

Enzyme supplementation of dry and wet wheat-based feeds for broiler chickens: performance and gut responses

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To test whether the improvements in digestive efficiency due to either wetting of the food or inclusion of enzymes are accompanied by the same changes in gut function, foods with a high content of wheat were fed to broiler chicks from 1–42 d old. Twenty-four birds were caged individually while a further sixty-four were in group pens in experiments of 2 × 2 factorial design with two levels of enzyme (0 or 1 g/kg, designed for wheat) and two levels of water addition (0 and 1300 g/kg). Food intake and live-weight gain were significantly increased by wet feeding (from 89.3 to 153.4 g/d and from 39.7 to 65.4 g/d respectively), the differences increasing with age, while the enzyme had no significant effect (120.5 and 122.2 g/d and 51.9 and 53.1 g/week respectively). The viscosity of digesta was greatly reduced both by wetting (from 4.40 to 2.64 kPa · s) and enzyme (from 4.47 to 2.57 kPa · s) but there was a significant interaction with age in which the viscosity was low throughout in the wet only, enzyme only and wet + enzyme treatments but declined with age from a very high level in the dry, no enzyme treatment (11.5 kPa · s at 14 d). While wetting increased weight and length of digestive tract and thickness of some parts of the gut, enzyme had no significant effect, tending to reduce gut wall thickness. Crypt cell proliferation rate (CCPR) was significantly reduced by wet feeding (from 39.4 to 28.7 cells/crypt per 2 h) and by enzyme supplementation (from 38.9 to 29.2 cells/crypt per 2 h). Therefore, while both wetting and enzyme addition to the food reduced digesta viscosity and CCPR to a similar extent, the former had marked stimulatory effects on food intake and weight gain while the latter had little effect. The mode of action of wet feeding is therefore deduced to be not primarily through its effects on viscosity and CCPR.

Growth: Gut histo-morphology: Wet food: Broiler chickens

Wetting cereal-based foods caused significant improvements in food intake, weight gain and/or feed conversion efficiency (FCE) of broiler chickens (Yalda & Forbes, 1995, 1996; Yasar & Forbes, 1999). This was accompanied by changes in the epithelial structure of the gut (Yasar, 1998). Although wet feeding of cereal-based foods caused significant reduction in digesta viscosity, birds given wet foods did not convert food to live weight much more efficiently than those given dry foods (Yasar & Forbes, 1999), in contrast to the results of Yalda & Forbes (1995, 1996) with commercial standard foods. Inclusion of a high proportion of wheat in commercial broiler diets introduces an appreciable amount of viscous-gel-forming NSP pentosans into the intestinal lumen (Annison, 1991). Apart from problems such as sticky droppings that cause management difficulties in poultry production, an increase in viscosity of digesta due to NSP retards the passage of food throughout the tract (Edwards *et al.* 1992; Almirall & Garcia, 1994) and renders digestive

enzymes and secretions less able to penetrate the food particles efficiently, thereby causing poor utilisation of nutrients during digestion and absorption (Choct & Annison, 1990, 1992; Van Der Klis *et al.* 1993*a, b*).

Numerous studies have reported improvements in the nutritional value of cereal-based foods with enzyme addition through reductions in digesta viscosity, suggesting partial degradation of NSP in the gut by endogenous feed enzymes (Moran *et al.* 1969; Fengler & Marquardt, 1988; Fengler *et al.* 1988; Petterson & Aman, 1989; Pawlic *et al.* 1990; Bedford *et al.* 1991; Bedford & Classen, 1992). Recently it has been found that when enzyme addition to a standard dry food, which improved performance of broilers with an improvement in FCE, was coupled with wet feeding there was a significant additive response in performance (Yalda & Forbes, 1996). This was proposed to be mediated by a reduction in digesta viscosity. If highly viscous digesta is a cause of poor nutrient utilisation of

Abbreviations: CCPR, crypt cell proliferation rate; FCE, feed conversion efficiency.

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high-cereal foods by poultry, then using enzymes and/or wet feeding to reduce viscosity should improve nutritional value of high-cereal foodstuffs even more than they improve utilisation of foods low in cereals.

In order to test this hypothesis an experiment was carried out with broiler chickens offered wet and dry wheat-based foods with or without the addition of enzyme.

Materials and methods

Animals and management

Male broiler chicks (1 d old) of a commercial strain (Ross; Mayfield Hatcheries, Rossendale, Lancs., UK) were kept in floor pens in groups on a commercial starter crumb (J. Bibby Agriculture Ltd. Adderbury, Banbury, Oxon, UK; crude protein 230 g/kg, crude fibre 30 g/kg, total oil 53 g/kg) at an environmental temperature of 33°C for 7 d. They were then transferred to cages in a room in which the temperature was decreased by 4°C every week to reach 22°C by 23 d old, and then maintained at about 22°C to 42 d old with continuous lighting.

For individual monitoring of food and water intakes, and excreta outputs, twenty-four birds with similar live weights were transferred into individual cages at 7 d old. They were divided into four treatments, each with six birds and with similar average live weight. The design of the experiment was a 2×2 factorial with two levels of enzyme addition (0 and 1 g/kg food) and two water additions (dry and 1.3 kg water/kg air-dry food).

At the same time, additional birds were reared in floor pens to be killed every week of the experiment. Birds (*n* 64, 7 d old) were kept in four floor pens, each containing sixteen birds, and offered the experimental foods from 7 to 42 d old. Four birds from each pen were killed at 14, 21, 28 and 35 d old for collection of gut contents for viscosity determination and measurement of gut size. Tissue samples from various digestive segments were obtained from the four birds from each treatment killed at 28 d old for determination of crypt cell proliferation rate (CCPR) and histo-morphologic variables.

Feeds and feeding

All grains were ground through a hammer mill (Fritsch Industriestr. 8, 6580 Idar-Oberstein, W. Germany) to pass through a 4 mm sieve.

Wheat-based food was offered to 7 d old broiler chicks in two forms: air-dried or wet, unsupplemented or supplemented with the appropriate enzyme. The enzyme was added to food by first mixing it with a small proportion of ground cereal grain which was then mixed with the rest of the food ingredients. The experimental food was wheat-based, and its nutrient composition is given in Table 1. Wheat grain was obtained from Dalgety Agriculture Ltd (Bristol, UK), estimated to produce a high viscosity. The feed enzyme used (with activities of 2700 U xylanase/g, < 70 U β-glucanase/g, 700 U bacillus protease/g, included at 1 g/kg, the level of inclusion recommended by the suppliers, Finnfeeds International, Marlborough, Wilts., UK) was of fungal origin. The appropriate porridge-like

Table 1. Composition of experimental foods

Ingredients (g/kg)	Starter diet	Finisher diet
Wheat	626	700
Soyabean meal (523 g CP/kg)	200	164
Fish meal (678 g CP/kg)	100	80
Corn oil	48	30
Methionine	1.0	1.0
Tricalcium orthophosphate	10.0	10.0
Vitamin and mineral mixture*	12.0	12.0
Chromic oxide†	3.0	3.0
Estimated composition (per kg DM)		
Crude protein (g)	243	214
Metabolisable energy (MJ)	12.7	12.8

CP, crude protein.

*Vitamin and mineral premix supplied the following (per kg): vitamin A 1440 mg; vitamin D₃ 30 mg; vitamin E 10 000 mg; Ca 268 g; Na 80 g; Cu, 4000 mg as cupric sulphate; Se 80 mg as sodium selenite; Mo 400 mg as sodium molybdate.

† Experimental food with Cr₂O₃ as a marker to measure digestibility of food nutrients (results not reported in this paper).

consistency of the wet foods was achieved by adding 1.3 kg water/kg food. Food and water intake were recorded daily and live weight weekly throughout the experimental period, from 7 to 42 d old.

Wet foods were prepared fresh daily by adding 1.3 kg tap water to each kg of air-dry food and offered to the chicks immediately after achieving a homogeneous mixture. The time interval between wetting and delivery of food to the birds was about 15 min and wet food would therefore have an average of 12 h soaking period during each 24 h. This amount of water gave an appropriate semi-liquid porridge consistency as more than this resulted in a water layer above the bulk, deterring chicks from feeding.

Food refusals were discarded each day at about 09.00 hours to avoid mould growth, and clean containers were used every day. Samples of the refusals of wet food were collected once per week to study the effect of evaporation on DM content of wet food and allow calculation of actual DM intakes. Any waste food was collected from the trays underneath the cages and food intake corrected accordingly.

Food intake and water intake from nipple bottles were calculated daily. Total daily water intake was expressed as apparent daily water intake plus water taken in through the food eaten during a 24 h period. Live weight of birds was recorded at the start of each experiment, and then every week.

Dry matter retention

The excreta of the birds in cages were collected on metal trays for four consecutive days in every week and then frozen. All excreta from each 4 d collection were thawed, mixed and dried at 105°C for the measurement of the apparent retention of DM as a proportion of DM intake.

Post-mortem measurements

After killing birds by immersion in CO₂ the entire digestive tract was removed and divided into crop, proventriculus plus gizzard, duodenum (pancreatic loop only), proximal third, middle third and distal third of small intestine, caecum and colon. The length and full weight of each

segment was recorded, its contents milked out, and the segments re-weighed empty. Where DM of digesta were determined, some of the mixed digesta was placed in Al evaporating dishes to dry in an oven at 107°C. The remaining digesta sample from each segment was centrifuged at 900 g for 30 min after which approximately 0.5 ml supernatant was placed in a digital, cone-plate viscometer (model LVTD-CP-40; Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA), and the viscosity (kPa · s) measured at 41°C (deep body temperature of bird) at 50 r.p.m. It was not possible to obtain clear supernatant from digesta from caeca and colons.

CCPR was measured in five birds per treatment by injecting vincristine sulfate in distilled water (1 mg/kg body weight) intraperitoneally and killing 2 h later. Vincristine sulfate is a stathmokinetic (metaphase arrest) drug which interacts with the mitotic spindle to block cells in metaphase within the epithelial crypt of where cell renewal occurs in the intestine. The accumulation of these arrested metaphases represents the rate of cell proliferation and is expressed as cells/crypt per 2 h (Goodlad & Wright, 1982).

Two tissue samples each 10 mm long were cut from the midpoints of the digestive segments, one of which was fixed in formal-saline, dehydrated with ethanol in increasing concentrations, embedded, sectioned (5 µm) and stained with haematoxylin and eosin. Slides were examined under a light microscope (up to ×400 magnification) and the thickness of tissue layers, villi heights, crypt depths and thickness of tunica muscularis were measured at six or nine points of each slide using a graticule (100 µm divisions) placed in the eye-piece of the microscope. Although the mucosa lining folds in the wall of the caecum and colon are not villi in the strict sense, they have been called such by Bell & Freeman (1971) and Turk (1982).

The second samples were prepared for estimate of CCPR by counting of darkly-stained dividing cells for twelve entire crypts in each sample (broken or overlapping crypts were ignored).

Statistical analysis

The data from the individually-caged birds were statistically analysed using GLM model (Minitab for Windows, Statistical software, release 9.2 (1993); Minitab Inc., PA, USA) of

ANOVA with a factorial arrangement of 2×2 (level of enzyme×form of food) with day of experiment as a repeated measure. The data from the groups were similarly analysed using ANOVA (two levels of wetness×two levels of enzyme) with repeated measures (week).

Results

Food intake, live-weight gain, feed conversion efficiency, dry matter retention and water intake

The effect of wetting wheat-based food on food intake and live-weight gain of broiler chickens was found to be highly significant ($P < 0.001$), whereas supplementation of the same food with enzyme did not significantly affect food intake or live-weight gain (Table 2).

FCE (g weight gain/g food eaten) and DM retention were not significantly ($P > 0.05$) affected by wetting or enzyme addition. There was a significant interaction between the effects of wetness and week on food intake and live-weight gain: Fig. 1 clearly indicates that the difference between intake for dry food and wet food increased as the experiment progressed and this was also true for live-weight gain (Fig. 2).

Total water intakes (from food and drinker) were proportional to dry food intakes, with values of 188 and 110 g/d for wet- and dry-fed birds respectively ($P < 0.001$, SEM 2.53); the overall means with and without enzyme were 145 and 154 respectively ($P < 0.05$, SEM 2.53). The water:food ratios were thus 1.36, 1.26, 1.28 and 1.34 g/g (NS, SEM 0.03) for dry food, wet food, food without enzyme and food with enzyme respectively.

Digesta viscosity

Wetting wheat-based food significantly ($P < 0.001$) reduced the viscosity of the gut contents, compared with dry food with the same nutrient composition; the values were 2.64 and 4.40 kPa · s (SEM 0.18) for wet food and dry food respectively. Addition of enzyme to wheat-based food also significantly ($P < 0.001$) reduced digesta viscosity in gut contents over the whole digestive tract, from a mean of 4.47 for food without enzyme to one of 2.57 kPa · s for food with enzyme respectively (SEM 0.18).

Table 2. Feed intake, live-weight gain, feed conversion efficiency and dry matter retention in broiler chickens from 7 to 35 d post-hatching, given a wet and dry wheat-based diet with or without enzyme†

(Mean values for six broiler chickens per group)

Treatment . . .	Food type		Enzyme		Significance of interaction of wetting × age (ANOVA)	
	Dry	Wet	Without	With	SEM	P
Feed intake (g air-dried feed/d)	89.3	153.4***	120.5	122.2	1.72	< 0.001
Live-weight gain (g/week)	277.7	457.8***	363.5	372.0	14.5	< 0.05
Feed conversion efficiency (live-weight gain (g)/feed intake (g))	0.446	0.427	0.432	0.441	0.01	NS
Dry matter retention (g/kg food DM)	750.1	757.5	743.5	764.1	16.6	NS

Mean values were significantly different from those of dry-diet group: *** $P < 0.001$.

† For details of diet see Table 1 and p. 298. The wet diet was made by addition of 1.3 kg water to 1 kg air-dried diet. The feed enzyme was included at 1 g/kg and contained (U/g): xylanase 2700; β-glucanase < 70; bacillus protease 700 (Finnfeeds International, Marlborough, Wilts., UK).

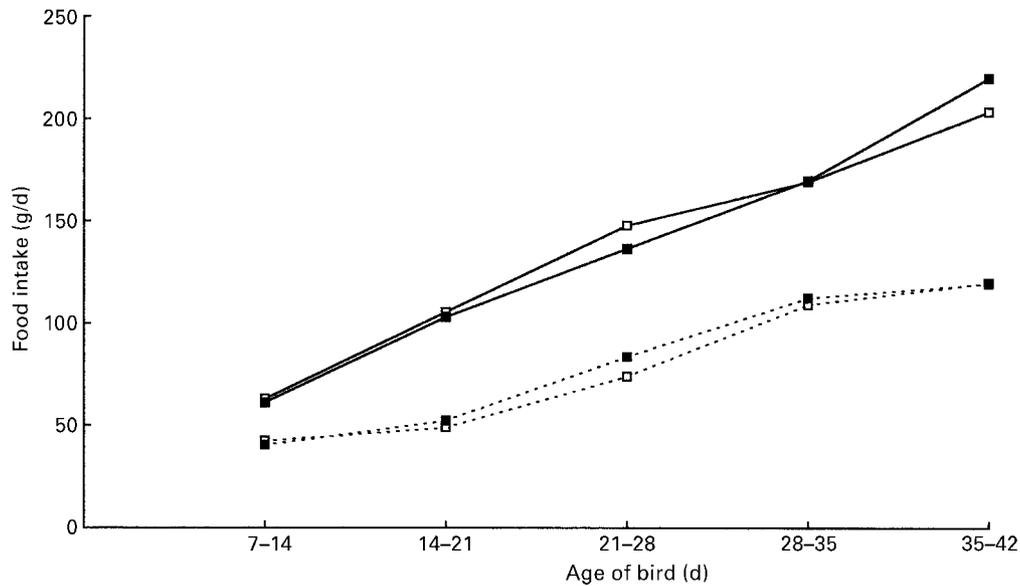


Fig. 1. Food intake (g DM/d) of broiler chicken (n 6 per group) fed on a wheat-based diet given in dry (---) or wet (—) form with (■) or without (□) added enzyme; SEM 1.72. For details of the diet see Table 1 and p. 298. The wet diet was made by addition of 1.3 kg water to 1 kg air-dried diet. The feed enzyme was included at 1 g/kg and contained (U/g): xylanase 2700; β -glucanase < 70; bacillus protease 700 (Finnfeeds International, Marlborough, Wilts., UK).

The interaction between wetting and enzyme addition was found to be significant on the overall viscosity values of the gut contents, and this was seen throughout the experimental period, from 14 d to 35 d old (Fig. 3). The viscosity of digesta in the gut was greatly increased in broiler chickens given dry food without enzyme addition, while offering dry or wet foods with addition of enzyme significantly reduced the viscosity of digesta. Fig. 3 also shows that the digesta viscosity in the gut for the dry no-enzyme treatment declined markedly as the birds got

older so that there was comparatively little difference between treatments from 28 d.

The viscosity of the chyme in the upper segments of digestive tract (crop, proventriculus plus gizzard, and duodenum) was similar in all treatment groups, whereas in the lower segments of the tract (proximal, middle and distal parts of the small intestine) the birds receiving dry food without enzyme produced significantly higher viscosities than those given wet food with or without enzyme and dry food with enzyme; Fig. 4 presents a summary of the

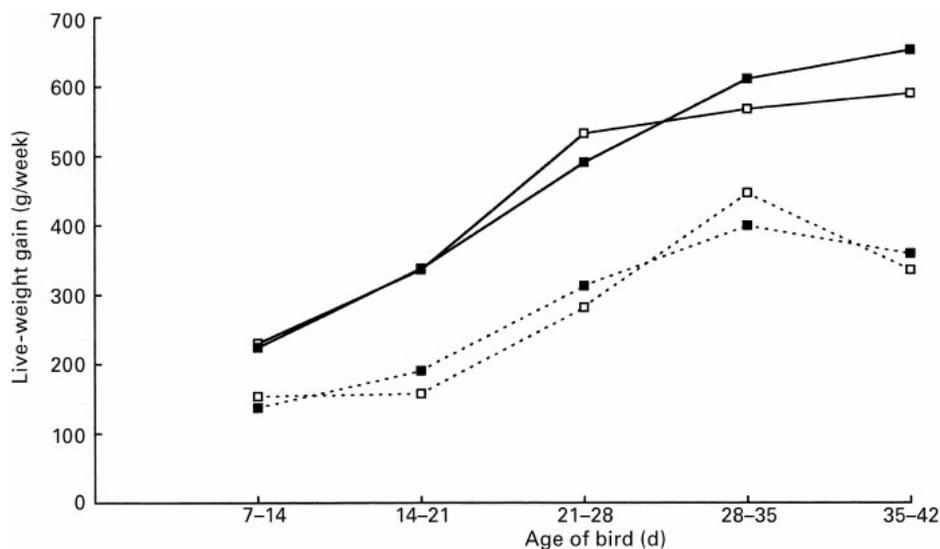


Fig. 2. Live-weight gain (g/week) of broiler chickens (n 6 per group) fed on a wheat-based diet given in dry (---) or wet (—) form with (■) or without (□) added enzyme; SEM 14.5. For details of the diet see Table 1 and p. 298. The wet diet was made by addition of 1.3 kg water to 1 kg air-dried diet. The feed enzyme was included at 1 g/kg and contained (U/g): xylanase 2700; β -glucanase < 70; bacillus protease 700 (Finnfeeds International, Marlborough, Wilts., UK).

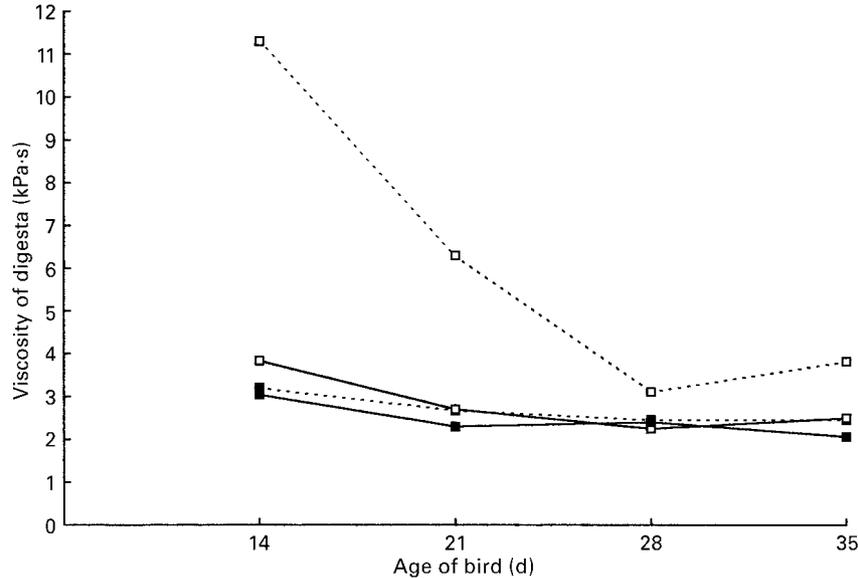


Fig. 3. Digesta viscosity of the gut contents (mean values for six segments) of broiler chickens (n 4 per group). Fed on a wheat-based diet given in dry (---) or wet (—) form with (■) or without (□) added enzyme; SEM 0.18. For details of diet see Table 1 and p. 298. The wet diet was made by addition of 1.3 kg water to 1 kg air-dried diet. The feed enzyme was included at 1 g/kg and contained (U/g): xylanase 2700; β -glucanase < 70; bacillus protease 700 (Finnfeeds International, Marlborough, Wilts., UK).

results as means for birds killed at 14, 21, 28 and 35 d post-hatching.

Gut dimensions

The length of the entire gut was significantly ($P < 0.05$) increased by wetting but not by addition of enzyme (Table 3) but there was a significant interaction whereby addition of enzyme to dry food significantly increased the gut length from 154.8 cm for dry food without enzyme to 162.2 cm (SEM 3.05) while a decrease in the gut length from 168.0 for wet food without enzyme to 162.4 cm for wet

food with enzyme (SEM 3.05) was not significant. The effect of enzyme addition and wetting on the length of individual segments was, however, not found to be significant (Table 3).

The main effects of treatments on the full and empty weight of the whole gut in broiler chickens are given in Table 3, from which it can be noted that wetting significantly ($P < 0.05$) increased both full and empty weight of the gut while the effect of enzyme addition was not significant. The interaction between enzyme and wetting was significant on empty-gut weight but not on full-gut weight: enzyme addition to dry-wheat food significantly

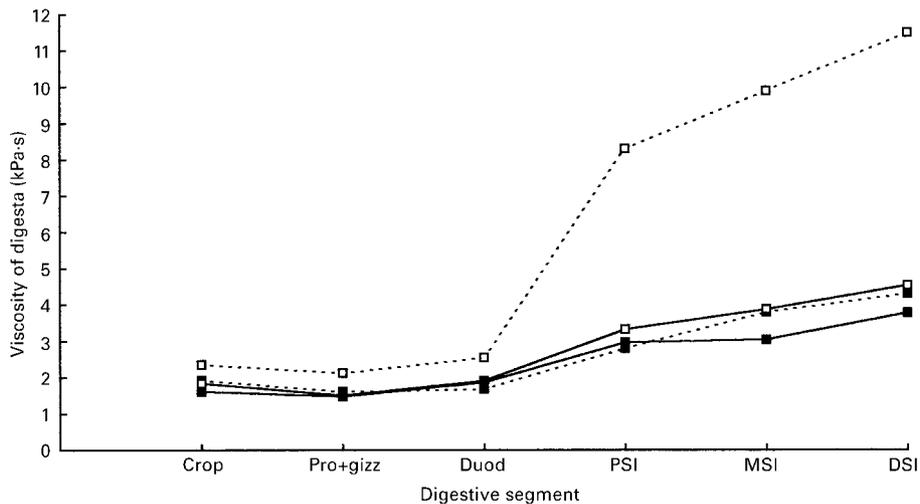


Fig. 4. Digesta viscosity in different gastrointestinal segments of broiler chickens (n 4 per group) fed on a wheat-based diet given in dry (---) or wet (—) form with (■) or without (□) added enzyme. Values are means from birds killed at 14, 21, 28 and 35 d post-hatching (SEM 0.18). Pro+ gizz, proventriculus and gizzard; Duod, duodenum; PSI, proximal small intestine; MSI, middle small intestine; DSI, distal small intestine. For details of diet see Table 1 and p. 298. The wet diet was made by addition of 1.3 kg water to 1 kg air-dried diet. The feed enzyme was included at 1 g/kg and contained (U/g): xylanase 2700, β -glucanase < 70; bacillus protease 700 (Finnfeeds International, Marlborough, Wilts., UK).

Table 3. Treatment means and results of statistical analyses for gut length (cm), full gut weight (g), empty gut weight (g) relative empty gut weight in broiler chickens given wet and dry food of a wheat-based diet supplemented with or without enzyme† (Mean values for four broiler chickens per group)

Treatment . . .	Food type		Enzyme		SEM	Significance of interactions‡
	Dry	Wet	Without	With		
Gut length (cm)	158.5	165.2*	161.4	162.3	2.15	§
Full-gut weight (g)	133.4	168.7*	144.6	157.5	4.72	
Empty gut weight (g)	78.60	85.72*	81.55	82.80	2.06	¶
Relative gut weight (g/kg body weight)	84.4	82.6	85.6	81.4*	1.1	**
Length of digestive segment (cm)						
Crop	9.54	10.3	9.61	10.22	0.51	
Proventriculus + gizzard	9.53	9.73	9.6	9.73		
Duodenum	24.2	25.3	24.8	24.6		
Proximal SI	37.5	39.3	38.5	38.2		
Middle SI	37.5	38.4	38.5	38.2		
Distal SI	37.5	38.4	38.5	38.2		
Caecum	13.36	13.4	13.4	13.2		
Colon	7.65	7.02	7.04	7.25		
Empty weights (g)						
Crop	5.75	7.5	6.21	7.04	0.44	
Proventriculus + gizzard	30.23	33.21	31.9	31.52		
Duodenum	7.54	8.22	7.85	7.92		
Proximal SI	10.43	11.46	10.8	11.08		
Middle SI	8.84	9.84	9.45	9.24		
Distal SI	7.57	8.45	7.88	8.14		
Caecum	5.14	4.73	4.8	5.07		
Colon	3.00	2.52	2.73	2.74		
The amount of digesta in digestive segment (g)						
Crop	14.30	34.81*	19.64	29.47	1.97	
Proventriculus + gizzard	13.36	15.77	14.47	14.66		
Duodenum	1.66	1.73	1.58	1.80		
Proximal SI	7.32	9.26	7.92	8.67		
Middle SI	6.76	9.23*	8.14	7.85		
Distal SI	4.35	6.00*	5.17	5.17		
Caecum	4.97	4.91	4.66	5.22		
Colon	1.92	1.60	1.69	1.82		

SI, small intestine.

Mean values were significantly different from that of dry-diet group: * $P < 0.05$.

† For details of diet see Table 1 and p. 298. The wet diet was made by addition of 1.3 kg water to 1 kg air-dried diet. The feed enzyme was included at 1 g/kg and contained (U/g): xylanase 2700; β -glucanase < 70; bacillus protease 700 (Finnfeeds International, Marlborough, Wilts., UK).

‡ The only other significant differences and interactions were as shown (see p. 000 for details).

§ Significant main effect of age ($P < 0.001$), interaction between effects of wetting \times enzyme ($P < 0.05$), enzyme \times age ($P < 0.05$).

|| Significant main effect of age ($P < 0.001$), interaction between effects of enzyme \times age ($P < 0.05$).

¶ Significant main effect of age ($P < 0.001$), interaction between effects of wetting \times enzyme ($P < 0.01$).

** Significant interaction between effects of enzyme \times week ($P < 0.05$).

($P < 0.05$) increased empty-gut weight compared with dry-wheat food without enzyme (73.6 v. 83.6 g/bird respectively), whereas there was a significant decrease in empty-gut weight with enzyme addition to wet-wheat food compared with wet-wheat food without enzyme (89.45 v. 82.0 g, respectively). Empty weights of individual digestive segments tended to increase with wet feeding and enzyme supplementation, but the differences in these treatment groups were not found to be significant (Table 3). The relative empty-gut weight (empty-gut weight/kg live weight) was found to be unaffected by wetting. However, the values were numerically lower in birds given wet foods than those given dry foods (Table 3). There was a significant ($P < 0.05$) reduction in the relative gut weight with food with added enzyme, compared with food without enzyme.

Wetting wheat-based food resulted in significant increases in the amount of fresh digesta in the gut (54.8 for dry-wheat food v. 83.0 g for wet-wheat food (SEM 5.6) respectively). This showed that the gut capacity increased by 33 % with wet food compared with dry food. Although

enzyme addition had no significant effect on fresh digesta contents, the birds given food with enzyme tended to have more fresh digesta in the gut than the birds given food without enzyme (63.0 v. 74.7 g (SEM 5.6) respectively). The amount of fresh digesta in the crop, the middle third and the distal third of small intestine was found to be significantly higher in wet-fed birds than in dry-fed birds (Table 3).

Gut histo-morphology

The thickness of proventricular tissue wall, proventricular gland, mucosa lining folds and muscle layer were significantly ($P < 0.05$) increased by wetting food but not by enzyme supplementation. However, the interaction between effects of wetting and enzyme addition were found to be significant ($P < 0.05$) on the thickness of these proventricular tissue layers, except the thickness of muscle layer (Table 4). A decrease in the thickness of all tissue layers in the proventriculus by enzyme addition was, however, not found to be significant ($P > 0.05$). Enzyme addition to dry

Table 4. Thickness (μm) of proventricular tissue wall, glands, mucosa lining; tunica muscularis thickness (μm) of gizzard glands and gizzard-lining; villi height (μm), crypt depth (μm), the thickness of tunica muscularis (μm), and the number of crypts per villi of the epithelium of the small intestine in broiler chickens given wet and dry food of a wheat-based diet supplemented with or without enzyme†
(Mean values for four broiler chickens per group)

Treatment . . .	Food type		Enzyme		Significance of treatment effects					
	Dry	Wet	Without	With	SEM	W	E	S	W × E	W × E × S
Proventriculus										
Wall (μm)	4542	4752‡	4687	4607	54.2	**	NS			***
Glands (μm)	3410.7	3489.0‡	3484	3415	22.07	**	NS			***
Mucosa lining folds (μm)	624	693‡	653.7	663.6	17.89	**	NS			**
Gizzard										
Tunica muscularis (μm)	507.3	570.0‡	549.0	528.3	14.24	**	NS			NS
Glands (μm)	454.3	492.8	546.5	400.6‡	17.09	NS	***			NS
Mucosal lining (μm)	474.4	528.3	562.1	440.6‡	25.76	NS	**			NS
Small intestine										
Villi height (μm)	748.6	799.7‡	769.6	778.8	6.91	***	NS	***	**	***
Crypt depth (μm)	95.0	115.1‡	104.1	105.9	1.7	**	NS	***	**	*
Thickness of tunica muscularis (μm)	279.4	320.0‡	290.1	309.1‡	4.01	***	**	***	***	NS
Crypts per villus (<i>n</i>)	3.35	3.78‡	3.60	3.53	0.048	***	NS	***	***	***

W, wetness; E, enzyme; S, segments.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Mean values were significantly different from those of the dry-diet group or no-enzyme group: ‡ $P < 0.05$.

† For details of diet see Table 1 and p. 298. The wet diet was made by addition of 1.3 kg water to 1 kg air-dried diet. The feed enzyme was included at 1 g/kg and contained (U/g): xylanase 2700; β -glucanase < 70; bacillus protease 700 (Finnfeeds International, Marlborough, Wilts., UK).

foods was found to cause a significant increase in the thickness of all tissue layers (except muscle layer) in the proventriculus, whereas its presence in wet foods significantly ($P < 0.05$) reduced the thickness of tissue wall and glands of proventriculus, but not the thickness of other tissue layers (muscle and lining folds) (Table 4).

Wet feeding did not affect the thickness of gizzard glands or of the tissues lining the gizzard while addition of enzyme to dry or wet foods significantly ($P < 0.05$) reduced the thickness of both gizzard tissue layers, with no interaction between enzyme addition and wetting (Table 4).

The height of villi, depth of crypts, the thickness of muscular layer and the number of crypts per villus of the gut, as a mean of digestive segments from duodenum to colon were found to be significantly elevated in the treated animals ($P < 0.05$) (Table 4). The effect of enzyme addition on the thickness of gut tissue layers was not found to be significant, whereas there was a significant interaction between wetting and enzyme addition (Table 4): enzyme addition to dry foods significantly ($P < 0.05$) increased the heights of villi, the depths of crypt layers, the thickness of tunica muscularis and the crypts: villi ratios, whereas the presence of enzyme in wet foods tended to reduce the thickness of the tissue layers, but not significantly so (Table 4).

Crypt cell proliferation rate

Wetting significantly ($P < 0.001$) reduced the mean CCPR over the whole digestive tract in the crypts in chickens aged 28 d, from 39.4 in the dry control group to 28.7 cells/crypt per 2 h (SEM 0.36). Enzyme supplementation was also found to significantly reduce CCPR, from 38.90 in the control group without enzyme to 29.2 cells/crypt per 2 h (SEM 0.36). A significant interaction was observed between the effects of wetting and enzyme in which CCPR was significantly ($P < 0.05$) higher in birds given dry food without enzyme supplementation, compared with those given dry food with

enzyme or wet food with or without enzyme. Enzyme addition to dry food significantly reduced CCPR whereas the effect of enzyme was not found to be significant on CCPR in birds given wet foods, and CCPR was significantly lower with wet food without enzyme than dry food without enzyme.

Discussion

In the present experiment, significantly higher food intake and live-weight gain were observed in birds receiving wheat-based food with the addition of 1.3 kg water/kg air-dried food, compared with those given the same food in the dry form. Wetting reduced digesta viscosity as did the addition of exogenous enzyme, but the latter did not significantly affect food intake or live-weight gain. Possible reasons for this discrepancy will be explored.

Wet feeding

The increased live-weight gain in broiler chickens with wet food based on a high proportion of ground wheat was not accompanied by a significant improvement in FCE, suggesting that the birds with wet food grew faster because they ate more than those with dry food. This agreed with the results of Yasar & Forbes (1999) under similar conditions but was in contrast to the earlier findings (Yalda & Forbes, 1995, 1996) where broiler chickens given wet commercial pelleted feed had a significantly higher FCE, with little or no increase in food intake. However, during the period in the present experiment in which the wet-fed birds gained 2285 g, those on dry food gained only 1400 g. During the time the wet-fed birds were gaining 1400 g their FCE would, presumably, have been much higher than those fed dry food and it is commercial practice to 'correct' FCE for differences in body weight. Therefore, it is reasonable to reinterpret the data as suggesting that wet feeding did increase FCE, compared with dry feeding, when equivalent

periods of growth are compared. Possible causes of this are discussed later.

There are several possible reasons for the increased food intake with wet feeding: increased rate of passage; improved palatability; improved nutrient balance; restricted water intake.

Rate of passage. Wet feeding has been shown to be associated with faster rate of passage of digesta through the tract (Gohl & Gohl, 1977), linked to a reduction in digesta viscosity (Gohl *et al.* 1978). The evidence for increased rate of passage is indirect in the present experiment as it was not measured. Comparison of the results in Tables 2 and 3 shows that daily food intake was increased more by wet feeding than was the weight of digesta in the whole digestive tract, which would suggest a 13% faster feed passage with wet food. The shorter time spent by digesta in the gut would tend to reduce digestion and absorption which might have approximately equaled the potential increase in absorption expected from wet feeding. The work of Almirall & Garcia (1994) showed a positive relationship between the rate of feed passage and feed intake in young broilers but the extent to which this was a causal relationship is not known. Van Der Klis & Van Voorst (1993) noted a slowing of digesta passage when carboxymethyl cellulose was incorporated into the diet to greatly increase digesta viscosity and it is reasonable to conclude that the reduction in viscosity caused the increased rate of passage. However, it is not clear why the exogenous enzyme did not increase voluntary food intake when it had a similar effect on rate of passage as did wetting.

Palatability. It is unlikely that the greater intake of food DM with wet feeding is due to an improvement in palatability. Palatability is a complex concept in which the preference shown for a food is, in the long-term, related to the learned association between the sensory properties of a food and the adequacy of nutrient supply and balance after eating that food (Forbes, 1995). Simply changing the flavour or texture of food does not result in long-lasting changes in intake or preference whereas changing the nutrient properties of the food frequently does affect intake and preference. Given a choice between wet and dry food from the same source, chicks initially preferred the one to which they had previously become accustomed, but within 1 week were eating almost exactly equal proportions of DM from each (AY Yalda and JM Forbes, unpublished results). Thus, however much it might be thought that wet food is attractive to chickens, there is nothing to suggest that this would cause the long-term large increases in food intake observed in this experiment.

Nutrient supply. The third possibility is that intake was increased by wet feeding due to a more appropriate balance of nutrients being absorbed compared with dry food. Given the very large increase in the weight of digesta in the crop due to wet feeding (Table 3), it is likely that there was appreciable digestion occurring in that organ, due both to activation of endogenous enzymes in the food and to fermentation by microbial action. This digestion could have included partial cleavage of NSP by endogenous phytase in the wheat and/or partial breakdown of cell walls, leading to the observed reduction in digesta viscosity and thus to a greater capacity to ingest food. The potential

for improved nutrient absorption was offset by the increased rate of passage with wet feeding, resulting in no overall effect of diet on the proportion of the food retained in the body.

Restriction of water intake. A fourth possibility to account for the large increase in food intake and live-weight gain with wet feeding is that the water intake of the dry-fed birds was restricted by the use of individual nipple drinkers. Water restriction depresses food intake, approximately in proportion to the degree of restriction (Kellerup *et al.* 1965) although it has been suggested that broiler chickens drink more than they need, perhaps due to boredom (Yeomans, 1986). The total water intakes of the dry-fed birds were significantly lower than those of wet-fed birds, even though the latter took less water from the drinkers as they had a high intake from that incorporated with the wet food. However, both treatments resulted in similar water intake : food intake ratios which might suggest that the dry-fed birds were not short of water, although it is worth noting that in some previous experiments on wet feeding the water intake : food intake ratios were significantly higher for wet-fed birds than for dry-fed birds (Yalda & Forbes, 1995). Previous work with broilers in the same conditions, including nipple drinkers, as the ones reported here did not result in such a marked depression in performance of the dry-fed birds (Yalda & Forbes, 1996) but, despite this, it is not possible to rule out water restriction as a reason for the poor performance of the broilers given food in the dry form in the present experiment.

Exogenous enzyme addition

In contrast to the large effect of wetting, enzyme addition to foods, whether dry or wet, did not result in significant increases in food intake, weight gain or FCE in the present experiment. This was in contrast to earlier results (Yalda & Forbes, 1996) and the expectation that addition of an enzyme with high arabinoxylanase activity to a wheat-based diet would result in significant improvement in bird performance. Enzyme addition to commercial pelleted foods has often been found to increase live-weight gain with a significant improvement in FCE although these responses to enzyme were highly variable between studies (Rose & Arscott, 1962; Burnett, 1966; Adams & Naber, 1969; White *et al.* 1981; Classen *et al.* 1985; Fengler *et al.* 1988; Pawlic *et al.* 1990; Bedford *et al.* 1991; Friesen *et al.* 1992; Graham & Petterson, 1992; Almirall *et al.* 1995), probably related to the rate of enzyme inclusion and the type and inclusion rate of ground cereal grains (summarised by Yasar, 1998).

Birds fed on diets with added enzymes have been found not to increase food intake while there is most often a significant increase in FCE which has been speculated to be due to the partial degradation of viscous, gel-forming compounds present in the grain cell-wall materials by enzymes (for review see Bedford, 1995). In a recent study, despite significant reductions in digesta viscosity with wheat-based diets due to enzymes, there appeared to be no significant effect on weight gain or food intake but a significant increase in FCE accompanied by an increase in

the metabolisability of dietary energy (Allen *et al.* 1997). In the present case trends for higher FCE and weight gains obtained with enzyme were not significant even though there was significantly lower digesta viscosity.

Lack of response to the enzyme supplementation in the present case may have been due to an insufficient rate of enzyme inclusion, as Willingham *et al.* (1959), Rose & Arscott (1962) and Mannion (1981) found that increasing the inclusion level of enzyme resulted in significant improvement in FCE and weight gain. However, the activity of the enzyme (arabinoxylanase) seems to have been appropriate for this wheat-based diet as it significantly reduced viscosity. In addition, the enzyme added had some protease activity but we did not measure N retention in this experiment so cannot say whether or not the protease had a beneficial effect.

Ritz *et al.* (1995a) found that enzyme supplementation did not affect the rate of digesta passage although there was a reduction in digesta viscosity. Bedford (1993) and Choct *et al.* (1996) also found that reduction in digesta viscosity with enzyme is most probably associated with a partial degradation of NSP, which in turn results in improved FCE, rather than an increase in the rate of passage that may cause food intake to increase. These findings were, however, in contrast to the results of Almirall & Garcia (1994), in which enzyme reduced the digesta viscosity but also increased the rate of feed passage and the food intake.

The effect of enzyme on gut size was not very consistent; enzyme addition gave an increase in the gut length and weight with dry food, but a decrease with wet food, although the relative gut weights (g/kg body weight) significantly decreased with enzyme addition to both dry and wet food. The latter is frequently found with enzyme supplementation (Brenes *et al.* 1993; Viveros *et al.* 1994; Ritz *et al.* 1995b).

Enzyme supplementation of wheat-based diet, whether dry or wet, reduced total water intake. Bedford (1995) observed that enzyme addition to broiler foods significantly reduced the water in faecal output by approximately 23% compared with control foods while Van Der Klis & Van Voorst (1993) found that feeding NSP caused an increase in the water content of the digesta. Enzyme supplementation and water treatment have been reported to alleviate the problems of high water consumption by birds and the production of sticky droppings associated with viscous grains (Gohl *et al.* 1978; McNab & Smithard, 1992), presumably by reducing the amounts of NSP in the digesta.

Structure and function of digestive tract

Savory (1975) and Savory & Gentle (1976a, b) stated that increased levels of fibre in the broiler diet lead to compensatory increases in food intake and as a consequence of this the digestive tract increases in length and weight although there is no change in the villi heights. Such differences in the gut size between birds given low- or high-fibre diets are most probably due to the differences in food intake rather than dietary fibre *per se*. In the present case, the birds given wet food ate significantly larger amounts of food DM and had longer and heavier digestive tracts than those given the same food in dry form. These increases in dimensions of

the digestive tract are approximately proportional to the increase in body weight and might, therefore, be a general consequence of faster growth, rather than a mechanism for the achievement of that growth.

As the bird ages it seems to be able to cope better with NSP and such treatments as wetting and exogenous enzymes might be most beneficial in young birds. The viscosity of digesta was low at all ages at which it was measured in those birds which had water and/or enzyme added to the food (mean of all segments < 4 kPa · s, Fig. 3). In contrast, those given dry food without enzyme had very high digesta viscosities (about 11 kPa · s) at 14 d, falling to < 4 kPa · s at 28 d. This supports the observation that wet feeding is more effective in improving DM retention and/or efficiency in younger birds (Yasar & Forbes, 1999).

The structure of the gut as well as its size appeared to be affected by the intestinal environment. In the proventriculus and the gizzard, wetting significantly increased the thickness of all tissue layers, in parallel with an increase in live-weight gain and gut size. The effect of enzyme was, however, found to be different, depending on the segment of the foregut and the form of food. In the proventriculus, the thickness of tissue layers increased with dry food and there was a decrease with wet food in the presence of enzyme. However, enzyme supplementation reduced the thickness of tissue layers in the gizzard with both dry and wet foods. Viscous NSP have been shown to decrease the density of crypts and villi as well as their thickness, but to increase the rates of enterocyte division in rats (Johnson *et al.* 1984) and chickens (Viveros *et al.* 1994), as was found with broiler chickens in the present experiment. Wet feeding increased the height of villi, the thickness of crypt and muscle layers in the intestinal segments and significantly reduced the CCPR. Enzyme supplementation increased the thickness of intestinal tissue layers in dry-fed birds whereas there was a reduction in these variables in wet-fed birds, reflecting the similar trend in the gut size with enzyme. This effect of enzyme was found to be more pronounced for the duodenum, proximal, middle and distal part of the small intestine than the caecum and colon. The increased digestive tract size, villi heights and crypt depths with the use of enzyme in dry food in our experiment is in contrast to the results of Viveros *et al.* (1994). However, the results obtained in the present study from the wet foods with enzyme were found to be quite similar to the results of Viveros *et al.* (1994). Similarly, enzyme addition has been found to reduce CCPR in broiler chickens given rye-based diets (Silva & Smithard, 1996).

In terms of digestive function, these various changes in gut dimensions and cell proliferation support the notion of an increased rate of passage with wet feeding. The increased height of villi and number of crypts per villus and crypt depth point to increased secretion of succus entericus and increased absorptive area. The thicker muscular layer points to increased propulsive function and the reduced CCPR indicates reduced costs of maintaining gut integrity. That CCPR was reduced even though the layers of the gut wall were thicker in wet-fed birds emphasises the magnitude of this effect and the large potential saving in nutrients for maintenance of digestive function due to wet feeding. The significant interaction between effects of wetting and

enzyme addition on the weights and lengths of the digestive tract, heights of villi, the depths of crypt layer, the thickness of tunica muscularis, the crypts : villi ratio and CCPR, in which there was no additional effect of enzyme addition in wet-fed birds, supports the notion stated earlier, that pre-digestion in the crop with wet food replaces many of the actions of exogenous enzyme.

In conclusion, wet feeding caused very large and significant increases in food intake and growth rate, but with no change in FCE. Enzyme supplementation had little effect on intake and growth, perhaps because the inclusion level was not sufficient for the high-wheat food. Both wetting and enzyme reduced CCPR and digesta viscosity but neither of these could be the cause of the increase in intake and growth with wet feeding as such increases were not seen with the enzyme.

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