

Effects of xylanase supplementation of corn-soybean meal-dried distiller's grain diets fed to first-cycle laying hen performance, metabolizable energy and body composition

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## SUMMARY

The increased cost of energy for laying hen diets has resulted in the use of dietary enzymes such as xylanase to increase energy digestibility and reduce the need for supplemental dietary energy. A 24-wk-long experiment was conducted using 432 17 wk old Hy-Line W36 first-cycle laying hens to determine the effects of energy concentrations and xylanase supplementation (Hostazym<sup>®</sup> X100) on hen performance, metabolizable energy and body composition. Three concentrations of dietary energy fed with and without xylanase supplementation and arranged as a 3 x 2 factorial: Control (C); C-77 kcal/kg diet and C-154 kcal/kg diet were fed with and without xylanase supplementation. Egg production, feed intake, body weight, egg weight, egg mass, feed efficiency and egg solids data were collected over the 24 wk experimental period. Nitrogen corrected apparent metabolizable energy was determined at 12 and 24 wk and body composition (crude fat, crude protein and ash) was determined at 24 wk. Hens fed dietary xylanase resulted in increased hen-day egg production over wk 1-16 and 1-24 ( $P < 0.05$ ). Feed intake was increased with reduced dietary energy ( $P < 0.05$ ). Egg mass and feed efficiency were improved with xylanase over the experiment, but significance was only achieved at select time periods. Hen body weight, egg weight and egg solids were generally not different among treatments. Main effects at wk 12 showed that reduced energy resulted in reduced AMEn and xylanase increased AMEn. In contrast, AMEn at wk 24 resulted in reduced AMEn with xylanase treatment. There were no differences in carcass protein or ash composition, but carcass fat resulted in an interaction as xylanase increased carcass fat in the control diet but reduced it in the C-77 kcal/kg diet. Overall, supplementation of xylanase to laying hens increased egg production, egg mass and feed efficiency over the 24 wk period although AMEn and body composition data were mixed.

**Key words:** laying hen, xylanase, production, energy, body composition

## **DESCRIPTION OF PROBLEM**

Ethanol and biofuel production diverts corn and oil from animal agriculture, resulting in an increased cost of dietary energy for laying hens [1]. Dietary enzymes have been developed to increase energy and nutrient utilization for poultry and other species [2, 3]. Xylanase enzymes have been suggested to increase the production of soluble, fermentable oligomers, which results in enhanced cecal fermentation and improved utilization of grains such as barley and wheat in poultry which contain a high content of soluble non-starch polysaccharides (NSPs) [4]. Xylanase preparations were first demonstrated efficacious in high soluble-NSP diets by reducing viscosity and improving nutrient utilization, digestion and performance [5]. However, xylanase effectiveness in corn-soybean meal (SBM) diets has taken longer to demonstrate due to differences in constituent NSP [3]. Xylanases improved bodyweight gain in broilers fed corn-rye-based diets, and increased egg weight and feed conversion in laying hens fed corn-oat-wheat middling-based diets [6]. A 12 wk experiment with second-cycle layers showed xylanase increased hen body weight and egg weights inconsistently over the experimental period [7]. The objective of the present experiment was to determine the effects of dietary energy concentration and xylanase supplementation on laying hen production, metabolizable energy and body composition of hens fed corn-SBM-dried distillers grains with solubles (DDGS)-based diets.

## **MATERIALS AND METHODS**

### ***Experimental Design***

The experiment was approved by the Institutional Animal Care and Use Committee at Iowa State University (ISU, Ames, Iowa) and was conducted at the ISU Poultry Research and Teaching Unit. A total of 432, 17-wk old Hy-Line W36 hens (Hy-Line International, Dallas Center, IA) were fed a standard diet over a 2 wk transition period after which they were provided *ad libitum* access to one of the six experimental diets and water. Initial photostimulation was 12 h light: 12 h dark and the photoperiod was increased 0.5 h of light/wk until 16 h light: 8 h dark was achieved and maintained. Hens were monitored twice daily with mortality removed from the cages and recorded as they occurred. Each experimental unit (EU) was defined as three adjacent cages of three hens (439 cm<sup>2</sup> per hen), resulting in eight EU for each of the six treatment groups (72 hens per dietary treatment). Experimental diets were corn-SBM-DDGS-based and were arranged as a 3x2 factorial by reducing energy (soybean oil) from the control diet to provide three levels of energy (control, control – 77kcal/kg and control – 154 kcal/kg; Table 1) with or without the addition of 0.01% Hostazym<sup>®</sup> X 100 (xylanase; activity level of 15,000 EPU/g) [8]. Diets were re-formulated over time, resulting in 3 dietary phases fed during 20-30, 31-40, and 41-44 wk of age, respectively.

Eggs were collected daily at approximately 1100 h and egg production data were recorded by EU. Feed intake was determined weekly by calculating feed offered minus feed refused. Hens were weighed by cage and pooled to average hen week per EU at the initiation of the experiment and every subsequent 4 wk period. Egg weight, egg mass, and egg solids were determined every 4 wk by combining 5 d of egg production [7, 9]. Feed efficiency was calculated as the ratio of egg mass to feed intake and calculated every 4 wk. Excreta samples were collected over the last 48 h of wk 12 and 24 for AMEn determination [9]. On the last day of the experiment, 3 hens per

EU (1 per cage) were euthanized using carbon dioxide asphyxiation. Hens were de-feathered before being ground for wet chemistry body composition analysis to reduce variance due to differences in feather coverage [13]. Data were analyzed using JMP statistical software [14].

## RESULTS AND DISCUSSION

### *Hen Performance*

Hen day egg production was increased by 2.65 and 2.12% with xylanase supplementation over the 20-36 and 20-45 wk periods, respectively (Table 2,  $P \leq 0.05$ ). Dietary energy had no effect on hen day egg production and no interactions were noted for any time periods. Feed intake was significantly increased in hens fed low energy diets versus control fed birds over both the 20-36 and 20-45 wk periods (Table 2,  $P \leq 0.05$ ). Enzyme supplementation had no effect on feed intake and no significant interactions were observed. It is interesting to note that W36 laying hens fed reduced dietary energy were able to increase feed intake and maintain egg production over the 24 wk experimental period, in contrast to previous reports [15, 16]. Feeding second cycle W36 laying hens fed diets containing four concentrations of energy resulted in decreased feed consumption, as energy increased, although these results were with large differences in dietary energy (198 kcal/kg) and the hens would have increased body weight in the second cycle [7]. Similar data in other strains of laying hens have also been reported. Bovans White and Dekalb White first-cycle laying hens were able to adjust dietary intake to maintain energy intake [17]. In the current experiment, the increased feed intake for the lowest energy diet (control–154 kcal/kg) resulted in reduced feed efficiency over the 20-36 wk period of the experiment. Although the feed efficiency effect was significant during wk 28, it was consistent over the other wk measured (Table 3). Feed efficiency was not different at wk 40 and 44, possibly due to

increased feed intake for the control fed hens later in the experiment. Xylanase supplementation resulted in numeric increases in feed efficiency over all weeks with only wk 36 resulting in a significant increase ( $P \leq 0.05$ ). Similar to these data, hens fed corn-SBM diets with a xylanase + beta-glucanase enzyme preparation resulted in significant improvements in feed conversion ratio and weight gain due to enzyme supplementation, although egg production, egg weight, and egg mass were not affected [6]. There were no interactions between enzyme and dietary energy for egg production, feed intake or feed efficiency suggesting that the effects of xylanase are independent of dietary energy.

There were no significant main effects for egg weight (data not shown) as egg weights increased from 54.6 g/egg to 61.4 g/egg over the experimental period. There were significant energy x enzyme interactions at wk 28 and 36 as reduced energy without xylanase increased egg weight, but xylanase treatment reduced egg weight in those same reduced energy diets. These interactions were inconsistent with egg weight data in the remaining experiment. Egg mass (g egg/hen per day) was significantly increased with xylanase treatment over wk 32 and 36 of the experiment and approached significance in wk 28 and 40 (Table 4). At 36 and 40 wk, the control diet without xylanase supplementation resulted in the lowest egg mass, resulting in a significant interaction (Figure 2). This is possibly explained by reduced feed intake in hens fed control diets, reducing egg mass in comparison to hens fed the other diets. Previous work with xylanase supplementation in hens has shown to increase egg weight and mass [18]. In the experiment presented here, egg mass was increased due to increased egg production and not increased egg weight.

No differences in hen body weight were noted for the duration of the experiment. Hen body weights (kg) were as follows: wk 20, 1.32; wk 24, 1.44; wk 28, 1.48; wk 32, 1.48; wk 36, 1.50; wk 40, 1.51; wk 44, 1.51. In total, 5 mortalities were reported for the experiment; 3 in the control diet without xylanase, and 1 each in the reduced energy diets without xylanase. Over the experimental period egg solids were approximately 25.4%. Xylanase treatment significantly increased egg solids at wk 24 but no differences were reported at any later time point (data not shown). A significant interaction resulted at wk 32, where hens fed the lowest dietary energy with xylanase resulted in increased egg solids versus all hens without xylanase ( $P \leq 0.05$ ). Both the main effect at wk 24 and the interaction at wk 32 were inconsistent with general responses over time and their biological relevance is questionable.

#### ***Nitrogen Corrected Apparent Metabolizable Energy (AMEn)***

Results for AMEn data were mixed between wk 32 and 44. At 32 wk, reducing dietary energy significantly reduced AMEn, whereas the addition of xylanase resulted in a significant increase in AMEn (main effects of both energy and enzyme on AMEn,  $P < 0.05$ , Table 5). At 44 wk, xylanase reduced AMEn ( $P \leq 0.05$ ) and there were no significant effects due to energy. A trend was noted as the C-77 kcal/kg diet resulted in the lowest AMEn and although not significant, an interaction was noted due to the C-77 kcal/kg diet with xylanase resulting in the lowest response lowering both the C-77 kcal/kg treatment and the xylanase treatment. The contrary results between 32 and 44 wk are perplexing, although increased variability in feed efficiency and egg mass at wk 44 might indicate short term health or stress issues that could adversely affect AMEn measured at wk 44. The data reported in wk 32 agree with previous reports as xylanase has

significantly improved AMEn and metabolizability coefficients of amino acids in laying hens fed wheat/rye/SBM diets [19], albeit these diets have a greater level of soluble NSPs.

### ***Body Composition***

Wet chemistry analysis of hen body composition resulted in no differences in carcass protein or ash, but reductions in dietary energy reduced carcass fat ( $P \leq 0.05$ , Table 6). Hens fed control diets had the highest carcass fat (41.0%), whereas those fed C-77 and C-154 kcal/kg, resulted in 39.6 and 37.3% carcass fat, respectively. There was a significant interaction between dietary energy and xylanase treatment due to increased carcass fat in birds fed the control diet with xylanase and the C-77 kcal/kg without xylanase treatments (Figure 2). The high energy (control) diet with the xylanase treatment could be expected to result in higher carcass fat if excess energy is stored after productive energy requirements are met, but the higher carcass fat from the C-77 kcal/kg diet without xylanase is unexplained. Although few data have been published regarding the effects of dietary energy on hen body composition it appears that carcass fat content may be a more sensitive indicator of dietary energy status than egg production. These data are in agreement with previous research showing hens reduced energy storage in the abdominal fat pad before reducing egg production, when limit fed diets with a 90 kcal/kg reduction in dietary energy [15].

## **CONCLUSIONS AND APPLICATIONS**

1. The addition of xylanase (Hostazym® X100) increased egg production and consistently, if not significantly, improved feed efficiency of first-cycle Hy-Line W36 laying hens regardless of dietary energy.



2. Hy-Line W36 laying hens were sensitive to dietary energy as both 77 and 154 kcal/kg reductions in dietary energy content resulted in increased feed intake over the experiment.
3. AMEn was responsive to both dietary energy and xylanase at 32 wk, whereas 44 wk data were either flat or contrary to the 32 wk data.
4. High energy-xylanase diets increased carcass fat, suggesting this variable is more sensitive to dietary energy than is egg production.

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8. Enzymes was added at manufacturer's recommendations (minimum 15,000 EPU xylanase activity per gram, derived from *Trichoderma longibrachiatum*; Huvepharma, Sofia, Bulgaria)
9. The collected excreta samples were frozen at -20°C before they were oven dried at 65°C for 3 d to determine dry matter. Feed samples were corrected to a dry matter basis by measuring 5.0g of each diet, drying them in an oven at 100°C for 24 hr, and calculating the ratio between the dry weight and pre-dry weight. The excreta samples were then ground through a 1mm screen and the feed samples were ground using a 0.5mm screen (Brinkmann Instruments Company, Westbury, NY). Feed and excreta samples were assayed for the AMEn by determining the gross energy content using an adiabatic oxygen bomb calorimeter and nitrogen content by the micro-Kjeldahl method using a Kjeltech 1028 distilling unit (U.S. Tecator, Inc., Herndon, PA). [10]. Titanium dioxide was determined [11] in the excreta and feed samples to calculate the AMEn [12] as follows:  
AMEn = Diet GE - [Excreta GE x Diet Ti/Excreta Ti - 8.22 x (Diet N - Excreta N x Diet Ti/Excreta Ti)]
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13. Ground carcass samples were freeze dried before being analyzed for crude protein (LECO: LECO Tru-Mac N; Ash: AOAC 1980 Method 7.003; and crude fat: AOAC Method 960.39 (39.1.05 in the 18th, 2005 edition).
14. JMP (SAS Institute, Cary, NC). Three consecutive cages within a dietary treatment were considered a replicate group with a randomized complete block design. Hens were assigned to treatment groups using blocks within house and a recognized randomization technique for treatments treatments were randomized within block. A two-way ANOVA as a 2x3 factorial was

used to analyze data, and if ANOVA was significant, means were separated by a protected student's t-test ( $\alpha = 0.05$ ;  $t = 1.987$ ) to separate LS means. Data were considered significantly different if  $P \leq 0.05$ .

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## TABLES AND FIGURES

Table 1. Formulation of dietary treatments and nutrient composition of laying hen diets: control (C), C – 77kcal/kg (C-77) and C – 154 kcal/kg (C-154), with or without 0.01% xylanase.

Ingredient, %	Wk 20-30			Wk 31-40			Wk 41-44		
	C	C-77	C-154	C	C-77	C-154	C	C-77	C-154
Corn	43.11	44.70	46.30	50.51	52.11	53.70	48.37	49.96	50.90
Soybean meal	25.55	25.4	25.25	17.74	17.59	17.44	16.34	16.19	16.64
Corn DDGS <sup>1</sup>	10.00	10.00	10.00	10.00	10.00	10.00	15.00	15.00	15.00
Bakery meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Soy oil	4.26	2.82	1.39	3.44	2.00	0.56	2.98	1.55	0.20
Salt	0.24	0.24	0.24	0.19	0.19	0.19	0.16	0.16	0.16
DL-methionine	0.19	0.19	0.19	0.12	0.12	0.11	0.10	0.10	0.09
Bio-Lys <sup>2</sup>	0.06	0.06	0.06	0.09	0.09	0.10	0.11	0.12	0.09
Limestone, small	4.89	4.89	4.89	4.90	4.90	4.90	4.93	4.93	4.93
Limestone, large	4.89	4.89	4.89	4.90	4.90	4.90	4.93	4.93	4.93
Di-calcium phosphate	1.21	1.20	1.20	1.26	1.25	1.25	1.16	1.15	1.15
Choline chloride 60	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin-mineral premix <sup>3</sup>	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
E. coli phytase	0.006	0.006	0.006	0.006	0.006	0.006	0.06	0.06	0.06
Celite	-	-	-	1.00	1.00	1.00	-	-	-
Titanium dioxide	-	-	-	0.25	0.25	0.25	0.25	0.25	0.25
Nutrient Profile, %									
Crude protein	19.29	19.33	19.37	16.12	16.16	16.19	16.62	16.66	16.91
Metabolizable energy, kcal/kg	2,900	2,823	2,746	2,875	2,798	2,721	2,850	2,773	2,696
Calcium	4.10	4.10	4.10	4.10	4.10	4.10	4.10	4.10	4.10
Non-phytate phosphorus	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Digestible sulfur amino acids	0.74	0.74	0.74	0.60	0.60	0.60	0.60	0.60	0.60
Digestible threonine	0.66	0.67	0.67	0.55	0.55	0.55	0.56	0.56	0.57
Digestible lysine	0.89	0.89	0.89	0.71	0.71	0.71	0.71	0.71	0.71

<sup>1</sup> Dried distiller's grains with solubles

<sup>2</sup> Bio-Lys (50.7% L-Lysine)

<sup>3</sup> Vitamin and mineral premix contained the following per kilogram diet: Vitamin A-6605 IU; Vitamin E-14.31IU; Cholecalciferol-2200 IU; Menadione-880 µg; Vitamin B12-9.3 µg; Biotin-33.0 µg ; Choline-357 mg; Folic acid-1,100 µg; Niacin-33.0 mg; Pantothenic acid-8.81 mg; Pyridoxine-0.88 mg; Riboflavin-4.4 mg; Thiamine-1.1 mg; Iron-102.5 mg; Magnesium-10 mg; Manganese-100 mg; Zinc-100 mg; Copper-10 mg; Iodine-0.7; Selenium-200 µg.

Table 2. Hen day egg production (%) and feed intake (g/hen/d) of hens fed three dietary energy concentrations with and without xylanase.

Energy	Enzyme	Hen-day egg production (%)			Feed intake (g/hen/d)		
		20-28	20-36	20-44	20-28	20-36	20-44
Wk of age							
Control (C)		79.0	87.2	88.3	88.4	95.6 <sup>b</sup>	98.3 <sup>b</sup>
C-77 kcal/kg		77.5	87.4	89.2	89.8	97.7 <sup>a</sup>	100.2 <sup>a</sup>
C-154 kcal/kg		78.2	86.6	88.6	90.9	98.6 <sup>a</sup>	101.4 <sup>a</sup>
SEM		1.48	0.94	0.78	0.77	0.64	0.61
	None	77.1	77.2 <sup>b</sup>	87.8 <sup>b</sup>	89.6	97.2	100.0
	Xylanase	79.3	79.3 <sup>a</sup>	89.7 <sup>a</sup>	89.9	97.5	100.0
SEM		1.21	0.76	0.64	0.63	0.53	0.50
		<i>P-value</i>					
Energy		0.77	0.83	0.72	0.07	0.01	0.01
Enzyme		0.22	0.05	0.04	0.70	0.69	0.95
Energy x Enzyme		0.71	0.83	0.75	0.37	0.18	0.17

<sup>ab</sup> Columns without similar superscript letters denote significant difference ( $P < 0.05$ ).

Values shown are means of 8 EU observations per treatment.

Table 3. Feed efficiency (g of egg/kg feed intake) of hens fed three dietary energy concentrations with or without xylanase.

Energy	Enzyme	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44
Control (C)		551	564 <sup>a</sup>	546	515	542	508
C-77 kcal/kg		538	571 <sup>a</sup>	547	517	543	505
C-154 kcal/kg		522	544 <sup>b</sup>	528	496	545	491
SEM		10.6	8.0	6.4	7.4	7.1	18.8
	None	529	555	539 <sup>b</sup>	502	537	499
	Xylanase	546	565	551 <sup>a</sup>	517	549	504
SEM		8.7	6.6	5.2	6.0	5.8	15.4
		<i>P-value</i>					
Energy		0.38	0.05	0.06	0.09	0.93	0.78
Enzyme		0.17	0.28	0.01	0.07	0.15	0.83
Energy x Enzyme		0.83	0.71	0.86	0.70	0.47	0.12

<sup>ab</sup> Columns without similar superscript letters denote significant difference ( $P < 0.05$ ).

Abbreviations: Wk denotes wk of age of the laying hens.

Values shown are means of 8 EU observations per treatment.

Table 4. Egg mass (g of egg/hen/d) of hens fed three dietary energy concentrations with or without xylanase.

Energy	Enzymes	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44
Control (C)		50.1	54.8	55.8	55.4	56.4	52.5
C-77 kcal/kg		48.3	56.5	56.8	57.0	57.1	54.9
C-154 kcal/kg		50.2	55.0	55.5	55.2	57.6	55.5
SEM		1.07	0.70	0.54	0.62	0.57	1.01
	None	48.7	54.7	54.9 <sup>b</sup>	54.8 <sup>b</sup>	56.5	53.8
	Xylanase	50.4	56.1	57.1 <sup>a</sup>	56.9 <sup>a</sup>	57.6	54.8
SEM		0.87	0.58	0.44	0.51	0.47	0.83
		<i>P-value</i>					
Energy		0.38	0.20	0.21	0.09	0.36	0.07
Enzyme		0.17	0.10	0.01	0.01	0.10	0.41
Energy x Enzyme		0.83	0.37	0.85	0.04	0.03	0.13

<sup>ab</sup> Columns without similar superscript letters denote significant difference ( $P < 0.05$ ).

Abbreviations: Wk denotes wk of age of the laying hens.

Values shown are means of 8 EU observations per treatment.

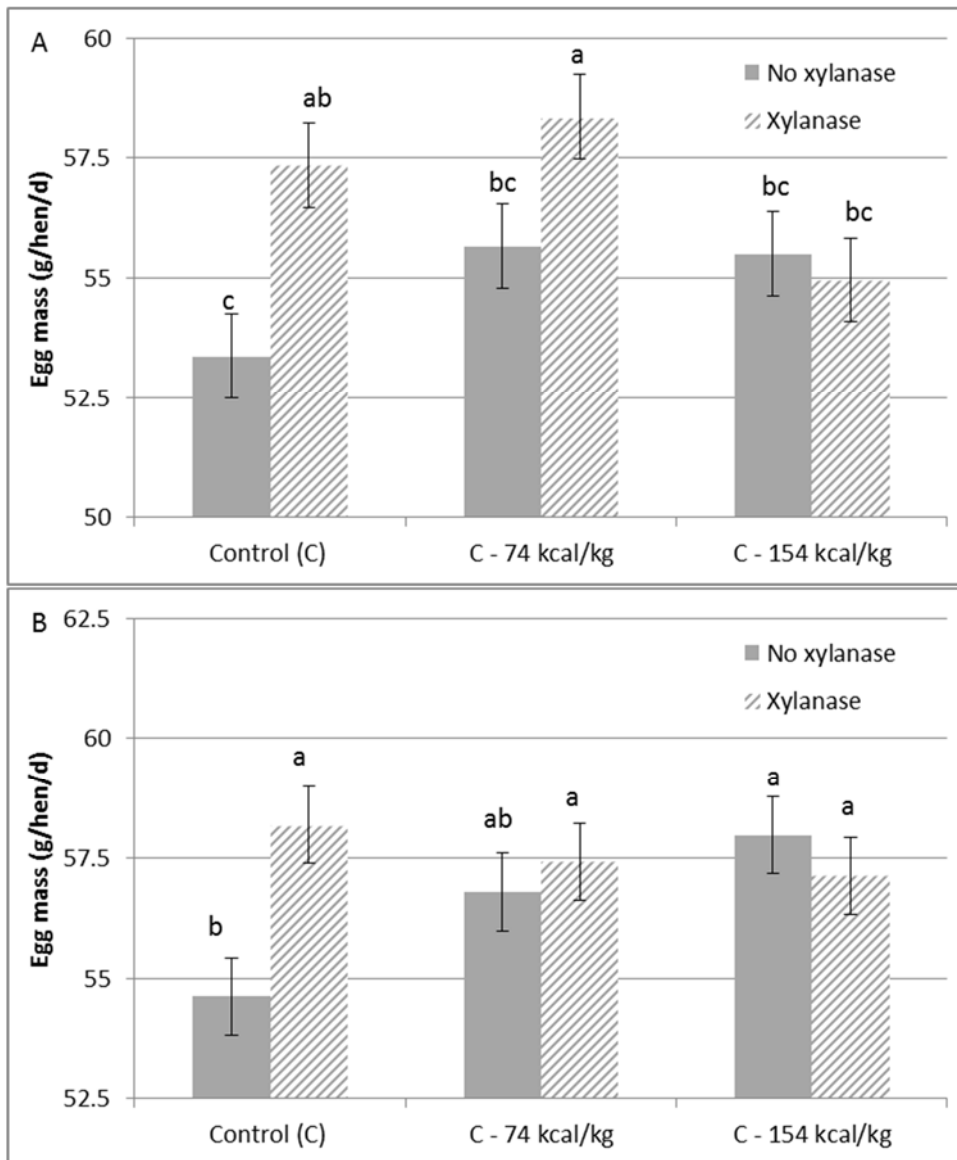


Figure 1. Egg mass (g/hen per d) of hens fed three energy concentrations with or without xylanase at 36 (panel A) and 40 (panel B) wk of age. Values shown are means of 8 EU observations per treatment.

<sup>a-c</sup> Bars without similar superscript letters denote significant difference ( $P \leq 0.05$ ).



Table 5. Nitrogen corrected Apparent Metabolizable Energy (AMEn; kcal/kg) of first cycle laying hens fed various dietary energy concentrations with or without xylanase at 32 and 44 wk of age.

Energy	Enzyme	Wk 32	Wk 44
Control (C)		3042 <sup>a</sup>	3112
C-77 kcal/kg		3002 <sup>a</sup>	3057
C-154 kcal/kg		2945 <sup>b</sup>	3123
SEM		19.2	21.1
	None	2953 <sup>b</sup>	3123 <sup>a</sup>
	Xylanase	3039 <sup>a</sup>	3071 <sup>b</sup>
SEM		15.7	17.2
<i>P-value</i>			
Energy		0.01	0.07
Enzyme		0.01	0.04
Energy x Enzyme		0.95	0.09

<sup>ab</sup> Columns without similar superscript letters denote significant difference ( $P < 0.05$ ).

Values shown are means of 8 EU observations per treatment.

Table 6. Body composition of de-feathered hens fed three dietary energy concentrations with or without xylanase.

Energy	Enzyme	Carcass Protein	Carcass Fat	Carcass Ash
		(%)	(%)	(%)
Control (C)		43.8	41.0	9.25
C-77 kcal/kg		43.9	39.6	9.29
C-154 kcal/kg		45.1	37.3	9.42
SEM		0.77	0.72	0.279
	None	44.5	39.3	9.24
	Xylanase	44.0	39.3	9.40
SEM		0.63	0.59	0.228
		<i>P-value</i>		
Energy		0.43	0.01	0.90
Enzyme		0.51	0.95	0.64
Enzyme x Energy		0.52	0.01	0.39

<sup>ab</sup> Columns without similar superscript letters denote significant difference ( $P < 0.05$ ).

Values shown are means of 3 hens/EU (1 per cage) for each dietary treatment.

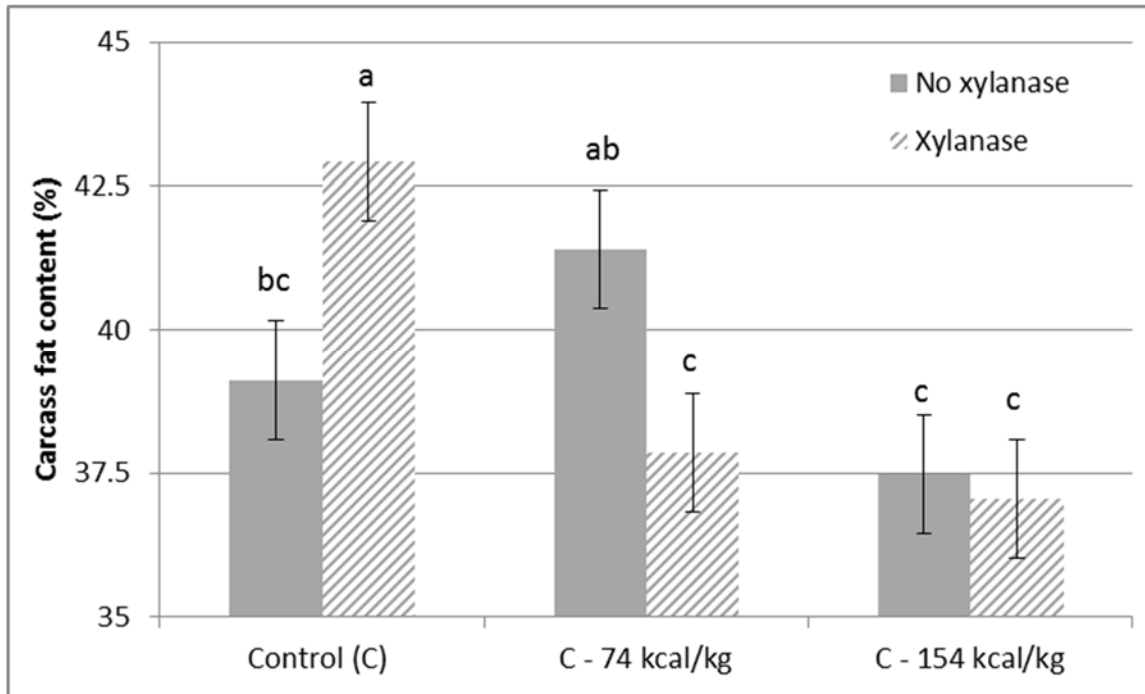


Figure 2. Carcass fat content (%) of first cycle laying hens fed three concentrations of dietary energy and xylanase at wk 44 of age. Values shown are means of 3 hens/EU (1 hen per cage) for each dietary treatment.

<sup>abc</sup> Bars without similar superscript letters denote significant difference ( $P < 0.05$ ).