

Influence of Enzyme Supplementation and Heat Processing of Barley on Digestive Traits and Productive Performance of Broilers¹

M. García, R. Lázaro, M. A. Latorre,² M. I. Gracia,³ and G. G. Mateos⁴

Department of Animal Science, Universidad Politécnica de Madrid, Ciudad Universitaria, 28040 Madrid, Spain

ABSTRACT We studied the influence of enzyme supplementation (ES) of the diet and heat processing (HP) of barley on digestive traits and productive performance of broilers from 1 to 42 d of age. There were 6 diets arranged factorially with 2 doses (0 and 500 ppm) of a fungal enzyme complex with β -glucanase and xylanase activity and 3 HP of barley (raw, micronized, and expanded). In addition, a control diet based on raw corn without ES was also included from 1 to 21 d of age. Enzymes reduced intestinal viscosity (IV) at all ages ($P \leq 0.001$) and water intake at 21 d of age ($P \leq 0.01$) and increased DM of the ileal contents at 28 d ($P \leq 0.001$). Also, ES increased total tract apparent retention of nutrients and BW gain and feed conversion ratio from 1 to 42 d of age ($P \leq 0.001$). Heat processing of barley increased

IV at 7 and at 28 d of age, and DM of ileal contents ($P \leq 0.05$) at 28 d of age. In addition, HP improved feed intake ($P \leq 0.01$) and BW gain ($P \leq 0.001$) from 1 to 7 d of age, but the effects disappeared after 21 d of age. From 1 to 7 d of age, chicks fed micronized barley had higher IV, gained less weight, and had poorer feed conversion ratio than chicks fed expanded barley ($P \leq 0.05$). It is concluded that barley with enzymes can substitute for all of the corn in diets fed to broilers from 1 to 21 d of age. Enzymes improved digestive traits, retention of nutrients, and broiler performance from 1 to 42 d of age, and HP of barley improved performance from 1 to 7 d of age. The effects of HP of barley on broiler performance were more evident with expansion than with micronization.

Key words: barley, broiler performance, enzyme supplementation, heat processing, nutrient digestibility

2008 Poultry Science 87:940–948
doi:10.3382/ps.2007-00266

INTRODUCTION

The use of barley in poultry diets is limited, because its high content in nonstarch polysaccharides (NSP) results in increased intestinal viscosity (IV), reduced litter quality, and poor productive performance (Herstad and McNab, 1975). Most of the adverse effects of barley feeding have been attributed to the content of β -glucans (Ravindran et al., 2007), although xylans are also a contributing factor (Gracia et al., 2003b). The NSP fraction of the cereal protects lipids, starch, and protein, thereby compromising the access of digestive enzymes to dietary components. Enzyme supplementation (ES) reduces IV and improves total tract apparent retention (TTAR) of nutrients and feed intake (Lázaro et al., 2003b, 2004), thereby increasing broiler productivity.

Heat processing (HP) of cereals at temperatures above 90°C is a common practice to improve nutrient digestibility and productive performance in piglets (Medel et al., 2004; Mateos et al., 2007). However, the effects of HP in broilers are not well documented (Vukic-Vranjes et al., 1994; González-Alvarado et al., 2007). Heating disrupts the structures of the cell, liberating the lipids contained in the oil bodies (Huang, 1992) and releasing the starch from the protein matrix, thereby increasing the susceptibility of starch to α -amylase. On the other hand, an excess of heat might increase Maillard reactions and the proportion of resistant starch, a fraction of starch that is less available to enzyme activity (Vicente et al., 2008). In addition, HP may increase the solubility of the dietary fiber increasing IV and reducing nutrient digestibility (Mateos et al., 2002).

Two methods widely used to process cereals in practice are micronization and expansion. These 2 methods use a different set of conditions and, therefore, affect the structure of the dietary components of barley differently. Consequently, micronization and expansion might have different effects on digestive physiology and broiler performance. To our knowledge, there is no comparative data on the influence of these 2 procedures on performance of broilers fed barley diets.

©2008 Poultry Science Association Inc.
Received June 26, 2007.

Accepted January 23, 2008.

¹Supported by funding through Project AGL 2005-03724, Ministerio de Ciencia y Tecnología, Madrid, Spain.

²Current address: CITA, Avda Montañana 930, 50059 Zaragoza, Spain.

³Current address: Imasde Agroalimentaria, Pozuelo, Madrid, Spain.

⁴Corresponding author: gonzalo.gmateos@upm.es

The aim of this research was to examine the effects of ES to diets based on raw, micronized, or expanded barley on digesta characteristics, water intake, nutrient retention, and productive performance of broilers from 1 to 42 d of age.

MATERIALS AND METHODS

All experimental procedures used in this research were approved by the Animal Ethics Committee of the Universidad Politécnica de Madrid and were in compliance with the Spanish guidelines for the care and use of animals in research (Boletín Oficial Estado, 2005).

Ingredients and Diets

A batch of barley (8.2% moisture content) was obtained from a commercial supplier (Essasa, Cabezón de Pisuerga, Valladolid, Spain) and split into 3 portions. The first portion was passed through a hammer mill (model DFZC-635, Bühler AG, Uzwil, Switzerland) provided with a 2.5-mm screen and used in the manufacture of the diets. Vapor was added to the second portion until reaching 19.3% humidity, macerated for 24 h, and then passed through the micronizer (Microred 20, Micronizing Company, Framlingham, UK). The temperature and humidity of the grains in the last section of the machine were $74 \pm 2^\circ\text{C}$ and 11.2%, respectively. Afterwards, the barley was flaked through riffled rolls, finely ground (2.5-mm screen), and used to make the feeds. The third portion was finely ground (2.5-mm screen), expanded at 120°C and 30 bars of pressure during 5 s in a hydrothermal reactor (model AK38-1, Amandus Kahl, Reinbek, Germany), and used then as for the other 2 batches.

Six diets containing 50% barley were prepared in mash form. The diets combined 2 doses (0 or 500 ppm) of an enzyme complex (Endofeed, GNC Bioferm Inc., Saskatoon, Saskatchewan, Canada) produced from *Aspergillus niger* that contained by duplicate analysis 869 IU of xylanase (EC 3.2.1.8) and 850 IU of β -glucanase (EC 3.2.1.6) per gram of commercial product and 3 methods of processing the barley (unprocessed, micronized, and expanded). The diets were formulated according to the nutrient composition of ingredients of Fundación Española Desarrollo Nutrición Animal (2003) and met or exceeded the nutrition recommendations of the NRC (1994) for broilers. In addition, a control diet containing 50% corn and similar nutritive value to the barley diets was formulated. Because of lack of finishing cages, this diet was tested only from 0 to 21 d of age (see below). Chromium oxide was included at 0.4% in all diets (22 to 42 d of age) and was used as a marker. The ingredient composition, estimated nutrient content, and determined chemical values of the experimental diets are shown in Table 1.

Husbandry and Experimental Design

A total of 588 one-day-old straight-run chicks (Cobb 500) were obtained from a commercial hatchery (Cobb

España, Alcalá de Henares, Madrid, Spain), placed in a windowless, environmentally controlled room, and randomly allotted to 42 battery cages (Avícola Grau, Madrid, Spain; 1.0×0.4 m). Room temperature was kept at 32°C during the first 3 d of the trial and was then reduced gradually according to age until reaching 25°C at 21 d. The chicks received a 23 h/d light program and had free access to feed in mash form and water throughout the trial. At 21 d of age, the chicks were moved to a finisher barn that contained 36 cages (Avícola Grau, Madrid, Spain; 1.2×0.8 m). Due to lack of sufficient cages, all the replicates belonging to the corn control diet were withdrawn from the trial. Therefore, from 21 to 42 d, there were only 6 diets based on barley.

Laboratory Analyses

Feeds were analyzed in duplicate for moisture by the oven-drying method (930.15), ash by a muffle furnace (942.05), N by the Kjeldahl method (954.01), ether extract by Soxhlet fat analysis after 3 N HCl acid hydrolysis (920.39), and Ca by the dry ash method (927.02) as described by AOAC International (2000). Gross energy was determined using an adiabatic bomb calorimeter (IKA-4000, Janke & Kunkel, Staufen, Germany). The excreta was analyzed in duplicate for moisture, N, and GE, and the ileal contents were analyzed for N following the same methods previously described for feeds. Chromium content of feeds, excreta, and ileal contents were analyzed by atomic absorption spectrophotometry (Smith-Hieftje 22, Thermo Jarrell Ash Corporation, Franklin, MA), using predosed samples of excreta to prepare common-matrix standards (García et al., 1999). Before analysis, the samples were ashed at 550°C for 6 h and digested by boiling with a solution of nitric acid (1.5 M) and potassium chloride (3.81 g/L). The activity of the commercial enzyme complex used was determined by the rate of release of reducing sugar (Miller, 1959) during incubation (5 or 10 min) with the appropriate substrate (β -glucan or xylan) at pH 4 and 30°C . Total NSP content of barley, diets, and excreta were analyzed following the method described by Theander et al. (1995).

Digestive Traits

Three (7 d of age), 2 (28 d of age), and 1 (42 d of age) chicks per cage were randomly selected, weighed, and euthanized by cervical dislocation. The jejunum (defined as the region from the pancreas to Meckel's diverticulum) was dissected aseptically, and the digesta contents were collected and pooled by replicate as described by Lázaro et al. (2004). The digesta was homogenized, and 2 Eppendorf tubes were filled (1.5 g of sample) and centrifuged ($12,000 \times g$, 3 min). The viscosity (in centipoises, cP) of a 0.5-mL aliquot obtained from the supernatant solution was determined at 7, 28, and 42 d of age with a digital viscosimeter (model DV-III, Brookfield Engineering Laboratories Inc., Stoughton, MA) at 37°C . Each sample was read twice at the speed of 12 rpm, and the

Table 1. Composition and chemical analyses of the experimental diets (% , as-fed basis unless stated otherwise)

Item	0 to 21 d			22 to 42 d			
	Corn	Barley		Barley			
Ingredient							
Barley ¹	—	50.0		50.0			
Corn	50.0	3.7		7.5			
Lard	5.0	6.0		7.0			
Full-fat soybean meal, 36% CP	1.6	1.6		1.6			
Soybean meal, 47% CP	29.9	32.7		29.6			
Sunflower meal, 36% CP	9.4	2.0		—			
Limestone	0.93	1.11		1.04			
Dicalcium phosphate	1.82	1.60		1.67			
Sodium chloride	0.43	0.45		0.39			
DL-Met, 99%	0.25	0.28		0.29			
L-Lys-HCl, 78%	0.17	0.06		0.01			
Vitamin and mineral premix ²	0.50	0.50		0.50			
Chromium oxide	—	—		0.40			
Calculated analysis ³							
ME _n , kcal/kg	2,980	2,930		3,040			
CP	22.1	22.7		21.0			
Total Lys	1.30	1.30		1.15			
Total Met + Cys	1.00	1.00		0.95			
Total Thr	0.84	0.85		0.78			
Total Trp	0.26	0.27		0.25			
Ca	0.91	0.92		0.90			
Available P	0.43	0.43		0.43			
		UNP ⁵	MICR ⁶	EXP ⁷	UNP	MICR	EXP
Determined analysis ⁴							
DM	89.0	91.8	91.2	90.8	90.9	90.9	89.8
CP	21.8	22.0	22.6	22.4	21.5	21.3	19.7
Crude fiber	4.3	4.0	4.3	3.9	3.9	4.0	4.4
Ether extract	7.8	8.0	7.7	8.3	9.2	9.2	8.8
Total ash	5.5	5.7	5.4	5.8	5.5	5.6	6.0
Ca	0.96	0.94	0.91	0.89	0.91	0.88	0.94

¹The barley used was unprocessed, micronized, or expanded, either with or without 500 ppm of the enzyme complex (Endofeed, GNC Bioferm Inc., Saskatoon, Saskatchewan, Canada), according to treatment.

²Provided the following (per kg of diet): vitamin A (transretinyl acetate), 7,500 IU; vitamin D₃ (cholecalciferol), 3,000 IU; vitamin E (all-*rac*-tocopherol acetate), 10 IU; riboflavin, 5.3 mg; pantothenic acid (D-calcium pantothenate), 8 mg; pyridoxine (pyridoxine·HCl), 1.8 mg; folic acid, 0.5 mg; vitamin K (bisulfate menadione complex), 2 mg; thiamin (thiamin mononitrate), 2 mg; vitamin B₁₂ (cyanocobalamin), 12.5 µg; D-biotin, 0.15 mg; niacin, 24 mg; choline (choline chloride), 350 mg; Se (Na₂SeO₃), 0.15 mg; I (KI), 1 mg; Cu (CuSO₄·5H₂O), 6 mg; Fe (FeSO₄·7H₂O), 30 mg; Zn (ZnO), 50 mg; Mn (MnSO₄·H₂O), 80 mg; and ethoxyquin, 150 mg.

³According to Fundación Española Desarrollo Nutrición Animal (2003).

⁴In duplicate samples.

⁵Unprocessed barley diet.

⁶Micronized barley diet.

⁷Expanded barley diet.

average value was used for the statistical analysis. Percentage of pasted vents, expressed as the proportion of chicks showing adhered droppings to the perineal region, was evaluated at 7 and 10 d of age by visual observation of the chicks, by a single monitor blind to treatment to ensure homogeneity of criteria.

Water intake for the last 24 h was measured by replicate at 21 and 35 d of age, and the DM content of ileal digesta and excreta were determined at 28 d of age by using representative samples of ileal digesta (4 to 5 g) and excreta (220 to 250 g). In addition, 2 g of crop content was collected immediately after slaughter at 42 d and mixed with 5 mL of distilled water to measure the pH using a pH meter fitted with a glass electrode (Crison 507, Crison Instruments S.A., Barcelona, Spain).

TTAR of Nutrients and Apparent Ileal Digestibility of N

At 21 d of age, 2 chicks per replicate were moved to metabolism cages and fed their respective experimental diets from 21 to 42 d of age. At 28 d of age, representative samples of excreta produced during the previous 48 h were collected by replicate, homogenized, oven-dried (60°C for 72 h), and ground with a laboratory hammer mill (1-mm screen, Riechst model Z-I, Stuttgart, Germany). The TTAR of N, organic matter (OM), and NSP and the AME_n of the diets were estimated by the indigestible marker method as described by Gracia et al. (2003a). Also, the ileal contents of the 2 chicks per replicate, euthanized at 28 d of age to study digesta traits, were col-

Table 2. Composition (% on DM basis) of the total nonstarch polysaccharides (NSP) of barley grain and barley diets

Item	Barley			Diet ¹		
	Raw	Micronized	Expanded	Raw	Micronized	Expanded
Rhamnose	0.05	0.04	0.05	0.18	0.15	0.16
Fucose	0.02	0.02	0.03	0.12	0.11	0.11
Arabinose	2.09	2.11	1.97	1.75	1.84	1.84
Xylose	3.16	3.34	2.91	1.91	2.17	1.96
Mannose	0.34	0.41	0.39	0.56	0.56	0.60
Galactose	0.32	0.37	0.42	1.83	1.78	1.94
Glucose	8.87	8.90	7.83	6.27	6.38	5.87
Uronic acids	0.37	0.36	0.42	1.26	1.14	1.28
Total NSP	15.23	15.56	14.01	13.89	14.12	13.76
Total β -glucans	3.51	3.62	3.14	—	—	—
Total xylans	6.04	6.35	5.47	—	—	—
Soluble xylans	0.39	0.38	0.39	—	—	—

¹Data corresponds to the nonenzyme-supplemented diets fed to broilers from 1 to 21 d of age.

lected, pooled, frozen (-20°C), and stored. Ileal samples were freeze-dried, ground, and analyzed for N, and the apparent ileal digestibility of N (AIDN) was calculated.

Productive Performance Traits

Body weights of chicks and feed consumption were recorded by cage at 1, 7, 14, 21, 28, 35, and 42 d of age, and BW gain (BWG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated from these data by period (1 to 21 d and 21 to 42 d of age) and for the overall experiment. Birds that died during the experiment were weighed, and their BWG was included in the calculations of FCR. Feed wastage was observed to be negligible and was not recorded.

Statistical Analysis

Data were analyzed as a completely randomized design with type of cereal, ES of the barley diets, and HP of barley as main effects. From 1 to 21 d of age, the effects of type of cereal were analyzed by performing a contrast between the corn diet and the average of the 6 barley diets. A nonorthogonal contrast was also made to compare the corn control diet and the 2 raw barley diets with and without ES in this period. To reduce type I error, the Bonferroni test was used for this comparison. In addition, the effects of ES and HP of barley were analyzed according to a 2×3 factorial arrangement by using a 2-way ANOVA of the 6 barley diets (SAS Institute, 1990). When HP of barley effect was significant, treatment means were separated using Tukey's test (Steel and Torrie, 1980). From 22 to 42 d of age, data of the 6 barley diets were analyzed as a 2-way ANOVA, as previously described. All differences were considered significant at $P \leq 0.05$.

RESULTS

The determined values for CP, ether extract, crude fiber, total ash, and Ca of the experimental diets were close to formulated values (Table 1). Total NSP, β -glucans, xylans, and soluble xylans of raw barley were 15.23, 3.51, 6.04, and 0.39% on a DM basis, respectively (Table 2). Thus,

the NSP composition of the barley used in the current trial was similar to the composition determined by Lázaro et al. (2003a), whereas the total xylan content of the raw barley was slightly below the 8.5% reported by Henry (1987). Heat processing of barley did not modify NSP values to any great extent. The proportion of soluble xylans with respect to total xylans was low and little affected by HP of barley (6.5, 6.0, and 7.1% for raw, micronized, and expanded barley, respectively). The predominant monosaccharides in raw barley (DM) were glucose (8.87%), xylose (3.16%), and arabinose (2.09%).

Digestive Traits

Effect of Cereal. Intestinal viscosity and percentage of pasted vents decreased with age (Table 3). In fact, no pasted vents were observed in any bird after 7 d of age. At 7 d of age, broilers fed the raw barley diet without ES had higher IV (171 vs. 8 cP; $P \leq 0.05$) and higher incidence of pasted vents (81.0 vs. 3.6%; $P \leq 0.05$) than broilers fed the corn diet. However, ES of the raw barley diet reduced IV and the incidence of pasted vents to 6 cp and 7.1%, respectively. Also, water intake at 21 d of age was greater in broilers fed raw barley without ES than in broilers fed corn or raw barley supplemented with enzymes (107 vs. 92 and 95 mL/d, respectively; $P \leq 0.05$).

Effect of ES. The ES of the barley diets reduced IV at all ages (110 vs. 8 cP) and the incidence of pasted vents at 7 d (73.4 vs. 10.0%). The decrease in IV with ES was more pronounced at early ages (311 vs. 8 cP at 7 d and 14 vs. 5 cP at 42 d). Enzyme supplementation reduced water intake at 21 d (106 vs. 98 mL/d), but no differences were detected at 35 d of age. Also, ES increased DM of ileal content at 28 d of age (16.2 vs. 19.0%) but did not affect DM of the excreta. Dietary treatment had no effect on crop pH at 42 d of age.

Effect of HP. Heat processing of barley increased IV at 7 d of age (270, 121, and 89 cP for micronized, expanded, and raw barley, respectively), but the effects disappeared with age (11, 6, and 11 cP at 42 d of age; Table 3). Intestinal viscosity at 7 and at 28 d of age was higher for micronized than for expanded barley diets (270 vs. 121 cP at 7 d and

Table 3. Influence of enzyme supplementation (ES, ppm) of the diet and heat processing (HP) of barley on water intake and digestive traits of broilers¹

Treatment	Intestinal viscosity (cP) ²				Pasting vents ³ (%)	Water intake		DM (%)		ph of the crop
	7 d	28 d	42 d	Average		7 d	21 d	35 d	Ileal, 28 d	
Cereal										
Corn	8	—	—	—	3.6	92	—	—	—	—
Barley	160	24	9	59	41.7	102	172	17.6	22.8	5.7
SEM	47.7	—	—	—	5.4	3.6	—	—	—	—
ES (ppm)										
0	311 ^a	39 ^a	14 ^a	110 ^a	73.4 ^a	106 ^a	184	16.2 ^b	22.5	5.8
500	8 ^b	9 ^b	5 ^b	8 ^b	10.0 ^b	98 ^b	160	19.0 ^a	23.2	5.6
HP										
Raw	89 ^b	25 ^{ab}	11	42	44.1	101	177	16.7 ^b	23.0	5.7
Micronized	270 ^a	32 ^a	11	84	38.7	102	173	17.2 ^b	22.9	5.7
Expanded	121 ^b	17 ^b	6	51	42.4	103	167	19.0 ^a	22.6	5.8
ES × HP										
Raw	171 ^b	36 ^b	18	74	81.0	107	190	16.1	23.9	6.2
Raw + ES	6 ^c	13 ^c	4	10	7.1	95	163	17.3	22.1	5.2
Micronized	529 ^a	55 ^a	17	160	67.9	107	178	15.5	20.3	5.7
Micronized + ES	10 ^c	8 ^c	5	8	9.5	97	168	18.8	25.5	5.7
Expanded	234 ^b	26 ^b	6	95	71.4	103	184	17.0	23.2	5.7
Expanded + ES	8 ^c	7 ^c	5	7	13.4	102	149	20.9	21.9	5.9
SEM	51.9	4.1	4.2	15.8	5.7	3.1	17.7	1.0	2.4	0.3
	Probability									
Effect ⁴										
Cereal	**	—	—	—	***	*	—	—	—	—
ES	***	***	*	***	***	**	NS	***	NS	NS
HP	*	**	NS	NS	NS	NS	NS	*	NS	NS
ES × HP	**	*	NS	NS	NS	NS	NS	NS	NS	NS

^{a-c}Means within a column and main effect not sharing a common superscript are significantly different ($P \leq 0.05$).

¹There were 6 cages of 12 broilers each per diet. Cage means were used to calculate dietary averages.

²cP = centipoise.

³Proportion of chicks showing adhered droppings to the perineal region.

⁴A nonorthogonal contrast was performed to compare the corn diet to the average of the 6 barley diets. For the 6 barley diets, the significance of the main effects (ES, HP) and interaction (ES × HP) were determined by 2-way ANOVA.

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

32 vs. 17 cP at 28 d), but no differences were observed at 42 d of age. The DM of the ileal contents was higher with expanded barley than with micronized and raw barley (19.0 vs. 17.2 and 16.7%, respectively), but no effect was found on the DM of the excreta. Also, DM of ileal, but not of excreta content, was higher with expansion than with micronization (19.0 vs. 17.2%).

A few interactions between HP of the barley and ES of diet were detected. At 7 d of age, ES reduced IV more with HP barley than with raw barley ($P \leq 0.01$). Also, the decrease in IV observed at 7 d ($P \leq 0.01$) and at 28 d ($P \leq 0.05$) was higher in broilers fed micronized barley than in broilers fed expanded barley.

TTAR and AIDN

Effect of ES. Enzymes increased TTAR of N (62.1 vs. 59.4%; $P \leq 0.05$), OM (73.7 vs. 71.3%; $P \leq 0.001$), and NSP (28.3 vs. 25.1%; $P \leq 0.05$) and AME_n of the diets (3,122 vs. 3,051 kcal/kg; $P \leq 0.001$; Table 4). In contrast, AIDN was not affected by ES (82.7 vs. 81.5%).

Effect of HP. Heat processing of barley increased TTAR of NSP (26.8 vs. 29.9 vs. 23.4% for micronized, expanded, and raw barley, respectively; $P \leq 0.001$). However, HP did not affect digestibility of any other dietary component

or AME_n of the diets while AIDN was higher for expanded than for micronized barley diets (83.8 vs. 80.7%; $P \leq 0.05$).

Productive Performance

Mortality during the trial was low (2.1%) and was not related to treatment (data not shown). Most of the mortality (>70%) occurred during the first week of trial.

Effect of Cereal. From 1 to 7 d of age, broilers fed the corn diet had greater BWG than broilers fed the raw barley diet with or without enzymes (15.2 vs. 14.3 and 14.2 g/d, respectively; Table 5). From 1 to 21 d of age, broilers fed corn or raw barley with ES grew faster (31.3 and 31.8 vs. 28.7 g/d) and had better FCR (1.55 and 1.51 vs. 1.65 g/g) than broilers fed the raw barley without ES (Table 6).

Effect of ES. Enzyme supplementation of barley diets improved most of the productive traits studied throughout the trial. From 1 to 7 d of age, broilers fed enzymes grew faster (15.5 vs. 14.6 g/d; $P \leq 0.05$) and had better FCR (1.15 vs. 1.20 g/g; $P \leq 0.05$) than broilers fed the control nonsupplemented diets, and the differences were maintained from 1 to 21 d (32.2 vs. 27.9 g/d for BWG and 1.52 vs. 1.69 g/g for FCR; $P \leq 0.001$) and from 1 to

Table 4. Influence of enzyme supplementation (ES, ppm) of the diet and heat processing (HP) of barley on apparent ileal digestibility of N (AIDN, %), total tract apparent retention of nutrients (TTAR, %), and AME_n (kcal/kg) content of the diets of broilers at 28 d of age¹

Treatment	AIDN	TTAR			AME _n
		N	OM ²	NSP ³	
ES (ppm)					
0	81.5	59.4 ^b	71.3 ^b	25.1 ^b	3,051 ^b
500	82.7	62.1 ^a	73.7 ^a	28.3 ^a	3,122 ^a
HP					
Raw	81.9 ^{ab}	60.7	72.1	23.4	3,084
Micronized	80.7 ^b	60.8	73.0	26.8	3,099
Expanded	83.8 ^a	60.8	72.4	29.9	3,077
ES × HP					
Raw	82.2	59.2	71.0	23.4	3,047
Raw + ES	81.7	62.1	73.2	23.4	3,120
Micronized	79.1	59.6	72.1	24.6	3,070
Micronized + ES	82.3	62.0	73.8	28.9	3,128
Expanded	83.3	59.5	70.8	27.2	3,037
Expanded + ES	84.3	62.1	74.0	32.5	3,117
SEM	1.14	1.49	0.70	1.51	22
		P-value			
Effect					
ES	NS	*	***	*	***
HP	*	NS	NS	***	NS
ES × HP	NS	NS	NS	NS	NS

^{a,b}Means within a column and main effect not sharing a common superscript are significantly different ($P \leq 0.05$).

¹There were 6 cages of 12 broilers each per diet. Cage means were used to calculate dietary averages.

²Organic matter.

³Nonstarch polysaccharides.

* $P \leq 0.05$; *** $P \leq 0.001$.

42 d of age (57.4 vs. 53.7 g/d for BWG and 1.65 vs. 1.71 g/g for FCR; $P \leq 0.001$).

Effect of HP. From 1 to 7 d of age, HP of barley improved ADFI (18.2 and 18.0 vs. 16.8 g/d for expanded, micronized, and raw barley diets, respectively; $P \leq 0.01$). Also, from 1 to 7 d, broilers fed expanded barley grew faster (15.9 vs. 15.0 vs. 14.3 g/d) and had better FCR (1.15 vs. 1.21 vs. 1.18 g/g) than broilers fed micronized or raw barley. After 7 d of age, no improvements in productive performance of broilers were observed because of HP of barley. An interaction ES × HP was observed for ADFI from 1 to 7 d of age; ES reduced ADFI in the raw barley diets but increased it in the HP barley diets ($P = 0.087$; Table 5).

DISCUSSION

Digestive Traits

Intestinal viscosity decreased with age, similar to previous reports (Petersen et al., 1999; Gracia et al., 2003b). Also, the percentage of pasted vents decreased with age, which suggests that chicks adapt quickly to the NSP content of the diet. In fact, no pasted vents were observed at 10 d of age.

Enzymes reduced IV at all ages and the incidence of pasted vents at 7 d of age, which agrees with Pettersson et al. (1991) and Esteve-García et al. (1997). In addition,

ES reduced water intake at 21 d of age and increased the DM of the ileal content at 28 d of age. Also, Vukic-Vranjes and Wenk (1995) reported that ES reduced water intake from 7 to 21 d of age in chicks fed barley, and Pettersson et al. (1991) found an increase in DM of ileal content in broilers fed barley supplemented with enzymes.

Heat processing of barley increased IV at 7 d of age, an observation that agrees with that of Vukic-Vranjes and Wenk (1995) and Svihus et al. (2000). Also, ADFI from 1 to 7 d of age was higher in broilers fed HP barley than in broiler fed raw barley in spite of the increased IV. These results agree with Gracia et al. (2003b), who observed that HP of barley increased digesta viscosity in broilers from 1 to 8 d of age by 66% but also feed intake by 10.5%. Probably, HP increased the solubility of the fibrous portion of the barley, and in consequence, IV was increased. However, in spite of the increase in IV, HP did not affect the incidence of pasted vents, which might explain the lack of effect of the increase in IV on ADFI. The procedure used in HP the barley influenced digesta viscosity in broilers at 7 and 28 d of age; IV was greater for micronized than for expanded barley. Consequently, the procedure used in HP the cereal portion of the diet might influence digesta traits in young chicks, which in turn might affect broiler performance at these ages. In fact, at 28 d of age, ileal DM content and AIDN were lower for micronized than for expanded barley diets.

The reduction observed in IV with ES at 7 d of age was greater with HP than with raw barley diets, results that agree with Vukic-Vranjes and Wenk (1995), who reported that the effects of ES on IV, nutrient availability, and broiler performance were greater with HP than with raw barley diets. Heat processing might solubilize part of the fibrous components of the cereal, and therefore, the probability for enzymes to improve digestive traits is higher with HP than with raw barley diets.

TTAR and AIDN

Enzymes consistently improved the TTAR of all nutrients in agreement with previous research (Ankrah et al., 1999; Gracia et al., 2003b; Ravindran et al., 2007). Also, Vukic-Vranjes and Wenk (1995) reported that ES increased the AME_n of extruded diets. However, in the current trial, ES did not modify AIDN, in contrast to the observation of Ravindran et al. (2007), who found that supplementation of barley diets with β -glucanase improved AIDN and of most amino acids. Probably, the variability for AIDN values observed (Table 4) precluded the detection of differences between supplemented and nonsupplemented diets.

The influence of HP on nutrient digestibility of poultry diets is the subject of debate (Mateos et al., 2002; González-Alvarado et al., 2007). Vukic-Vranjes et al. (1994) and Plavnik and Sklan (1995) have found that HP of barley improved nutrient digestibility. Wiseman (2006) indicated that HP might denature the α -amylase inhibitors contained in the cereals, thereby improving starch digestion. Plavnik and Sklan (1995) observed that HP of barley

Table 5. Influence of enzyme supplementation (ES, ppm) of the diet and heat processing (HP) of barley on growth performance of broilers from 1 to 21 d of age¹

Treatment	1 to 7 d			7 to 14 d			14 to 21 d		
	BWG ²	ADFI ³	FCR ⁴	BWG	ADFI	FCR	BWG	ADFI	FCR
Cereal									
Corn	15.2	17.7	1.17	29.5	48.5	1.65	49.3	79.3	1.61
Barley	15.0	17.7	1.18	29.5	47.6	1.62	45.6	78.2	1.74
SEM	0.39	0.53	0.022	0.92	1.23	0.047	1.30	1.63	0.066
ES (ppm)									
0	14.6 ^b	17.6	1.20 ^a	27.6 ^b	46.8	1.70 ^a	41.4 ^b	76.4 ^b	1.85 ^a
500	15.5 ^a	17.8	1.15 ^b	31.4 ^a	48.5	1.55 ^b	49.8 ^a	80.1 ^a	1.62 ^b
HP									
Raw	14.3 ^b	16.8 ^b	1.18 ^{ab}	28.7	46.9	1.64	47.7 ^a	79.2	1.67
Micronized	15.0 ^b	18.0 ^a	1.21 ^a	29.6	48.2	1.65	44.4 ^b	77.8	1.78
Expanded	15.9 ^a	18.2 ^a	1.15 ^b	30.3	47.8	1.59	44.7 ^b	77.6	1.77
ES × HP									
Raw	14.2	17.4	1.22	27.5	46.2	1.68	44.3	78.4	1.77
Raw + ES	14.3	16.2	1.13	29.9	47.6	1.60	51.0	80.0	1.57
Micronized	14.2	17.4	1.23	27.0	47.1	1.75	38.9	75.6	1.95
Micronized + ES	15.8	18.7	1.19	32.2	49.3	1.54	49.9	80.1	1.60
Expanded	15.6	18.0	1.16	28.4	47.1	1.67	41.0	75.1	1.84
Expanded + ES	16.3	18.5	1.13	32.2	48.4	1.51	48.4	80.1	1.69
SEM	0.40	0.56	0.023	0.94	1.22	0.045	1.31	1.57	0.068
	P-value								
Effect ⁵									
Cereal	NS	NS	NS	NS	NS	NS	*	NS	NS
ES	*	NS	*	***	NS	***	***	**	***
HP	***	**	*	NS	NS	NS	**	NS	NS
ES × HP	NS	NS	NS	NS	NS	NS	NS	NS	NS

^{a,b}Means within a column and main effect not sharing a common superscript are significantly different ($P \leq 0.05$).

¹There were 6 cages of 12 broilers each per diet. Cage means were used to calculate dietary averages.

²BW gain (g).

³Average daily feed intake (g).

⁴Feed conversion ratio (g of feed/g of BW gain).

⁵A nonorthogonal contrast was performed to compare the corn diet to the average of the 6 barley diets. For the 6 barley diets, the significance of the main effects (HS, EP) and interaction (ES × HP) were determined by 2-way ANOVA.

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

increased AME_n of the diets significantly, but the improvement was only 2.2% for extrusion and 2.7% for expansion. However, HP of barley did not influence TTAR of any nutrient other than NSP (Table 4). Heat processing might have solubilized part of the NSP contained in the cereal, thereby improving fiber digestibility. Francesch et al. (1994) and Vukic-Vranjes and Wenk (1995) did not find any effect of HP on energy utilization in broilers. In the current experiment, the expansion of barley improved AIDN, whereas no effect was observed for micronization. The reason of this interaction is unknown, but probably the conditions of time and temperature applied during micronization were excessive for barley, thereby reducing the digestibility of some nutrients as has been demonstrated by González-Alvarado et al. (2007) and Vicente et al. (2008) in broilers and piglets fed HP rice.

Productive Performance

From 1 to 21 d of age, BWG was reduced 9.1% and FCR was impaired 6.1% when corn was substituted by raw barley. This information agrees with Yu et al. (1998),

who observed that BWG of broilers decreased linearly with increasing levels of barley in the diet. However, when the barley diet was supplemented with enzymes, the differences between the barley and the corn diets disappeared, indicating that, from 1 to 21 d of age, barley can be substituted for all the dietary corn provided that enzymes are used. These results agree with Lázaro et al. (2003a), who reported similar performance (egg production, egg weight, and egg mass) of laying hens when 35% of the corn was substituted by barley supplemented with enzymes. In contrast, Yu et al. (1998) found that broilers fed barley diets supplemented with enzymes had poorer performance than broilers fed a control diet based on corn.

Enzyme supplementation of barley diets improved BWG and FCR of broilers at all ages, results that are consistent with previous research (Ankrah et al., 1999; Gracia et al., 2003b). However, the beneficial effects of ES were more pronounced from 1 to 21 d of age than from 22 to 42 d of age, which agrees with most published information (Pettersson and Aman, 1988; Gracia et al., 2003a).

Heat processing of barley improved BWG in broilers from 1 to 7 d of age by 8.4%, which was consistent with

Table 6. Influence of enzyme supplementation (ES, ppm) of the diet and heat processing (HP) of barley on growth performance of broilers from 1 to 42 d of age¹

Treatment	1 to 21 d			21 to 42 d			1 to 42 d		
	BWG ²	ADFI ³	FCR ⁴	BWG	ADFI	FCR	BWG	ADFI	FCR
Cereal									
Corn	31.3	48.5	1.55	—	—	—	—	—	—
Barley	30.0	47.8	1.60	80.6	138.2	1.71	55.5	93.2	1.68
SEM	0.66	0.91	0.037	—	—	—	—	—	—
ES (ppm)									
0	27.9 ^b	46.9 ^b	1.69 ^a	79.1	136.6	1.73	53.7 ^b	92.0	1.71 ^a
500	32.2 ^a	48.8 ^a	1.52 ^b	82.2	139.8	1.70	57.4 ^a	94.4	1.65 ^b
HP									
Raw	30.2	47.7	1.58	80.4	139.4	1.74	55.5	93.9	1.69 ^a
Micronized	29.7	48.0	1.63	80.4	138.3	1.72	55.1	93.3	1.70 ^a
Expanded	30.3	47.9	1.59	81.2	136.9	1.69	56.1	92.4	1.65 ^b
ES × HP									
Raw	28.7	47.3	1.65	78.1	138.0	1.77	53.7	93.2	1.74
Raw + ES	31.8	48.0	1.51	82.7	140.9	1.71	57.3	94.6	1.65
Micronized	26.7	46.7	1.75	81.2	139.3	1.72	54.0	93.1	1.73
Micronized + ES	32.6	49.4	1.51	79.6	137.4	1.73	56.2	93.6	1.67
Expanded	28.3	46.8	1.65	77.9	132.6	1.70	53.4	89.7	1.68
Expanded + ES	32.3	49.0	1.53	84.4	141.1	1.67	58.8	95.0	1.62
SEM	0.65	0.88	0.035	2.14	2.14	0.027	1.19	1.75	0.020
	P-value								
Effect ⁵									
Cereal	NS	NS	NS	—	—	—	—	—	—
ES	***	*	***	NS	NS	NS	***	NS	***
HP	NS	NS	NS	NS	NS	NS	NS	NS	*
ES × HP	NS	NS	NS	NS	NS	NS	NS	NS	NS

^{a,b}Means within a column and main effect not sharing a common superscript are significantly different ($P \leq 0.05$).

¹There were 6 cages of 12 broilers each per diet. Cage means were used to calculate dietary averages.

²BW gain (g).

³Average daily feed intake (g).

⁴Feed conversion ratio (g of feed/g of BW gain).

⁵A nonorthogonal contrast was performed to compare the corn diet to the average of the 6 barley diets. For the 6 barley diets, the significance of the main effects (HS, EP) and interaction (ES × HP) was determined by 2-way ANOVA.

* $P \leq 0.05$; *** $P \leq 0.001$.

the 7.7% increase in ADFI observed. No effect of HP of barley on broiler growth was detected after this age, which agrees with Gracia et al. (2003b), who observed that chicks fed HP barley performed better than chicks fed raw barley from 0 to 8 d of age and that the beneficial effects of HP disappeared with age. In contrast, Herstad and McNab (1975) and Vukic-Vranjes and Wenk (1995) concluded that HP of barley did not have any effect on performance of broilers at any age. In fact, Herstad and McNab (1975) observed that heating barley at 120°C increased BWG only in 1 of 4 trials. Heat processing of barley modifies the physical and chemical structure of the cereal, improving accessibility of enzymes to dietary components and facilitating its utilization. Therefore, HP is expected to be more beneficial during the first days of life, because the digestive capability of the gastrointestinal tract is limited in young chicks (Noy and Sklan, 1995).

In the pig, no differences in performance were observed with diets based on expanded or micronized barley (Medel et al., 2000) or corn (Medel et al., 1999). No data is available in the literature comparing the effects of these 2 HP procedures in broilers. The beneficial effects of HP of barley on broiler performance were limited to the first

week of age and were more pronounced with expansion than with micronization. The reason for the better feed efficiency observed with expanded barley from 1 to 7 d of age is not apparent. We hypothesize that the conditions imposed in the micronization process might have been too severe, increasing Maillard reactions and starch retrogradation and thereby reducing protein and energy availability (Vicente et al., 2008). In fact, Pettersson et al. (1991) and Plavnik and Sklan (1995) have shown that mild heating of barley, as occurred in our trial with barley expansion, improved nutrient digestibility and broiler performance.

We conclude that barley can be used in substitution of corn in prestarter broiler diets provided that enzymes are used. Enzyme supplementation of barley diets improves digestive traits, nutrient digestibility, and broiler growth at all ages. In general, HP of barley has little effect on broiler performance after 7 d of age, and expansion might be a better procedure to process barley destined to broiler diets than micronization.

REFERENCES

Ankrah, N. O., G. L. Campbell, R. T. Tyler, B. G. Rossnagel, and S. R. T. Sokhansanj. 1999. Hydrothermal and β -glucanase

- effects on the nutritional and physical properties of starch in normal and waxy hull-less barley. *Poult. Sci.* 81:205–219.
- AOAC International. 2000. *Official Methods of Analysis of AOAC International*. 17th ed. AOAC Int., Gaithersburg, MD.
- Boletín Oficial del Estado. 2005. Real Decreto 1201/200588 sobre protección de los animales utilizados para experimentación y otros fines científicos. BOE 252:34367–34391.
- Esteve-García, E., J. Brufau, A. Pérez-Vendrell, A. Miquel, and K. Duven. 1997. Bioefficacy of enzyme preparations containing β -glucanase and xylanase activities in broiler diets based on barley or wheat, in combination with flavomycin. *Poult. Sci.* 76:1728–1737.
- Francesch, M., A. M. Pérez-Vendrell, E. Esteve-García, and J. Brufau. 1994. Effects of cultivar, pelleting and enzyme addition on nutritive value of barley in poultry diets. *Br. Poult. Sci.* 35:259–272.
- Fundación Española Desarrollo Nutrición Animal. 2003. Normas FEDNA para la formulación de piensos compuestos. C. de Blas, G. G. Mateos, and P. G. Rebollar, ed. *Fund. Esp. Desarro. Nutr. Anim.*, Madrid, Spain.
- García, J., R. Carabaño, and J. C. de Blas. 1999. Effect of fiber source on cell wall digestibility and rate of passage in rabbits. *J. Anim. Sci.* 77:898–905.
- González-Alvarado, J. M., E. Jiménez-Moreno, R. Lázaro, and G. G. Mateos. 2007. Effect of type of cereal, heat processing of the cereal, and inclusion of fiber in the diet on productive performance and digestive traits of broilers. *Poult. Sci.* 86:1705–1715.
- Gracia, M. I., M. J. Aranibar, R. Lázaro, P. Medel, and G. G. Mateos. 2003a. α -Amylase supplementation of broiler diets based on corn. *Poult. Sci.* 82:436–444.
- Gracia, M. I., M. A. Latorre, M. García, R. Lázaro, and G. G. Mateos. 2003b. Heat processing of barley and enzyme supplementation of diets for broilers. *Poult. Sci.* 82:1281–1291.
- Henry, R. J. 1987. Pentosan and (1-3)(1-4)- β -glucan concentrations in endosperm and whole grain of wheat, barley, oats and rye. *J. Cereal Sci.* 6:253–258.
- Herstad, O., and J. M. McNab. 1975. The effect of heat treatment and enzyme supplementation on the nutritive value of barley for broiler chicks. *Br. Poult. Sci.* 16:1–8.
- Huang, A. H. C. 1992. Oil bodies and oleosins in seeds. *Ann. Rev. Plant Physiol.* 43:177–200.
- Lázaro, R., M. García, M. J. Aranibar, and G. G. Mateos. 2003a. Effect of enzyme addition to wheat-, barley- and rye-based diets on nutrient digestibility and performance of laying hens. *Br. Poult. Sci.* 44:256–265.
- Lázaro, R., M. García, P. Medel, and G. G. Mateos. 2003b. Influence of enzymes on performance and digestive parameters of broilers fed rye-based diets. *Poult. Sci.* 82:132–140.
- Lázaro, R., M. A. Latorre, P. Medel, M. I. Gracia, and G. G. Mateos. 2004. Feeding regime and enzyme supplementation to rye-based diets for broilers. *Poult. Sci.* 83:153–160.
- Mateos, G. G., R. P. Lázaro, and M. I. Gracia. 2002. The feasibility of using nutritional modifications to replace drugs in poultry feeds. *J. Appl. Poult. Res.* 11:437–452.
- Mateos, G. G., E. López, M. A. Latorre, B. Vicente, and R. P. Lázaro. 2007. The effect of inclusion of oat hulls in piglet diets based on raw or cooked rice and corn. *Anim. Feed Sci. Technol.* 135:100–112.
- Medel, P., M. García, R. Lázaro, J. C. De Blas, and G. G. Mateos. 2000. Particle size and heat treatment of barley in diets for early-weaned piglets. *Anim. Feed Sci. Technol.* 84:13–21.
- Medel, P., M. A. Latorre, J. C. De Blas, R. Lázaro, and G. G. Mateos. 2004. Heat processing of cereals in mash or pellet diets for young pigs. *Anim. Feed Sci. Technol.* 113:127–140.
- Medel, P., S. Salado, J. C. De Blas, and G. G. Mateos. 1999. Processed cereals in diets for early-weaned piglets. *Anim. Feed Sci. Technol.* 82:145–156.
- Miller, G. L. 1959. Use of dinitrosalicilic acid reagent for determination of reducing sugars. *Anal. Chem.* 31:426–428.
- NRC. 1994. *Nutrient Requirements of Poultry*. 9th rev. ed. Natl. Acad. Press, Washington, DC.
- Noy, Y., and D. Sklan. 1995. Digestion and absorption in the young chick. *Poult. Sci.* 74:366–373.
- Petersen, S. T., J. Wiseman, and M. R. Bedford. 1999. Effects of age and diet on the viscosity of intestinal contents in broiler chicks. *Br. Poult. Sci.* 40:364–370.
- Pettersson, D., and P. Åman. 1988. Effects of enzyme supplementation of diets based on wheat, rye or triticale on their productive value for broiler chickens. *Anim. Feed Sci. Technol.* 20:313–324.
- Pettersson, D., H. Graham, and P. Åman. 1991. The nutritive value for broiler of pelleting and enzyme supplementation of a diet containing barley, wheat and rye. *Anim. Feed Sci. Technol.* 33:1–14.
- Plavnik, I., and D. Sklan. 1995. Nutritional effects of expansion and short time extrusion on feeds for broilers. *Anim. Feed Sci. Technol.* 55:247–251.
- Ravindran, V., Z. V. Tilman, P. C. H. Morel, G. Ravindran, and G. D. Coles. 2007. Influence of β -glucanase supplementation on the metabolisable energy and ileal nutrient digestibility of normal starch and waxy barleys for broiler chickens. *Anim. Feed Sci. Technol.* 134:45–55.
- SAS Institute. 1990. *SAS/STAT User's Guide*. Version 6. 4th ed. SAS Inst. Inc, Cary, NC.
- Steel, R. G. D., and H. H. Torrie. 1980. *Principles and Procedures of Statistics*. McGraw-Hill Book Co., New York, NY.
- Svihus, B., D. H. Edvarsen, M. R. Bedford, and M. Gullord. 2000. Effect of methods of analysis and heat treatment on viscosity of wheat, barley and oats. *Anim. Feed Sci. Technol.* 88:1–12.
- Theander, O., P. Åman, E. Westerlund, R. Anderson, and D. Peterson. 1995. Total dietary fiber determined as neutral sugar residues, uronic acid residues, and Klason lignin (the Uppsala method): Collaborative study. *J. AOAC Int.* 78:1030–1044.
- Vicente, B., D. G. Valencia, M. Pérez-Serrano, R. Lázaro, and G. G. Mateos. 2008. The effects of rice feeding in substitution of corn and the degree of starch gelatinization of rice on the digestibility of dietary components and productive performance of young pigs. *J. Anim. Sci.* 86:119–126.
- Vukic-Vranjes, M., H. P. Pfirter, and C. Wenk. 1994. Influence of processing treatment and type of cereal on the effect of dietary enzymes in broiler diets. *Anim. Feed Sci. Technol.* 46:261–270.
- Vukic-Vranjes, M., and C. Wenk. 1995. The influence of extruded vs. untreated barley in the feed, with and without dietary enzyme supplement on broiler performance. *Anim. Feed Sci. Technol.* 54:21–32.
- Wiseman, J. 2006. Variations in starch digestibility in non-ruminants. *Anim. Feed Sci. Technol.* 130:66–77.
- Yu, B., J. C. Hsu, and P. W. S. Chiou. 1998. Effects of β -glucanase supplementation of barley diets on growth performance of broilers. *Anim. Feed Sci. Technol.* 70:353–361.