

ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF
POULTRY SCIENCE

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Response of Broiler Chicks to Xylanase Supplementation of Corn/Rye Containing Diets Varying in Metabolizable Energy

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Abstract: The contribution of enzyme (xylanase) supplementation (ES) to improving performance of broiler chicks fed decreased ME diets based on corn/ rye was investigated in the current study. Three hundred-sixty-one day old Arbor Acres chicks were randomly allocated in a 2x3x2 factorial design in twelve experimental groups, three replicates per group and ten birds per replicate. Experimental diets were based on two Cereal Sources (CS); corn alone or rye contributing to 10% of the whole diets that differed on dietary ME levels [positive control according to strain recommendations, negative control 1; 50 kcal/kg ME less than recommended and negative control 2; 100 kcal/kg ME less than recommended] at supplemental xylanase doses 0 or 16,000 U/kg diet. Feeding rye to broilers had no negative effect on total Feed Conversion Ratio (FCR) which could compensate for the significant decrease in accumulative live weight gain (BWG) compared to total corn grain feeding. Data on the effect of dietary ME level reveal a significant decrease in total BWG associated with a corresponding increase in total FI and a worsening in FCR as the dietary ME decreased. Inclusion xylanase significantly improved FCR without affecting total BWG and decreasing both abdominal fat content and liver weight. Dressing percentage was not affected by main factors investigated. Interactions studied show no significant effects on growth and most noticeable significant interactions are those for the total FCR between CSxME and CSxES. Treatments have a limited effect on the absence/presence of some pathogenic bacteria. So far it can be include rye in broiler chick diets at the rate of 10% with xylanase supplementation providing that the dietary ME level be kept within the recommendations. While enzyme supplementation is recommended in case of decreasing dietary ME level.

Key words: Broiler chicks, rye, xylanase, metabolizable energy, growth performance, microbiota

INTRODUCTION

Non-starch polysaccharides (NSPs) of cereals have anti-nutritional properties for the poultry. NSPs vary among cereal grains; it is low in non-viscous grains as in case of corn, the most used cereal grains in poultry diets in many parts around the world, which estimated by 97 g/kg, while it is high in viscous cereals, as in case of rye that contains 152 g/kg (Knudsen, 1997). The water soluble part of NSPs are comprised mainly of arabinoxylans and beta-glucan that depress poultry performance by either encapsulating nutrients and inhibit the access of digestive enzymes to the starch, fat and protein molecules and increasing the viscosity of the intestinal contents of the gut and depress the overall nutrient digestibility through gastro-intestinal modifications. The depression in digestion of nutrients results in a decrease of the AME and thereby leading to poor poultry performance (Bedford and Schulze, 1998; Hetland *et al.*, 2004). Hence inclusion of rye in poultry diets is limited because of its adverse effects on the performance (Lazaro *et al.*, 2003a, b).

Supplementation of xylanase and beta-glucanase enzymes, especially to viscous cereals containing reduced the viscosity of intestinal content and the weight of digestive organs (Lazaro *et al.*, 2004) and accelerated

the rate of passage of digesta through gastrointestinal tracts of birds (Danicke *et al.*, 1997; Lazaro *et al.*, 2003b). A decrease in transit time with enzyme supplementation reduced the growth of microflora in the intestine (Danicke *et al.*, 1999), which could counteract the negative effects of rye feeding on bile acid deconjugation (Mathlouthi *et al.*, 2002) and improved vitamins and mineral absorption and leg soundness (Grammar *et al.*, 1982). Pettersson and Aman (1989) reported that supplemental pentosanase preparation containing beta-glucanase and xylanase of rye based diet improved growth performance of broiler chicks with increasing supplemental enzyme level. Also, Lazaro *et al.* (2003b) reported that enzyme supplementation (beta-glucanase and xylanase) of rye-based diets improved BWG, FI and FCR of broiler chicks. In that study, little differences in weight gains were detected between corn and rye diets with enzyme supplementation. Jozefiak *et al.* (2007) reported that supplementing a feed grade enzyme containing xylanase and protease to rye-based diets improved the final body weight and FCR of broiler chicks compared to none supplementation. Also, enzyme supplementation could improve the AME of the poultry diet. Torres *et al.* (2003) evaluating a multiple enzymes' preparation, verified that lower AME diets supplemented

with the mixture provided the broilers a similar performance as those fed diets with optimum ME level. Youssef *et al.* (2011) concluded that a mixture of enzyme preparation containing xylanase and amylase added to corn-based diet could reduce the dietary energy level by about 150 kcal/kg without a negative effect on growth performance or carcass traits. The objective of the current study was to evaluate the response of broiler chicks to xylanase supplementation of corn/rye containing diet varied in dietary ME content.

MATERIALS AND METHODS

Growth performance: Three hundred-sixty-one day old Arbor Acres chicks were randomly allocated in a 2x3x2 factorial design in twelve experimental groups, three replicates per group and ten birds per replicate. Experimental diets were based on two Cereal Sources (CS); corn alone or rye contributing to 10% of the whole diets that differed on dietary ME levels [positive control according to strain recommendations, negative control 1; 50 kcal/kg ME less than recommended and negative control 2; 100 kcal/kg ME less than recommended] at supplemental xylanase doses 0 or 16,000 U/kg diet. The tested xylanase commercial enzyme was Econase XT25 which supplemented at level 100 mg/kg diet to provide 16000 U xylanase/kg diets. Econase XT25 is a product of AB Enzymes GmbH, Germany. Chicks were reared on wire battery cages and had the same managerial procedures throughout the growth trial term. Birds were

given *ad libitum* the diets described in Table (1, 2 and 3) and had access to water. Three-phased diets were formulated to meet the Arbor Acres chicks requirements; phase starter (1-14 days of age), phase grower (15-28 days of age) and finisher (29-40 days of age). Live body weight and FI were recorded for each growth phase and the corresponding BWG and FCR were calculated. At the termination of the growth trial period (40 days of age), three representing birds of each experimental group were overnight fasted, slaughtered and eviscerated. Dressing, liver and abdominal fat weights were proportioned to the live body weight upon slaughtering.

Microbiological studies: Three chicks from each group at day 10 of age were randomly selected and bacteriologically examined for detection of some pathogenic bacteria; *Echerichia coli*, Salmonella, *Staphylococcus aureus*, Campylobacter and *Clostridium perfringens*, via cloaca swabs. Isolation of *Echerichia coli* was carried out according to Collins *et al.* (1998). Isolation of Salmonella was carried out according to Ellis *et al.* (1976). Isolation of *Staphylococcus aureus* was carried out according to Brown (2005). Isolation of Campylobacter was carried out according to Oosterom *et al.* (1983). Isolation of *Clostridium perfringens* was carried out according to Holt *et al.* (1994). On day 40 of age, the same bacteriological analyses were carried out on the same bacterial species on 108 samples collected from cloaca, liver and spleen, equally.

Table 1: Ingredients and calculated chemical analyses of the starter diets

Ingredients	Recommended ME level		Lower 50 kcal ME/kg diet than recommended		Lower 100 kcal ME/kg diet than recommended	
	CS		CS		CS	
	Corn	Corn+10% Rye	Corn	Corn+10% Rye	Corn	Corn+10% Rye
Yellow corn	56.2	46.68	55.85	46.0	57.1	47.0
Rye	0.0	10.0	0.0	10.0	0.0	10.0
Soybean meal (44%)	30.0	28.2	33.46	31.8	33.26	31.82
Corn gluten	6.5	6.8	4.0	4.4	4.0	4.4
Soy oil	2.8	3.8	2.3	3.25	1.25	2.35
Di Calcium phosphate	2.0	2.0	2.0	2.0	2.0	2.0
Limestone	1.15	1.15	1.15	1.15	1.15	1.15
Vit. Min. Premix*	0.30	0.30	0.30	0.30	0.30	0.30
NaCl	0.24	0.24	0.24	0.24	0.24	0.24
NaHCO ₃	0.23	0.23	0.23	-0.23	0.23	0.23
L-lysine	0.34	0.36	0.24	0.29	0.24	0.27
DL-methionine	0.24	0.24	0.23	0.24	0.23	0.24
Chemical composition						
Crude protein (%)	22.02	21.91	21.86	21.88	21.87	21.95
ME (kcal/kg)	3047	3046	2974	2975	2923	2924
Ether extract (%)	5.53	6.40	5.01	5.92	4.01	4.96
Crude fiber (%)	3.61	3.57	3.81	3.77	3.82	3.79
Calcium (%)	0.97	0.97	0.98	0.98	0.98	0.98
Available P (%)	0.52	0.52	0.53	0.53	0.53	0.53
Lysine (%)	1.35	1.34	1.35	1.36	1.35	1.35
Methionine+Cystene (%)	1.02	1.0	0.98	0.98	0.98	0.98
* Each 3.0 kg containing		12000000 IU vit. A		2000000 IU vit. D ₃		10000 mg vit. E
1000 mg vit. K ₃		1000 mg vit. B ₁		5000 mg vit. B ₂		1500 mg vit. B ₆
10 mg vit. B ₁₂		30000 mg vit. PP		10000 mg vit. B ₅		50 mg B ₈
1000 mg B ₉		250000 mg choline chloride		60000 mg Mn		30000 mg Fe
50000 mg Zn		10000 mg Cu		1000 mg I		100 mg Se and 100 mg Co
CaCO ₃ up to 3 kg		CS: Cereal source				

Table 2: Ingredients and calculated chemical analyses of the grower diets

Ingredients	Recommended ME level		Lower 50 kcal ME/kg diet than recommended		Lower 100 kcal ME/kg diet than recommended	
	----- CS -----		----- CS -----		----- CS -----	
	Corn	Corn+10% Rye	Corn	Corn+10% Rye	Corn	Corn+10% Rye
Yellow corn	60.45	50.70	61.35	51.65	62.55	52.85
Rye	0.0	10.0	0.0	10.0	0.0	10.0
Soybean meal (44%)	23.84	22.00	23.84	22.00	23.94	22.00
Corn gluten	8.50	9.00	8.50	9.00	8.20	8.80
Soy oil	3.0	4.05	2.10	3.10	1.10	2.10
Di Calcium phosphate	1.75	1.80	1.75	1.80	1.75	1.80
Limestone	1.10	1.05	1.10	1.05	1.10	1.05
Vit.-Min. Premix*	0.30	0.30	0.30	0.30	0.30	0.30
NaCl	0.24	0.24	0.24	0.24	0.24	0.24
NaHCO ₃	0.23	0.23	0.23	0.23	0.23	0.23
L-lysine	0.40	0.43	0.40	0.43	0.40	0.43
DL-methionine	0.19	0.20	0.19	0.20	0.19	0.20
Chemical composition						
Crude protein (%)	20.9	20.9	21.0	21.0	20.9	20.9
ME (kcal/kg)	3143	3145	3098	3098	3045	3047
Ether extract (%)	5.84	6.75	4.98	5.84	4.02	4.88
Crude fiber (%)	3.30	3.25	3.32	3.27	3.35	3.30
Calcium (%)	0.88	0.87	0.88	0.87	0.88	0.87
Available P (%)	0.47	0.48	0.47	0.48	0.47	0.48
Lysine (%)	1.25	1.24	1.25	1.24	1.25	1.24
Methionine+Cystene (%)	0.95	0.96	0.96	0.96	0.95	0.96
* Each 3.0 kg containing		1200000 IU vit. A		2000000 IU vit. D ₃		10000 mg vit. E
1000 mg vit. K ₃		1000 mg vit. B ₁		5000 mg vit. B ₂		1500 mg vit. B ₆
10 mg vit. B ₁₂		30000 mg vit. PP		10000 mg vit. B ₅		50 mg B ₈
1000 mg B ₉		250000 mg choline chloride		60000 mg Mn		30000 mg Fe
50000 mg Zn		10000 mg Cu		1000 mg I		100 mg Se and 100 mg Co
CaCO ₃ up to 3 kg		CS: Cereal source				

Table 3: Ingredients and calculated chemical analyses of the finisher diets

Ingredients	Recommended ME level		Lower 50 kcal ME/kg diet than recommended		Lower 100 kcal ME/kg diet than recommended	
	----- CS -----		----- CS -----		----- CS -----	
	Corn	Corn+10% Rye	Corn	Corn+10% Rye	Corn	Corn+10% Rye
Yellow corn	65.00	54.62	66.00	55.77	67.00	56.77
Rye	0.0	10.0	0.0	10.0	0.0	10.0
Soybean meal (44%)	19.25	18.33	19.28	18.23	19.28	18.23
Corn gluten	8.00	8.0	8.00	8.00	8.00	8.00
Soy oil	3.28	4.55	2.25	3.50	1.25	2.5
Di Calcium phosphate	1.55	1.55	1.55	1.55	1.55	1.55
Limestone	1.58	1.57	1.58	1.57	1.58	1.57
Vit.-Min. Premix*	0.30	0.30	0.30	0.30	0.30	0.30
NaCl	0.24	0.24	0.24	0.24	0.24	0.24
NaHCO ₃	0.23	0.23	0.23	0.23	0.23	0.23
L-lysine	0.40	0.43	0.40	0.43	0.40	0.43
DL-methionine	0.17	0.18	0.17	0.18	0.17	0.18
Chemical composition						
Crude protein (%)	18.9	18.9	19.0	19.0	19.0	19.0
ME (kcal/kg)	3197	3199	3145	3148	3095	3097
Ether extract (%)	6.21	7.33	5.22	6.32	4.26	5.36
Crude fiber (%)	3.06	3.05	3.09	3.07	3.11	3.10
Calcium (%)	1.00	1.00	1.00	1.00	1.00	1.00
Available P (%)	0.42	0.42	0.42	0.42	0.42	0.42
Lysine (%)	1.12	1.13	1.12	1.13	1.12	1.13
Methionine+Cystene (%)	0.87	0.87	0.88	0.88	0.88	0.88
* Each 3.0 kg containing		1200000 IU vit. A		2000000 IU vit. D ₃		10000 mg vit. E
1000 mg vit. K ₃		1000 mg vit. B ₁		5000 mg vit. B ₂		1500 mg vit. B ₆
10 mg vit. B ₁₂		30000 mg vit. PP		10000 mg vit. B ₅		50 mg B ₈
1000 mg B ₉		250000 mg choline chloride		60000 mg Mn		30000 mg Fe
50000 mg Zn		10000 mg Cu		1000 mg I		100 mg Se and 100 mg Co,
CaCO ₃ up to 3 kg		CS: Cereal source				

Statistical procedures: Data were statistically analyzed using the general linear model producer (SAS, 1990)

with three way ANOVA model using grain source, enzyme supplementation and dietary energy level as

main effects. The means were compared by multiply range test according to Duncan (1955).

RESULTS AND DISCUSSION

Growth performance: Results in Table 4 indicate that feeding rye to broilers at the rate of 10% had no negative effect on FCR, which could compensate for the significant decrease in total BWG. Also, feeding corn alone as the sole cereal source resulted in significant improvements in BWG during starter and finisher periods and only a significant improvement in FCR during starter period which disappeared in consequent intervals. FI was decreased significantly during finishing and for total experimental periods with rye inclusion in the diets. Data on the effect of dietary ME level reveal a significant decrease in total BWG associated with a corresponding increase in total FI and a worsening in FCR as the dietary ME decreased. Results in Table 5 indicate the interaction between studied factors on growth variable, where no significant differences between treatments were detected. The inferiority in growth performance (lower BWG and higher FCR) due to the decrease in dietary ME level was significantly detected for each growth interval. Xylanase supplementation improved FCR during starter (7.59%), finisher (5.91%) and overall (3.26%) periods. Neither BWG nor FI was significantly responded to enzyme supplementation, except for the significant decrease in FI during starter period and the increase in BWG during finisher period. Statistics for the interactions studied show no significant effects on growth and most detected significant interactions are those for the total FCR between CS x ME and CS x ES.

Results in Table 6 indicate that any of the studied main factors (cereal source, dietary ME level or enzyme supplement) and their respective interactions had a significant effect on dressing percentage. Liver weight percentage as proportioned to live weight upon slaughtering was significantly affected and was higher in favor of decreasing dietary ME level and feeding broiler chicks diets without supplemental enzymes. Supplemental xylanase significantly decreased liver weight percentage (10.62%) and the abdominal fat content of the carcass (25.41%) compared to recorded values of un-supplemented groups. Figures also reveal that none of the investigated interactions brought a significant effect on studied variables.

Rye feeding impaired average daily gain as compared with corn feeding regardless of enzyme supplementation, which agrees with Marquardt *et al.* (1994), Lázaro *et al.* (2004), Józefiak *et al.* (2007), Mourao and Pinheiro (2009) who reported that feeding broiler chicks on rye-containing diets significantly decreased BWG, FI, FCR and carcass yield compared to corn-based diets. They added that the water-soluble and viscous beta-glucans and arabinoxylans present in rye

interfere with the mixing of digestive enzymes and nutrients and impede digesta movement and transport of hydrolysis products to the intestinal mucosa which resulted in a retard in poultry performance. The improvement in growth performance reported herein, irrespective of dietary grain source could be expected in light that xylanase supplementation could hydrolyse polysaccharides which are involved in encapsulation of starch or protein in cereal grains, reducing the barriers to nutrient digestion and utilization (Nian *et al.*, 2011). In the current study, xylanase supplementation improved FCR during starter, finisher and total experimental terms, irrespective of dietary cereal grain source. The interaction between dietary cereal grain source and enzyme supplementation significantly affected total FCR that in turn imply that xylanase supplementation could eliminate the negative impact of rye feeding on broiler performance seen with rye compared with corn feeding without enzyme supplementation. These results are supported by findings of Cowieson (2005) who reported that FCR was improved when broiler chicks were fed corn/soy-based diets supplemented with xylanase. In this context, Pettersson and Aman (1989) and Lázaro *et al.* (2003b) reported that enzyme supplementation (beta-glucanase and xylanase) of rye-based diets improved BWG and FCR of broiler chicks. Also, it has been well documented that application of xylanase in corn based diets can improve nutrient utilization and performance of broilers (Wyatt *et al.*, 1999; Jin, 2001; Iji *et al.*, 2003). In this concern, Cowieson (2005) suggested that although viscosity per se is unlikely to be a major problem, the use of xylanase may have beneficial effects in corn-soybean diets for poultry, perhaps by an improvement in nutrient digestibility coefficients. This is presumably mediated through changes in cell wall architecture achieved by hydrolysis of structurally important arabinoxylans, which may release encapsulated nutrients.

The decrease in growth performance with decreasing dietary energy level is in accord with results of Golian *et al.* (2010) that BWG and FCR were improved as the dietary energy level was increased. On the other hand, Downs *et al.* (2006) reported that decreasing dietary ME level about 100 kcal/kg during each growth phase resulted in a significant comparable BWG for broilers, while FCR was significantly improved with increasing dietary ME level. Carcass yield and abdominal fat percentages were not significantly affected by dietary ME level. Conversely, El Tazi *et al.* (2009) found that BWG, FCR and dressing percentage of broiler chicks were not significantly affected by dietary energy level. Supplementing xylanase improved growth performance of broiler chicks fed decreased ME diets. Youssef *et al.* (2011) concluded that a mixture of enzyme preparation containing xylanase and amylase added to corn-based diet could reduce the dietary energy

Table 4: Effect of cereal source (CS), metabolizable energy (ME) level and enzyme supplementation (ES) on growth performance of broiler chicks

Main effects	Starter (1-14 d)			Grower (15-28 d)			Finisher (29-40 d)			Overall (1-40 d)		
	BWG (g)	FI (g)	FCR	BWG (g)	FI (g)	FCR	BWG (g)	FI (g)	FCR	BWG (g)	FI (g)	FCR
Cereal source (CS)												
Corn	136 ^a	206	1.51 ^b	504	942	1.87	1269 ^a	2277 ^a	1.79	1909 ^a	3428 ^a	1.80
Corn+10% Rye	128 ^a	203	1.59 ^a	513	956	1.86	1209 ^b	2175 ^b	1.80	1850 ^b	3334 ^a	1.81
Metabolizable energy (ME)												
Recommended	137 ^a	196 ^b	1.43 ^b	542 ^a	936	1.73 ^b	1286 ^a	2152 ^b	1.67 ^c	1965 ^a	3284 ^a	1.67 ^c
-50 kcal/kg diet	136 ^a	203 ^b	1.49 ^b	505 ^b	964	1.91 ^a	1245 ^a	2213 ^b	1.78 ^b	1886 ^b	3380 ^b	1.79 ^b
-100 kcal/kg diet	130 ^a	216 ^a	1.66 ^a	478 ^b	944	1.97 ^a	1189 ^b	2313 ^a	1.95 ^a	1798 ^c	3473 ^a	1.93 ^a
Enzyme Supplementation (ES)												
Without	134	212 ^a	1.58 ^a	510	949	1.86	1213 ^b	2252	1.86 ^a	1857	3413	1.84 ^a
Xylanase (16000 U/kg)	135	197 ^a	1.46 ^b	506	948	1.87	1258 ^a	2199	1.75 ^b	1876	3346	1.78 ^b
Probability												
CS	0.01	0.47	0.05	0.39	0.43	0.83	0.00	0.01	0.99	0.00	0.04	0.86
ME	0.05	0.00	0.03	0.00	0.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ES	0.53	0.00	0.03	0.75	0.98	0.75	0.04	0.17	0.00	0.13	0.12	0.01
CSXME	0.10	0.51	0.08	0.48	0.19	0.91	0.79	0.00	0.00	0.40	0.00	0.00
CSXES	0.61	0.08	0.16	0.42	0.07	0.08	0.79	0.07	0.20	0.77	0.01	0.03
MEXES	0.87	0.00	0.02	0.75	0.45	0.47	0.03	0.38	0.33	0.10	0.43	0.64
CSXMESES	0.34	0.17	0.88	0.95	0.86	0.79	0.08	0.07	0.82	0.19	0.11	0.74
Pooled SEM	1.61	2.61	0.02	6.55	8.77	0.03	12.54	27.99	0.03	15.86	29.99	0.02

^{a-b}Mean values within a column with unlike superscript letters were significantly different

Table 5: Effect of experimental treatments on growth performance of broiler chicks

C.S	ES	ME (kcal/kg diet)	Starter (1-14 d)			Grower (15-28 d)			Finisher (29-40 d)			Overall (1-40 d)		
			WG (g)	FI (g)	FCR	WG (g)	FI (g)	FCR	WG (g)	FI (g)	FCR	WG (g)	FI (g)	FCR
Corn	Without	Recommended	140	192	1.37	535	928	1.73	1322	2154	1.63	1997	3276	1.64
		-50	134	206	1.54	527	961	1.82	1291	2255	1.75	1952	3422	1.75
		-100	139	234	1.68	468	887	1.90	1133	2394	2.11	1740	3615	2.02
	Xylanase	Recommended	139	200	1.44	544	937	1.72	1298	1995	1.54	1981	3132	1.58
		-50	133	201	1.51	483	1000	2.07	1295	2375	1.83	1911	3576	1.87
		-100	131	201	1.53	467	937	2.01	1276	2498	1.95	1874	3626	1.93
	Corn+10%	Recommended	123	201	1.63	540	954	1.77	1247	2237	1.79	1910	3392	1.78
		-50	134	210	1.57	502	983	1.96	1186	2235	1.88	1822	3428	1.88
		-100	127	229	1.80	490	978	2.00	1145	2239	1.96	1762	3447	1.96
	Rye	Recommended	125	190	1.52	548	927	1.89	1278	2219	1.74	1953	3337	1.71
		-50	122	194	1.59	509	915	1.80	1205	2189	1.81	1836	3298	1.80
		-100	127	197	1.55	491	977	1.99	1191	2132	1.79	1809	3305	1.83
p-value	-	-	0.34	0.17	0.88	0.95	0.86	0.79	0.08	0.07	0.82	0.19	0.11	0.74

C.S: Cereal source ES: Enzyme supplementation

Table 6: Effect of cereal source (CS), dietary metabolizable energy (ME) level and enzyme supplementation (ES) on some slaughtering traits of broiler chicks

Main effects	Dressing (%)	Liver (%)	AF(%)
Cereal source (CS)			
Corn	68.3	2.84	1.08
Corn+10% Rye	67.9	2.69	0.96
Metabolizable energy (ME)			
Recommended	68.1	2.57 ^b	0.99
-50 kcal/kg diet	67.6	2.85 ^a	1.09
-100 kcal/kg diet	68.6	2.87 ^a	0.98
Enzyme supplementation (ES)			
Without	67.7	2.92 ^a	1.17 ^a
Xylanase (16000 U/kg)	68.5	2.61 ^b	0.87 ^b
Probability			
CS	0.57	0.09	0.32
ME	0.46	0.00	0.92
ES	0.22	0.00	0.01
CS x ME	0.78	0.13	0.53
CS x ES	0.14	0.07	0.03
ME x ES	0.76	0.04	0.06
CS x ME x ES	0.92	0.19	0.22
Pooled SEM	0.32	0.06	0.07

^{a,b}Mean values within a column with unlike superscript letters were significantly different. AF: Abdominal fat

Table 7: Detection of some pathogenic bacteria from cloaca at 10 days of age

Treatments			<i>E. coli</i>	<i>Campylobacter</i>	<i>Staph. aureus</i>	<i>Salmonella</i>	<i>Clostridium perfringens</i>
Cereal source	Enzyme supplementation	ME (kcal/kg diet)					
Corn	Without	Recommended	+	+	+	-	-
		-50	+	+	+	-	-
		-100	+	+	+	-	-
	Xylanase (16000 U/kg)	Recommended	+	+	+	-	-
		-50	+	+	+	-	-
		-100	+	+	+	-	-
Corn+10% Rye	Without	Recommended	+	+	+	-	-
		-50	+	+	+	-	-
		-100	+	+	+	-	-
	Xylanase (16000 U/kg)	Recommended	+	+	+	-	-
		-50	+	+	+	-	-
		-100	+	+	+	-	-

level about 150 kcal/kg, as no significant differences in growth performance and carcass traits were observed. O'Neill *et al.* (2012) reported that decreasing energy in broiler diets (100 kcal less than recommendation) resulted in less growth performance than recommendation. Xylanase supplementation (16000 U/kg diet) improved feed conversion ratio but not live weight gain. Zou *et al.* (2013) concluded that supplementation with a mixture of xylanase and beta-Glucanase) improved FCR but not BWG and FI of broilers in corn-soybean meal diet by improving energy utilization (ME reduction 100 kcal/kg diet). While were not affected by the interaction.

Enzyme supplementation has resulted in improving the digestion of encapsulated nutrients and resulting in improved sthe diet ME and thereby leading to improved poultry performance (Bedford and Schulze, 1998; Hetland *et al.*, 2004).

Bacteriological study: Table 7 clears the presence of some pathogenic bacteria species (*E. coli*, *Campylobacter* and *Staph. aureus*) and the absence of some others (*Salmonella* and *Clostridium perfringens*)

in collected cloaca swabs at day 10 of feeding the experimental diets. These results are quite close to data obtained at day 40 of the study (Table 8), where *E. coli* was detectable in all cloaca and liver samples and spleen samples except in groups fed diets of 100 kcal less recommended/kg diet and of rye diet with 50 kcal less recommended/kg diet. *Campylobacter* detection was positive in case of all liver samples and in spleen samples except chicks fed the basal diet and diets of low ME level supplemented with xylanase. *Staph. aureus* bacteria was found in all cloaca samples, in liver samples except for treatments of 100 kcal less recommended/kg diet (corn with enzyme supplementation and rye without enzyme supplementation and rye diets with enzyme supplementation of recommended or 50 kcal less recommended/kg diet and in spleen except the basal diet, basal with 50 kcal diet less recommended/kg diet and rye diet with 100 kcal less recommended/kg diet. *Salmonella* and *Clostridium perfringens* were not detected in samples. Feed enzymes can reduce the bacterial activity in the ileum by reducing the amount of nutrients available for microbial fermentation (Silva and

Table 8: Detection of some pathogenic bacteria from some organs at 40 days of age

CS	ES	Treatments	ME (kcal/kg diet)	E. coli			Campylobacter			Staph. aureus			Salmonella			Clostr. perfringens		
				C	L	S	C	L	S	C	L	S	C	L	S	C	L	S
Corn	Without	Recommended	-50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Corn+10% Rye	Without	Recommended	-50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Corn	Xylanase (16000 U/kg)	Recommended	-100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Corn	Xylanase (16000 U/kg)	Recommended	-100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Corn	Xylanase (16000 U/kg)	Recommended	-50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Corn	Xylanase (16000 U/kg)	Recommended	-100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

ES: Enzyme supplementation CS: Cereal source C: Cloaca L: Liver S: Spleen

Smithard, 2002), especially that are available as an energy source for bacteria (Apajalahti *et al.*, 2004). More direct anti-bacterial effects of enzymes have been implied but not proven categorically, such as the hydrolysis of polysaccharides used by bacteria to attach themselves to the walls of the gut, with a consequential reduction in the capacity for bacteria to colonize the gastro-intestinal tract (Cowieson, 2002). In rye-based diets, total anaerobe colony-forming units throughout the gut were shown to decrease with xylanase supplementation (Danicke *et al.*, 1999). Specific groups of bacteria were also modified, but this depended upon the region of interest and the type of fat in the diet. Vahjen *et al.* (1998) reported that xylanase supplementation led to significantly lower colony forming units per gram of wet weight for total presumptive enterobacteria and total gram-positive cocci in luminal and tissue samples in the first 3 weeks. Lactobacillus spp. colony counts from tissue samples were higher for animals with the xylanase-supplemented diet, but luminal CFU were not. Fernandez *et al.* (2000) reported that xylanase enhances mucin output from the goblet cells by lowering the viscosity of the intestinal content. Furthermore the data suggested that xylanase modifies the N-acetylglucosamine residues of goblet cell mucins along the proximal and distal parts of the chick intestinal tract. The carbohydrate characteristics of intestinal mucins have been reported to prevent attachment by *Salmonella typhimurium* and *Yesinia enterocolitica* (Engstaber *et al.*, 1992).

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