

The kinetics of starch content in excreta were observed for approximately 6 h after refeeding with diet F or diet C. The results are shown in the Figure. Two periods could be observed: P1 and P2. For period P1, up to 3 h 20 min after refeeding, starch contents showed a peak and great variation (Figure (a)). They were high compared to the mean obtained for the total collection period (diet C: 13.8%; diet F: 10.0%). For this period there was no significant difference between diet C and diet F (Table 6). For the period P2, starch contents did not change significantly for diet C, and decreased dramatically ($P < 0.01$) for diet F. Thus, for period P2, starch contents were much lower ($P < 0.01$) for diet F than for diet C. Measurements of (arabinose + xylose) and protein contents in excreta of 4 birds (Figure (c) and (e)) showed less relative variation than starch content. A close relationship was observed between starch content and starch digestibility calculated with (arabinose + xylose) content used as an indigestible marker (Figure (b) and (d)).

DISCUSSION

Particle sizes in pellets were lower than those of the wheat meals before pelleting. Differences were probably due to a reduction of the coarsest particles by the pelleting process and also to the dilution of wheat by ground soybean meal. However, the diets were still different with a higher percentage of coarse particles in diet C than in diet F.

Despite a higher AME_N value for wheat with fine grinding, this did not result in significant

Table 5. Digestibilities of dry matter, starch, protein and lipid, water excretion, AME_N (J/g dry matter basis), and vent score in broilers (3 week old) fed on pelleted diets: effects of wheat grinding intensity before pelleting and food deprivation

	No food deprivation		With food deprivation		RSD ¹	Food deprivation Effect ²	Grinding Effect ²	Deprivation × Grinding Effect ²
	Wheat coarse grinding (n = 20)	Wheat fine grinding (n = 20)	Wheat coarse grinding (n = 28)	Wheat fine grinding (n = 28)				
Dry matter digestibility (marker method)	0.631	0.676	0.621	0.635	0.043	0.0065	0.0018	0.088
Starch digestibility (marker method)	0.854	0.925	0.861	0.896	0.042	0.25	0.0001	0.051
Starch digestibility (total collection method)	-	-	0.862	0.896	0.047	-	0.011	
Protein digestibility (marker method)	0.800	0.814	0.767	0.747	0.039	<0.0001	0.714	0.041
Lipid digestibility (marker method)	0.760	0.760	0.718	0.745	0.101	0.21	0.54	0.55
AME _N (J/g DM) (marker method)	12 369	13 027	12 086	12 567	718.0	0.02	0.0005	0.572
Water excretion/feed intake ³ from 21 d to 24 d	0.85	0.76	0.98	0.92	0.21	0.002	0.12	0.71
Vent score ⁴ at 24 d	2.6	2.9	2.5	2.5	0.960	0.240	0.54	0.48

¹Residual standard deviation.²P-value.³Feed dry matter.⁴Vent score was assessed by 4 different persons, from 1 = very clean to 5 = very dirty.**Table 6.** Comparison of starch content in dry excreta between excretion periods (see Figure (a)) and between pelleted diets (coarse or fine grinding) (mean ± SEM, n = 8)

Starch content (g/100g) of dry excreta	Starch content (g/100g) of dry excreta	
	Period 1 (P1)	Period 2 (P2)
Coarse	15.2 ± 1.62 ^{xa}	12.4 ± 1.13 ^{xa}
Fine	15.0 ± 2.54 ^{xa}	7.3 ± 0.67 ^{yb}

^{a,b}Means in the same row with different superscripts are significantly different (paired *t*-test) ($P < 0.05$).^{x,y}Means in the same column with different superscripts are significantly different ($P < 0.05$).

effect on weight gain, feed intake or gain:food ratio. This absence of effect of fine grinding of wheat in birds fed on pelleted diets has already been observed (Hamilton and Proudfoot, 1995; Svihus and Hetland, 2001; Engberg *et al.*, 2002). The same observations were made with wheat/maize-based pelleted diets (Hamilton and Proudfoot, 1995). However, in the current study, individual feed efficiencies were observed to be more closely correlated with individual protein digestibility than with individual AME_N. Thus, it can be hypothesised that a limitation existed for amino acids and not for energy, probably because the composition of diets resulted in a ratio of amino acids to AME_N that was a little low.

In birds with food deprivation, the starch digestibility values calculated on the basis of marker excretion were very similar to those calculated using the total collection method (Bourdillon *et al.*, 1990). This shows the reliability of data obtained with the marker method.

Starch digestibilities of the wheat sample were rather low as has previously been observed for other wheat samples (Mollah *et al.*, 1983; Rogel *et al.*, 1987; Choct *et al.*, 1995; Maisonnier *et al.*, 2001; Marron *et al.*, 2001; Svihus, 2001; Carré *et al.*, 2002). The positive effect of fine grinding on wheat starch digestibility is in agreement with the hypothesis that an access problem in coarse particles in part explains the low starch digestibility values observed with hard wheat. However, fine grinding did not result in starch digestibility values close to 100%. Thus, it remains to be known whether a more intense grinding would be necessary to achieve complete starch digestion or whether other factors are involved in this incomplete starch digestion. The positive effect of fine grinding could not be attributed to an effect of pellet durability or feed intake as both these factors were not affected by wheat grinding. It was observed that this positive effect was halved by food deprivation. This interaction may be explicable by a starch overload following refeeding after starvation, suggesting a digestive disorder. This observation emphasises the importance of the method used for digestibility measurement in an experiment testing the effect of feed processing on wheat starch digestibility. The classical total collection method (Bourdillon *et al.*, 1990) results in a risk of overload after refeeding and thus possibly leads to underestimation and high variability in digestibility. This may, in turn, hide the positive effects of processing methods, especially when they are rather low. The slaughter method with the measurement of digestibility in the terminal

ileum may result in conflicting results, as digestibilities may depend on the period of slaughter with risks of variation and interactions between period and dietary treatment. Thus, a balance experiment without starvation using an indigestible marker, with excreta being collected over an extended period, appears to be a suitable method resulting in low variability and no dependence on period of collection.

With mash diets, Nir *et al.* (1994) observed that coarse grinding of wheat increased gizzard weight significantly but decreased duodenum weight. Our results, with pelleted diets, were in accordance with these previous results for gizzard weight but not for duodenum weight. Some studies found an association between increased gizzard size (produced by inclusion of oat hulls, grit, etc.) and improved starch digestibility (Rogel *et al.*, 1987; Hetland and Svihus, 2001). Hetland *et al.* (2002) hypothesised that increased gizzard size may influence starch digestibility by a better mixing between nutrients and digestive juices. However, their experiments differed from the current one, in that the changes in particle size were obtained by addition of coarse particles with no change in cereal particle size.

The pattern of protein content in the excreta after refeeding did not show evidence of an overload similar to that observed for starch. The pattern of starch content also differed between fine and coarse wheat grinding, with responses being much more variable for fine than for coarse grinding. So, overload affected wheat more than soybean meal, and finely ground more than coarsely ground. Overload with fine particles is probably related to transit time. It was previously observed that diets composed of fine particles moved through the digestive tract more rapidly than diets with coarse particles (O'Dell *et al.*, 1959; Nir and Ptichi, 2001). So, with diet F, the low starch digestibility observed just after refeeding may be due to an overload increasing the rate of passage of particles from gizzard to the small intestine. With coarse grinding, retention of particles in the gizzard is longer, which may result in better regulation of transit time. For those particles quickly leaving the gizzard, the protein matrix surrounding starch granules (Barlow *et al.*, 1973) may escape pepsin hydrolysis. Thereafter, an accessibility problem would appear for the hydrolysis of starch granules by α -amylase.

The positive effect of fine grinding on AME_N was consistent with the starch digestibility results. The negative effect of food deprivation on AME_N probably resulted from a small negative effect on the digestibility of all nutrients. This effect could be explained by a digestive disorder and by an increase in the ratio of endogenous losses to food intake, especially for proteins.

The negative effect of food deprivation on the ratio of water excretion to food intake was probably due to the reduction in food intake with a small change in water excretion. No significant effect of wheat grinding on water excretion was found, while a previous experiment (Carré *et al.*, 2002) suggested that coarse grinding may result in decreased water excretion. However, a strong negative correlation was observed between individual AME_N and water excretion. Thus, the decrease in AME_N due to coarse grinding was probably so high in the present experiment that its positive effects on water excretion balanced other effects that could act negatively on water excretion.

CONCLUSIONS

In conclusion, the present experiment showed that, with a strong hardness cultivar, fine grinding of wheat before pelleting resulted in an increased AME_N value of diets. Starch was the main component involved in this improvement. A balance experiment with an indigestible marker and an excreta collection for 3 d without food deprivation periods was necessary to detect clear positive effects of fine grinding of wheat on AME_N and starch digestibility. An excreta collection with food deprivation at the beginning and end of the balance experiment resulted in higher variability and a smaller effect of fine grinding on starch digestibility. This may be attributable to an overload phenomenon at refeeding after food deprivation, as assessed by the high starch content observed in excreta after refeeding the diets based on finely ground wheat.

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REFERENCES

- AACC (1995) Method 39-70 A, in: *Approved Methods of the AACC* (St Paul, MN, American Association of Cereal Chemists).
- AFNOR (1977) *Norme V18-102* (Paris, association Française de Normalisation).
- ASAE (1983) Method of determining and expressing fineness of feed materials by sieving. ASAE standard S319, in: *Agricultural Engineers Yearbook of Standards*, p. 325 (American Society of Agricultural Engineers).
- BARLOW, K.K., BUTTROSE, M.S., SIMMONDS, D.H. & VESK, M. (1973) The nature of the starch-protein interface in wheat endosperm. *Cereal Chemistry*, **50**: 443-454.

- BLAKENEY, A.B., HARRIS, P.J., HENRY, R.J. & STONE, B.A. (1983) A simple and rapid preparation of alditol acetates for monosaccharides analysis. *Journal of Nutrition*, **113**: 291–299.
- BOEHRINGER MANNHEIM (1980) *Methods of Enzymatic Food Analysis* (Mannheim, Germany, Boehringer Mannheim).
- BOURDILLON, A., CARRÉ, B., CONAN, L., FRANCESCH, M., FUENTES, M., HUYGHEBAERT, G., JANSEN, W.M.M.A., LECLERCQ, B., LESSIRE, M., MCNAB, J., RIGONI, M. & WISEMAN, J. (1990) European reference method of *in vivo* determination of metabolisable energy in poultry: reproductibility, effect of age, comparison with predicted values. *British Poultry Science*, **31**: 567–576.
- BRILLOUET, J.M. & CARRÉ, B. (1983) Composition of cell walls from cotyledons of *Pisum Sativum*, *Vicia Faba* and *Glycine Max*. *Phytochemistry*, **22**: 841–847.
- CARRÉ, B. (2003) Causes for variation in digestibility of starch among feedstuffs. *World's Poultry Science Journal*, **60**: 76–90.
- CARRÉ, B., BEAUFILS, E. & MELCION, J.P. (1991) Evaluation of protein and starch digestibility and energy value of pelleted or unpelleted pea seeds from winter or spring cultivars in adult and young chickens. *Journal of Agricultural and Food Chemistry*, **39**: 468–472.
- CARRÉ, B., GOMEZ, J., MELCION, J.P. & GIBOULOT, B. (1994) La viscosité des aliments destinés à l'aviculture. Utilisation pour prédire la consommation et l'excrétion d'eau. *Productions Animales*, **7**: 369–379.
- CARRÉ, B., IDI, A., MAISONNIER, S., MELCION, J.P., OURY, F.-X., GOMEZ, J. & PLUCHARD, P. (2002) Relationships between digestibilities of food components and characteristics of wheats (*Triticum aestivum*) introduced as the only cereal source in a broiler chicken diet. *British Poultry Science*, **43**: 404–415.
- CARRÉ, B., MULEY, N., GOMEZ, J., OURY, F.X., LAFFITTE, E., GUILLOU, D. & SIGNORET, C. (2005) Soft wheat instead of hard wheat in pelleted diets results in high starch digestibility in broiler chickens. *British Poultry Science*, **46**: 66–74.
- CHOCT, M., HUGUES, R.J., TRIMBLE, R.P., ANGKANAPORN, K. & ANNISON, G. (1995) Non-starch polysaccharide-degrading enzymes increase the performance of broiler chickens fed wheat of low apparent metabolizable energy. *Journal of Nutrition*, **125**: 485–492.
- CHOCT, M., HUGHES, R.J. & BEDFORD, M.R. (1999) Effect of a xylanase on individual bird variation, starch digestion throughout the intestine, and ileal and caecal volatile fatty acid production in chickens fed wheat. *British Poultry Science*, **40**: 419–422.
- DAHLKE, F., RIBEIRO, A.M.L., KESSLER, A.M., LIMA, A.R. & MAIORKA, A. (2003) Effects of corn particle size and physical form of the diet on the gastrointestinal structures of broiler chickens. *Revista Brasileira de Ciencia Avicola*, **5**(1): 61–67.
- ENGBERG, R.M., HEDEMANN, M.S. & JENSEN, B.B. (2002) The influence of grinding and pelleting of feed on the microbial composition and activity in the digestive tract of broiler chickens. *British Poultry Science*, **43**: 569–579.
- FORBES, J.M. & COVASA, M. (1995) Application of diet selection by poultry with particular reference to whole cereals. *World's Poultry Science Journal*, **51**: 149–165.
- HAMILTON, R.M.G. & PROUDFOOT, F.G. (1995) Ingredient particle size and feed texture: effects on the performance of broiler chickens. *Animal Feed Science and Technology*, **51**: 203–210.
- HETLAND, H. & SVIHUS, B. (2001) Effect of oat hulls on performance, gut capacity and feed passage time in broiler chickens. *British Poultry Science*, **42**: 354–361.
- HETLAND, H., SVIHUS, B. & OLAISEN, V. (2002) Effect of feeding whole cereals on performance, starch digestibility and duodenal particle size distribution in broiler chickens. *British Poultry Science*, **43**: 416–423.
- MAISONNIER, S., GOMEZ, J., CHAGNEAU, A.M. & CARRÉ, B. (2001) Analysis of variability in nutrient digestibilities in broiler chickens. *British Poultry Science*, **42**: 70–76.
- MARQUARDT, R.R. (1983) A simple spectrophotometric method for the direct determination of uric acid in avian excreta. *Poultry Science*, **62**: 2106–2108.
- MARRON, L., BEDFORD, M.R. & MCCracken, K.J. (2001) The effects of adding xylanase, vitamin C and copper sulphate to wheat based diets on broiler performances. *British Poultry Science*, **42**: 493–500.
- MIGNON-GRASTEAU, S., MULEY, N., BASTIANELLI, D., GOMEZ, J., PÉRON, A., SELLIER, N., MILLET, N., BESNARD, J., HALLOUIS, J.M. & CARRÉ, B. (2004) Heritability of digestibilities and divergent selection for digestion ability in growing chicks fed on a wheat diet. *Poultry Science*, **83**: 860–867.
- MOLLAH, Y., BRYDEN, W.L., WALLIS, I.R., BALNAVE, D. & ANNISON, E.F. (1983) Studies on low metabolisable energy wheats for poultry using conventional and rapid assay procedures and the effects of processing. *British Poultry Science*, **24**: 81–89.
- NIR, I. & PTICHI, I. (2001) Feed particle size and hardness: influence on performance, nutritional, behavioral and metabolic aspects, in: *Advances in Nutritional Technology 2001. Proceedings of the 1st World Feed Conference*, Utrecht, the Netherlands, 7th to 8th November 2001, pp. 157–186.
- NIR, I., HILLEL, R., SHEFET, G. & NITSAN, Z. (1994) Effect of grain particle size on performance. 2. Grain texture interactions. *Poultry Science*, **73**: 781–791.
- NOIROT, V., BOUVAREL, I., BARRIER-GUILLOT, B., CASTAING, J., ZWICK, J.L. & PICARD, M. (1998) Céréales entières pour les poulets de chair: le retour? *INRA Productions Animales*, **11**: 349–357.
- O'DELL, B.L., NEWBERNE, P.M. & SAVAGE, J.E. (1959) An abnormality of the proventriculus caused by feed texture. *Poultry Science*, **38**: 296–301.
- REARDON, J., FOREMAN, J.A. & SEARCY, R.L. (1966) New reactants for the colorimetric quantitation of ammonia. *Clinica Chimica Acta*, **14**: 403–405.
- ROCHE, M. (1981) Feeding behaviour and digestive motility of birds. *Reproduction, Nutrition, Development*, **21**: 781.
- ROGEL, A.M., ANNISON, E.F., BRYDEN, W.L. & BALNAVE, D. (1987) The digestion of wheat starch in broiler chickens. *Australian Journal of Agricultural Researches*, **38**: 639–649.
- SCOTT, T.A. & HALL, J.W. (1998) Using acid insoluble ash marker ratios (diet:digesta) to predict digestibility of wheat and barley metabolizable energy and nitrogen retention in broiler chicks. *Poultry Science*, **77**: 674–679.
- SVIHUS, B. (2001) A consistent low starch digestibility observed in pelleted broiler chicken diets containing high levels of different wheat varieties (Research note). *Animal Feed Science and Technology*, **92**: 45–49.
- SVIHUS, B. & HETLAND, H. (2001) Ileal starch digestibility in growing chickens fed on a wheat-based diet is improved by mash feeding, dilution with cellulose or whole wheat inclusion. *British Poultry Science*, **42**: 633–637.