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Original article

Effects of xylanase and β-glucanase addition on performance, nutrient digestibility, and physico-chemical conditions in the small intestine contents and caecal microflora of broiler chickens fed a wheat and barley-based diet

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Abstract — Corn- or wheat and barley-based diets were supplemented or not with xylanase and β-glucanase (Quatrazyme HP, Nutri-Tomen, France) and fed to broiler chickens (n = 12 per group) from 3 to 25 days of age. The unsupplemented wheat and barley-based diet reduced ($P \leq 0.05$) weight gain and feed intake, and increased the feed conversion ratio as compared to the corn-based diet. Viscosity in the supernatant of the small intestine contents was increased ($P \leq 0.05$), whereas pH and osmolality values decreased ($P \leq 0.05$). Crude fat and protein digestibility were reduced as well as the apparent metabolizable energy ($P \leq 0.05$). Moreover, wheat and barley consumption, when compared with the corn-based diet, produced an increase in the microflora of the caeca, with 10.0 vs. 8.9 log CFU·g^{-1} for facultative anaerobic bacteria, 6.5 vs. 5.6 log CFU·g^{-1} for *E. coli* and 9.7 vs. 8.3 log CFU·g^{-1} for *Lactobacillus*. The addition of xylanase and β-glucanase to the wheat and barley-based diet significantly reduced the viscosity of the small intestine contents and improved ($P \leq 0.05$) weight gain, feed intake and feed conversion ratio. The digestibility of the nutrients, the apparent metabolizable energy and the osmolality of the small intestine contents were also increased without alteration in pH values. At the same time, the number of total facultative anaerobic bacteria and *E. coli* decreased significantly ($P \leq 0.05$). In conclusion, the addition of xylanase and β-glucanase improves the digestibility of a wheat and barley-based diet, probably by reducing the viscosity of the intestine content and by impeding the growth of bacteria (total facultative anaerobic bacteria, *E. coli*).

xylanase / β-glucanase / wheat / barley / broiler / microflora

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Abbreviations: **AME**, Apparent metabolizable energy; **AME_n**, Apparent metabolizable energy to zero nitrogen balance; **CFU**, Colony forming units; **DM**, Dry matter; **GE**, Gross energy; **HPAEC**, High performance anion exchange chromatography; **NSP**, Non starch polysaccharides; **RAV**, Real applied viscosity; **VFA**, Volatile fatty acids.

Résumé — Influence de la supplémentation du régime alimentaire par une association de xylanase et de β-glucanase sur la digestibilité des nutriments, les conditions physico-chimiques dans l'intestin grêle et l'équilibre de la flore caecale chez le poulet nourri avec un aliment à base de blé et d'orge. Trente-six poulets de chair (Ross) sont, entre le 3e et le 25e jour d'âge, nourris soit avec un aliment témoin à base de maïs soit un aliment expérimental contenant du blé et de l'orge supplémenté ou non avec une association de xylanase et de β-glucanase (Quatrazyme HP, Nutri-Tomen, France). Le gain de poids et l'efficacité alimentaire sont plus faibles ($P \leq 0,05$) chez les poulets nourris avec un régime à base de blé et d'orge que chez les témoins. La baisse des performances zootechniques est liée à une réduction de la pression osmotique, une diminution ($P \leq 0,05$) de la digestibilité des lipides et des protéines et une baisse de l'énergie. En revanche, la viscosité du contenu intestinal, le nombre de bactéries anaérobies facultatives (10,0 vs. 8,9 log CFU·g^{-1}), de *E. coli* (6,5 vs. 5,6 log CFU·g^{-1}) et de lactobacilles (9,7 vs. 8,3 log CFU·g^{-1}) sont augmentés ($P \leq 0,05$). La supplémentation de l'aliment expérimental avec l'association de xylanase et de β-glucanase améliore les performances zootechniques, augmente la digestibilité des nutriments ainsi que la pression osmotique et diminue la viscosité du contenu intestinal. Dans le même temps, elle diminue significativement le nombre de bactéries anaérobies facultatives et de *E. coli* sans modifier ni celui des lactobacilles ni la valeur du pH. Nous en concluons que l'association de xylanase et de β-glucanase dans l'aliment réduit les effets anti-nutritionnels du blé et de l'orge sur les performances de croissance et l'utilisation digestive des nutriments en diminuant la viscosité du contenu intestinal et en gênant la croissance microbienne (bactéries anaérobies facultatives, *E. coli*).

xylanase / β-glucanase / blé / orge / poulet / microflore

1. INTRODUCTION

The water-soluble non-starch polysaccharides (NSP) of the endosperm cell walls of wheat, barley, rye and oats have anti-nutritive properties in poultry. It has been clearly demonstrated that the presence of soluble β-glucans in barley [3] and soluble arabinoxylans in wheat [10] are the major cause of growth depression and poor feed conversion in poultry. The primary mechanism of the anti-nutritional effects of the soluble NSP activity is related to their viscous properties, which consequently affect the viscosity of the aqueous fraction in the small intestine contents [7]. The exaggerated intestinal viscosity affects the digestion and absorption of nutrients in chicks [5] by reducing glucose and sodium transport into the epithelial cells [16] and reducing the release rate of pancreatic enzymes [27] and bile acids [30]. The hydrolysis and digestion of nutrients are therefore decreased [18]. Moreover, several studies have demonstrated that the intestinal microflora play an important role in the anti-nutritive properties of water soluble NSP [25]. The counts of anaerobic bacteria in the small intestine are increased in chicks fed a diet containing rye or citrus pectin compared to those fed a corn-based diet [36]. More precisely, the number of *Clostridia* are increased in the small intestine of birds fed a diet containing water-soluble NSP [24]. Langhout et al. [25] recently demonstrated that an increase in digesta viscosity leads to increased microbial activity, particularly that of *Enterococci*, *Bacteroidaceae*, *Clostridia* and *E. coli* in the ileum of chicks. Furthermore, Choct et al. [11] reported that caecectomized broilers were less sensitive to the anti-nutritive properties of wheat pentosans than intact broilers. These investigators proposed that

when the ileal viscosity increases, bacteria originally from the caeca might invade the small intestine and enter into competition for nutrients with the host.

The addition of exogenous enzymes (xylanase and/or β-glucanase) to wheat- [13] and barley-based diets [3] can overcome the anti-nutritive effect of water soluble NSP. Numerous studies have reported the beneficial impact of exogenous enzymes on chick performance and nutrient digestibility [3, 13]. However, little information is available concerning the mechanism of action of these enzymes. One of the interesting areas of investigation is the evaluation of the impact of exogenous enzymes on microbial activity in the chick intestine. To our knowledge, there are few data available on this subject and these involve the impact of xylanase supplementation on some bacteria groups adhering to the intestinal epithelium [14].

In the present study, we compared the effects of the addition of xylanase and β-glucanase to a wheat and barley-based diet on zootechnical parameters, nutrient digestibility, the physic properties of intestinal contents and caecal microflora of broiler chickens to those of a corn-based diet.

2. MATERIALS AND METHODS

2.1. Animals and diets

Seventy one-day-old male broiler chickens (Ross, SICAMEN, France) were housed in suspended wire cages (size : 29 × 28.5 × 36.5 cm) and received a corn-based diet. All the chickens were fasted at 3 days of age and were weighed the following day. Thirty-six chickens were distributed into three homogenous experimental groups and were housed in individual cages. A plastic tray was slipped under each cage in order to collect excreta during the digestive balance experiments. The room temperature was gradually decreased from 32 °C at day 1 to 24 °C at day 25. The light was continuous during the first 3 days, then the lighting regimen was 23 hours per day. Two diets based on corn (610 $g \cdot kg^{-1}$) or wheat (397.5 $g \cdot kg^{-1}$) and barley (200 $g \cdot kg^{-1}$) were formulated according to the nutritional requirements for chickens [26] and were calculated using PORFAL software version 2.0 (ITP-INRA, France). The two basal diets (Tab. I) were isocaloric (12.54 $MJ \cdot kg^{-1}$) and isonitrogenous (200 $g \cdot kg^{-1}$). All diets were given in a mashed form and contained no growth factors, coccidiostats or antibiotics. The exogenous enzyme used in this trial was the commercial powdered preparation Quatrazyme HP (Nutri-Tomen, France) with xylanase and β-glucanase activities of 28 000 and 140 000 IU per g of product, respectively. The enzyme preparation was added to the wheat and barley-based diet at the rate of 20 mg (560 and 2 800 IU of xylanase and β-glucanase respectively) per kg of diet. Feed and water were supplied ad libitum throughout the experiment. Thus, the 3 dietary treatments were corn, wheat/barley and wheat/barley plus enzyme preparation. Twelve broiler chickens were allocated to each dietary treatment. The dietary treatments were administered to broiler chickens from 4 to 25 days of age. Body weights were determined at 4, 21, 24 and 25 days of age. Feed intake was recorded individually during the entire experiment.

2.2. Collection of samples

A digestion balance experiment was performed from 21 to 24 days of age. This consisted of 18 hours feed deprivation, followed by 54 hours with ad libitum access to feed and 18 hours final feed deprivation. Birds had free access to water throughout the balance experiment. Individual feed intakes and excreta were measured throughout the feeding and the final feed deprivation periods. Contamination, such

Table I. Formulation and calculated composition of broiler chick diets.

	Corn based diet	Wheat- and barley-based diet
Ingredients (g·kg^{-1})		
Corn	610	--
Rialto wheat	--	397.5
Scarlett barley	--	200
Rapeseed oil	30	54.4
Soybean meal	318	308.6
Calcium carbonate	11.6	13.2
Dicalcium phosphate	17.9	14.5
DL-methionine	1.6	1.5
Lysine	0.6	0.3
L-tryptophan	0.3	--
NaCl	4	4
Mineral mixture[1]	1	1
Vitamin mixture[2]	5	5
Nutrient composition[3] (g·kg^{-1})		
ME[4] (MJ·kg^{-1})	12.54	12.54
CP[5]	200	200
Lysine	11	11
Methionine	4.6	4.4
Methionine + Cystine	8.1	8.1
Tryptophan	2.6	2.6
Calcium	9.5	9.5
Available phosphorus	3.8	3.8
Viscosity[6] (mL·g^{-1} of DM)	0.52	2.03

[1]Mineral mixture supplied (mg·kg^{-1} of diet): Co, 0.33; Cu, 8.7; I, 1.2; Se, 0.2; Zn, 84; Fe, 44; Mn, 106.
[2]Vitamin mixture supplied per kg of diet: vitamin A, 10 000 IU; cholecalciferol, 1 500 IU; vitamin E, 15 mg; butylated hydroxytoluene, 125; menadione, 5 mg; thiamine, 0.5; riboflavin, 4 mg; calcium pantothenate, 8 mg; niacin, 25 mg; pyridoxine, 1 mg; vitamin B_{12}, 0.008; folic acid, 1 mg; biotin, 0.2 mg; choline chloride, 750 mg.
[3]Calculated using PORFAL software version 2 (ITP-INRA, France).
[4]ME: metabolizable energy.
[5]CP: crude protein.
[6]Viscosity: real applied viscosity.
DM: dry matter.

as down and scales, were carefully removed and the excreta were stored in containers at –20 °C. Birds were weighed at the end of each deprivation period. Excreta were freeze-dried, weighed, ground through a 0.5-mm sieve and stored in a sealed plastic bag at 4 °C until analysis.

At the end of the experiment (25 days of age) all birds were killed by intracardiac injection with 1.5 mL of 6 Sodium Pentobarbital solution (Sanofi Santé, Nutrition animale, La Ballastière, France) per kg of chick live weight. The digestive tract between the gizzard and Meckel diverticulum

was removed to obtain the duodenum plus jejunum. The ileum was isolated as the intestine between the Meckel diverticulum and the ileo-caecal junction. Caeca were removed and stored at –20 °C until microbial measurements. Intestinal contents were collected by gently finger – stripping the intestinal segments. After collection, the fresh samples of the duodenal plus jejunal and ileal contents were immediately centrifuged (Biofuge 175, Heraeus Sepatech, France) at 3 200 × *g* for 10 min at 20 °C. The supernatant was collected after centrifugation, the pH was measured and the samples were stored at –20 °C for measurement of osmolality and viscosity.

2.3. Analyses and measurements

For the viscosity measurements, corn- and wheat and barley-based diets and the four feedstuffs (corn, soybean meal, wheat and barley) were milled through a 0.5-mm sieve, then an aliquot (3.5 g) was homogenized with a 30 mL acetate buffer solution (0.2 mol·L^{-1} of sodium acetate, pH = 4.5) and incubated for 1 h at room temperature (20 °C). The diet and feedstuff suspensions were centrifuged at 1000 × *g* for 15 min and the supernatant was isolated. The viscosity of the supernatants was then measured as previously described [9]. The viscosity was expressed as real applied viscosity (RAV = Ln (η_r) per mL·g^{-1} where η_r represents the relative viscosity calculated as the ratio of the viscosity of the sample to the viscosity of the buffer). The supernatant of the duodenum plus the jejunum and ileum was thawed and pooled. An aliquot (3.5 mL) was diluted with distilled water (15.5 mL), and the viscosity was measured using the same method. The results were expressed as Ln (η_r) mPa·s.

Water, ash, crude fat and protein for the four feedstuffs were determined according to the Official European Methods [2] and starch according to Carré et al. [8]. The water insoluble cell wall content (cellulose, water insoluble hemi-cellulose, water insoluble pectin matter, lignin and proteins) was determined according to AFNOR [1]. Soluble sugars (arabinose and xylose) were measured as previously described [21]. Arabinoxylans were calculated as arabinose plus xylose. Soluble β-glucan contents in wheat, barley, corn and soybean meal were analyzed according to the methods of McCleary and Codd [28]. The sugars released after the action of enzyme preparation (20 mg·kg^{-1}) on pure arabinoxylans (4 mg·mL^{-1}, Megazyme, Ireland) were determined using High Performance Anion Exchange Chromatography (HPAEC) which was performed on a Dionex system with pulsed amperometric detection. Monomers (arabinose, xylose, from Sigma, St Louis, USA) and oligomers (xylobiose to xylohexaose from Megazyme, Ireland) were used as standards.

Total lipid contents of food and excreta were determined by extraction of samples with petroleum spirit after boiling samples in 3 mol·L^{-1} hydrochloric acid for 20 min. The determination of nitrogen in food and excreta was performed with the Kjeldahl method. Faecal nitrogen in the excreta was calculated as total nitrogen minus nitrogen in the uric acid. Uric acid was analyzed by the method of Terpstra and De Hart [32]. The apparent metabolizable energy (AME) of each diet was calculated from the gross energy (GE) values of the diet and freeze-dried excreta. Gross energy values were measured using an isoperibol oxygen bomb calorimeter (Kalorimeter C7000 prozesso, IKA Laboratechnik Janke & Kunkel-STR 79219, Staufen, Germany). The AME values were corrected to zero nitrogen balance (AME_n) as described by Hill and Anderson [23].

The osmolality of non-diluted small intestine supernatants was measured using an osmometer (Fiske Mark 3 Osmometer, Fiske Associates, USA).

The pH of non-diluted supernatants prepared from the contents of the duodenum

plus the jejunum and ileum was determined immediately after collection in order to minimize carbohydrate buffering by inserting a micro pH electrode (INLAB 427, Mettler, Toledo, wrdorf, Swiss) into the supernatant.

For microbial measurements, the caeca were thawed and 1 g of the contents was collected. A 1/10 dilution in a sterile saline solution (0.1 NaCl) was homogenized for 1 min in a plastic bag using a masticator (IUL Instruments, Barcelona, Spain). Several (1/10) dilutions were then performed in a 0.1 sterile NaCl solution using a biodilutor (IUL Instruments, Barcelona, Spain). Aliquots of 0.1 mL of each dilution were then spread on petri dishes containing the appropriate agar medium: BHIA medium (Difco Laboratories, USA) for total facultative anaerobic bacteria, Drigalski lactose medium with bromothymol blue and crystal violet for *E. coli* and MRS Broth medium (Difco Laboratories, USA) for *Lactobacillus*. The cultures were incubated at 37 °C for 24 hours for *E. coli* and 48 hours for *Lactobacillus* and facultative anaerobic bacteria. After incubation, the total number of colonies was counted and the results were expressed as the number of log of colony forming units (log CFU) per g of caeca contents.

2.4. Statistical analyses

The level of statistical significance was preset at $P \leq 0.05$. Data were statistically analyzed for treatment effect by the General Linear Models procedures of the Statview software for Windows 4.5 (1992-1996). Mean differences were determined using the Fisher test of least significance.

3. RESULTS

3.1. Nutritional characteristics of feedstuffs

Table II indicates that corn contained the lowest water insoluble cell walls. In contrast, soybean meal showed the lowest content of

Table II. Nutritional characteristics of wheat, barley, corn and soybean meal ($g \cdot kg^{-1}$ of DM).

Item	Rialto wheat	Scarlett barley	Corn	Soybean meal
Dry matter	877.5	871.7	878.6	868.5
Ash	18.1	20.0	12.9	72.4
Crude fat	22.5	27.4	48.2	16.8
Crude protein	124.2	102.8	97.5	506.2
Starch	740.4	662.4	727.6	101.7
Water insoluble cell wall	123.4	141.2	96.1	211.1
Soluble sugars				
Arabinose	2.4	1.4	0.2	1.1
Xylose	2.8	1.6	0.1	0.1
Soluble arabinoxylans[1]	5.2	3.0	0.3	1.2
Soluble β-glucans	2.4	24.3	0.5	0.6
Viscosity[2] ($mL \cdot g^{-1}$ of DM)	2.79	3.68	0.37	1.64

[1]Arabinoxylans: arabinose + xylose.
[2]Viscosity: real applied viscosity.
DM: dry matter.

starch and the highest content of water insoluble cell walls. Wheat and barley contained more arabinose and xylose compared to corn and soybean meal. Wheat was characterized by a high soluble arabinoxylan content compared to barley, which contained the highest soluble β-glucan level (Tab. II). Barley, characterized by the highest total soluble arabinoxylan and β-glucan (27.3 g·kg^{-1} of dry matter (DM)), had the highest viscosity level. Corn, however, had the lowest viscosity and contained the lowest total soluble arabinoxylans and β-glucans (0.8 g·kg^{-1}).

3.2. Impact of exogenous enzyme on broiler performance and nutrient digestibilities

The diet based on wheat and barley instead of corn (Tab. III) decreased weight gain (605 vs. 438 g) and reduced feed intake (899 vs. 714 g). The feed conversion ratio was also impaired ($P \leq 0.05$). The addition of xylanase and β-glucanase to the wheat and barley-based diet improved ($P \leq 0.05$) broiler chicken performance (Tab. III). Similar performances were observed when animals were fed a wheat and barley-based diet supplemented with enzyme or a corn-based diet (Tab. III). It is noteworthy that wheat and barley reduced the digestibility of crude protein and fat ($P \leq 0.05$) and the metabolizable energy level as compared to corn, and this was reversed ($P \leq 0.05$) when xylanase and β-glucanase were added to the diet (Tab. III). Thus, the improved broiler chicken performance in birds fed a wheat and barley-based diet supplemented with xylanase and β-glucanase was due to an increase in the digestibility of nitrogen, lipids and AME_n.

3.3. Impact of the exogenous enzyme on the viscosity, pH and osmolality of the supernatant of the small intestine contents

The addition of xylanase and β-glucanase reduced ($P \leq 0.05$) the viscosity of the supernatant of the small intestine in

Table III. Weight gain, feed intake, feed to gain ratio, digestibility of nitrogen and lipids and AMEn[1] in broiler chickens fed corn- or wheat- and barley-based diets supplemented or not with xylanase and β-glucanase from 4 to 20 days of age[2].

Variable	Dietary group			Level of significance		
	Corn-based diet (A)	Wheat- and barley-based diet (B)	Wheat- and barley-based diet+E[3] (C)	A, B	A, C	B, C
Weight gain (g)	605 ± 57	438 ± 98	619 ± 67	$P < 0.0001$	$P = 0.6765$	$P < 0.0001$
Feed intake (g)	899 ± 71	714 ± 138	924 ± 89	$P = 0.0002$	$P = 0.5841$	$P = 0.0002$
Feed/gain ratio	1.489 ± 0.046	1.645 ± 0.104	1.495 ± 0.030	$P < 0.0001$	$P = 0.8538$	$P = 0.0001$
Digestibility coefficients (%)						
Crude protein	86.34 ± 1.22	82.21 ± 3.71	86.81 ± 3.95	$P = 0.0018$	$P = 0.7057$	$P = 0.0015$
Crude fat	81.19 ± 1.72	77.88 ± 3.15	82.75 ± 1.34	$P = 0.0007$	$P = 0.0879$	$P < 0.0001$
AME_n (MJ·kg^{-1} DM)	13.56 ± 0.19	12.91 ± 0.33	13.32 ± 0.16	$P < 0.0001$	$P = 0.0507$	$P = 0.0014$

[1]AME_n: apparent metabolizable energy corrected to a zero nitrogen balance.
[2]Values are means ± SD of 12 birds per dietary group.
[3]E: xylanase and β-glucanase addition at the level of 20 mg·kg^{-1} of diet (Quatrazyme HP, Nutri Tomen, France).
DM: dry matter.

broiler chickens fed the wheat and barley-based diet without reaching the viscosity level of the corn-based diet. This was probably due to the low dose of xylanase and β-glucanase added (20 mg of Quatrazyme HP per kg of diet). The pH values of the supernatant of the duodenum plus jejunum and ileum contents decreased ($P \leq 0.05$) after consumption of the wheat– and barley-based diet supplemented or not with xylanase and β-glucanase as compared to the corn-based diet (Tab. IV). The osmolality values of the supernatant of the small intestine contents were lower ($P \leq 0.05$) in animals fed a wheat and barley-based diet than in birds fed a corn-based diet (Tab. IV). Consumption of the wheat and barley-based diet supplemented with xylanase and β-glucanase raised ($P \leq 0.05$) the osmolality values (Tab. IV).

3.4. Impact of exogenous enzyme on the caecal microflora of the broiler

The inclusion of wheat and barley in the diet increased ($P \leq 0.05$) the total number of facultative anaerobic bacteria and the number of *E. coli* and *Lactobacillus* compared to the corn-based diet. This overgrowth of the caecal microflora was associated with a depression in broiler chick performance, nutrient digestibility, osmolality and pH and an increase in the viscosity of the supernatant of the small intestine. The addition of xylanase and β-glucanase to the wheat and barley-based diet reduced the total number of aerobic counts and the number of *E. coli* ($P \leq 0.05$). However, the number of *Lactobacilli* was not significantly modified (Tab. V). These changes in caecal microflora activity with enzymes were accompanied by improvement in broiler chick performance and nutrient digestibility, associated with an increase in intestinal osmolality and reduced viscosity without modification of the pH.

3.5. In vitro arabinoxylan hydrolysis by xylanase and β-glucanase

To investigate the in vitro action of these enzymes further, soluble arabinoxylan was incubated with xylanase and β-glucanase. The products released were analyzed by HPAEC and the experiment showed the release of arabinose (18.95 $\mu g \cdot mL^{-1}$), xylose (171.18 $\mu g \cdot mL^{-1}$), xylobiose (171.18 $\mu g \cdot mL^{-1}$), xylotriose (114.93 $\mu g \cdot mL^{-1}$) and xylotetraose (12.74 $\mu g \cdot mL^{-1}$). However, some peaks were not identified.

Table IV. Viscosity and pH of the supernatant of small intestine contents in broiler chickens fed corn- or wheat- and barley-based diets supplemented or not with xylanase and β-glucanase[1].

Variable	Dietary group			Level of significance		
	Corn-based diet (A)	Wheat- and barley-based diet (B)	Wheat- and barley-based diet+E[2] (C)	A, B	A, C	B, C
Viscosity[3] (Ln(mPa·s))	1.074 ±0.169	1.683 ±0.270	1.306 ±0.131	$P < 0.0001$	$P = 0.0271$	$P = 0.0008$
pH						
Duodenum+jejunum	6.33 ±0.17	6.06 ±0.19	6.00 ±0.22	$P = 0.0010$	$P < 0.0001$	$P = 0.4223$
Ileum	7.50 ±0.39	6.74 ±0.560	6.89 ±0.80	$P = 0.0084$	P = 0.0311	P = 0.5458
Osmolality ($mOsmol \cdot kg^{-1}$)	437 ±32	401 ±22	454 ±34	$P = 0.0355$	$P = 0.3032$	$P = 0.0025$

[1]Values are means ±SD of 12 birds per dietary group.
[2]E: xylanase and β-glucanase addition at the level of 20 $mg \cdot kg^{-1}$ of diet (Quatrazyme HP, Nutri Tomen, France).
[3]Viscosity of the supernatant of small intestine contents.

Table V. Colony forming units of bacterial species (log CFU·g^{-1}) per g of caecal contents in broiler chickens fed corn- or wheat- and barley-based diets supplemented or not with xylanase and β-glucanase[1].

Variable	Dietary group			Level of significance		
	Corn-based diet (A)	Wheat- and barley-based diet (B)	Wheat- and barley-based diet+E[2] (C)	A, B	A, C	B, C
Total facultative anaerobic bacteria	8.9 ± 1.1	10.0 ± 0.9	8.9 ± 0.6	$P = 0.0099$	$P = 0.9629$	$P = 0.0140$
E. Coli	5.6 ± 0.6	6.5 ± 0.9	5.7 ± 0.9	$P = 0.0115$	$P = 0.8008$	$P = 0.0319$
Lactobacillus ssp	8.3 ± 0.6	9.7 ± 0.9	9.2 ± 0.6	$P = 0.0001$	$P = 0.0143$	$P = 0.0872$

[1]Values are means ±SD of 12 birds per dietary group.
[2]E: xylanase and β-glucanase addition at the level of 20 mg·kg^{-1} of diet (Quatrazyme HP, Nutri Tomen, France).

4. DISCUSSION

The present study has shown that, although the diets are isocaloric and isonitrogenous, feeding a wheat and barley-based diet instead of a corn-based diet depressed broiler performance. Similar results were previously obtained in broiler chickens fed wheat- [5, 10] or barley- [3, 35] based diets.

In the digestive tract, the feeding of a wheat and barley-based diet increased the viscosity and decreased the pH of the intestinal contents. However, the pH was not affected in the intestinal wall [34].

Consumption of the wheat and barley-based diet depressed the osmolality of the small intestine supernatant as compared to the corn-based diet. The differences were associated with an increased intestinal viscosity leading to lower efficiency of feed digestion and/or solubilization. Van der Klis and Geerse [33] showed that the digestibility of starch and fat significantly decreased when broiler chickens were fed diets based on high viscosity wheat.

The mechanisms involved in the negative effect of digesta viscosity on digestion are not fully understood. A change in the intestinal microflora may be one of these mechanisms [24, 25]. In the present study the wheat and barley-based diet increased the microflora activity in the caecal contents, particularly that of *Lactobacillus* and *E. coli*. In an earlier study, Langhout et al. [25] reported an increase in the number of *Enterococci*, *Bacteriodaceae*, *Clostridia* and *E. coli*, but not in *Lactobacillus* in the ileum content of broiler chickens fed a corn-based diet supplemented with high levels of methylated citrus pectin. Overgrowing bacteria may impair nitrogen [4] and fat digestibility [31]. Some bacteria strains are able to deconjugate bile acids and subsequently reduce their efficacy in solubilizing lipids [17]. The role of bile acids is essential in the digestion of long chain and saturated fatty acids [37], and to a lesser extent in short chain or unsaturated fatty acids [19]. Thus, since the fat source used in our experiments was a vegetable oil which is characterized by short chain and unsaturated fatty acids, the low digestibility of fat observed with the broilers fed the wheat and barley-based diet may be explained by bacterial bile acid deconjugation and by gut morphology change [38].

We clearly demonstrated that the addition of xylanase and β-glucanase to a wheat and barley-based diet decreased ($P \leq 0.05$) the viscosity of the supernatant of the intestinal

contents [6]. However, the pH of the intestinal contents in birds fed a wheat and barley-based diet was not affected by the addition of an exogenous enzyme and remained lower than that of animals fed a corn-based diet. This is most probably associated with the release of fermentable sugars after NSP degradation by xylanase and β-glucanase, thus producing volatile fatty acids (VFA), which avoided an increase in pH. This hypothesis corroborates with that of a previous study [12] that showed increased VFA concentration when exogenous enzymes were added to a diet containing the NSP fraction from wheat.

Furthermore, the consumption of a wheat and barley-based diet supplemented with xylanase and β-glucanase increased the osmolality of the supernatant of intestinal content. Our results contrasted with those presented by van der Klis et al. [34] who showed that the addition of xylanase to wheat-based diets does not affect the osmolality of the intestinal contents.

Caecal microflora activity was markedly affected by the addition of xylanase and β-glucanase to the wheat and barley-based diet. These exogenous enzymes significantly decreased the total aerobic counts as well as the number of *E. coli*. However the number of *Lactobacillus* was not affected and remained significantly higher ($P \leq 0.05$) than that found when animals were fed a corn-based diet. Gilbert et al. [20] showed that the addition of exogenous enzymes (xylanase and cellulase) decreased the abundance of Mycoplasma, *Clostridium perfringens* and *Campylobacter* in the caeca of chicks. It may be speculated from these findings that oligosaccharides, released after NSP degradation by exogenous enzymes, are fermented by some specific bacteria [22, 29], leading to a decrease in pH values, which inhibit the growth of gram-negative bacteria such as *E. coli* [15]. All these modifications of the small intestine physico-chemical conditions and of the microflora activity, induced by the addition of xylanase and β-glucanase to a wheat and barley-based diet, were associated with an improvement in weight gain, feed intake, feed conversion ratio and the digestibility of nitrogen (+5.6%) and lipids (+6.2%).

In conclusion, the addition of xylanase and β-glucanase to a wheat and barley-based diet improved the growth performance of broiler chickens. Lipid and protein digestibility as well as metabolizable energy values became similar to those obtained in animals fed a corn-based diet. Moreover, the addition of xylanase and β-glucanase reduced *E. coli* and total facultative anaerobic bacteria numbers. Further studies are needed to evaluate the growth of some bacteria strains in relation to the effect of fermentable sugars released by xylanase and β-glucanase enzymes.

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