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Using Protease and Xylanase Enzymes as Single or Combined Supplementation to Corn-Soybean Meal Broiler Diets

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Abstract

Two experiments (EXP 1 and 2) were conducted to study the effect of adding xylanase (X), protease (P) or their combination (XP) to corn-soybean meal broiler diets on growth performance and carcass quality. In both EXP 1 and 2, day-old unsexed Arbor Acres broiler chicks were subjected to three phases feeding program (1 - 40 d of age). The supplementation rates were 16000 U of X/kg diet (Econase XT25) and 300000U of P/kg diet (CIBENZA® DP100). In EXP1, 160 chicks were randomly allocated to 4 treatments; Chicks were fed corn-soybean meal standard diet (STD) without or with supplemental X, P or XP. Diets of STD were formulated to contain 3000, 3100 and 3200 kcal of metabolizable energy (ME)/kg and 23, 21 and 19 % crude protein (CP) during starter, grower and finisher periods, respectively. In EXP2, 400 chicks were allocated to (2 x 5 factorial design) 10 treatments. Chicks were fed one of 5 formulae of reduced levels of CP and ME from values of STD of each feeding phase without or with XP supplementation. The formulas (F) were: F1, (STD); F2, was lower 10% CP and 100 kcal ME; F3, was lower 10% CP and 150 kcal ME; F4, was lower 15% CP and 100 kcal ME; F5, was lower 15% CP and 150 kcal ME. All diets in both EXP1 and 2 contained the same total lysine/CP and methionine + cystine/CP of STD of each phase. Results of EXP1 showed significant improvement of FCR due to adding X, P or XP and the best value obtained by using XP (1.55) followed by P (1.58). Using XP increased dressing % (6.18%) and reduced meat contents of malondialdehyde (MDA) and low-density lipoprotein (LDL). Results of main effects of EXP2 showed improvement of FCR (13.2%), dressing % (3.8%), and meat quality due to supplemental XP. Using F2, F3, F4 or F5 resulted in gradual adverse effect on growth and meat drip loss, while meat contents of LDL and MDA decreased. Among treatments, supplementing F2 with XP improved growth performance, carcass traits, meat quality, and showed the highest saving of feeding cost/kg of body weight (9.1%) compared with STD value. These results recommend adding XP combination to lower 10% of CP and 100 kcal ME/kg broiler diet to reduce feeding cost and improve growth performance, carcass traits and meat quality.

Keywords: *Broiler; Xylanase; Protease; Growth Performance; Meat Quality*

Introduction

The nutrients requirements of broiler strains have increased over the past few decades because of genetic improvement of growth performance and production period. The endogenous production and excretion of digestive enzymes are insufficient to get complete digestion and absorption of nutrients especially at early age of broiler chicks [1]. This means that some of the consumed feed will be lost, resulting in economic and environmental problems such as increased cost of feeding [2], and nitrogen emission [3,4]. As a way to introduce a solution to this complicated situation, workers in the biotechnology field produce several enzymes products like phytase, endo-xylanases, endo-mannanases, beta glucanase and protease and researchers of poultry nutrition field have studied the response of broilers to those additives [5,6].

Many researchers have demonstrated the positive effect of xylanase, a carbohydrase enzyme, on growth performance of broilers [7-11] through successful hydrolysis of cell wall arabinoxylans (non starch polysaccharides, NSP) and increased access of endogenous digestive enzymes to cell contents [12-14]. Moreover, this effect of xylanase has been reported when a 50 to 150 kcal ME/kg low energy diets were applied [15-18]. In many studies, the optimum reduction level of ME which could be compensated by xylanase supplementation was 100 kcal ME/kg lower than strain recommendations [19,16,17]. O'Neill., *et al.* [19] examined the effect of xylanase supplementation to low energy broiler diets (lower 100 kcal/kg diet than recommendation) based on corn-soybean meal and reported significant improvement of growth performance after the first 21 days of age. Also, Saleh., *et al.* [11] concluded that dietary supplementation of xylanase and arabinofuranosidase combination had beneficial effect on the growth performance of broiler fed on low energy diet (lower 90 kcal/kg diet than control). They concluded that decreasing ME of broiler diets resulted in worse performance and xylanase supplementation might improve FCR.

In addition, several investigators examined the effect of protease enzymes supplementation on protein and amino acid utilization of broilers and reported increased values of ileal digestibility and availability [20-23]. Some reports also recorded additional increase of energy utilization by applying protease in broiler diets [21,24]. As a result of these improvements, growth performance parameters, feed conversion ratio and body weight gain, were enhanced [6,21,25,26]. Some authors recorded economic and environmental benefits when using proteases in broiler diets by using low protein diets [6,26]. Odetallah., *et al.* [6] reduced CP of corn-soybean meal in starter diets of broiler chicks to 18% and recorded improvement of growth performance by supplementing an enzyme preparation containing a broad-spectrum protease enzyme, PWD-1 keratinase at levels 0.05, 0.1 and 0.15 % wt/wt. Likewise, Selim., *et al.* [26] reported growth improvement of broilers fed on 20.7, 18.9 and 17.1% CP diets supplemented with the commercial product of the same enzyme at 3000000 U protease/kg during starting, growing and finishing periods, respectively.

Concerning the combination of xylanase and protease supplementation in broiler diets, few reports are available and results were varied. For example, Kalmendal and Tauson [27] failed to detect extra improvement of nutrient utilization of broiler chicks fed wheat-soybean meal based diets when supplemented with a combination of xylanase and protease compared with their positive effect on growth performance when supplied separately. On the other hand, Romero., *et al.* [22,23] reported that the addition of xylanases would increase the potential of exogenous proteases to digest proteinaceous components in the feed. This opinion is supported by results of Olukosi., *et al.* [24] and Amerah., *et al.* [18] which compared protease, xylanase and amylase in single or combined supplementation. Also, using multi-enzyme supplement containing protease and xylanase recorded better body wight gain (961 versus 858g) and feed conversion ratio (1.69 v 1.86) compared to control diets ($P < 0.01$) when supplemented on broiler diet containing 11% rice bran during finisher phase [28].

Effect of xylanase or protease supplementation to broiler diets on carcass traits have been investigated and different results were detected concerning dressing %, carcass cuts, and meat quality [16,17,26].

Therefore, this study aimed to compare the effect of xylanase (X), protease (P) and their combination on growth performance and carcass traits in broilers fed a standard diet, and to evaluate the response of broilers to supplemental XP combination when using different feed formulas of low protein and low energy.

Materials and Methods

The procedures of this study were designed and conducted with the approval of Animal Production Research Institute (APRI) and the Agricultural Research Center (ARC), Egypt. Two experiments on broiler chicks were conducted in Fayoum research station, while the laboratory and editorial procedures were carried out in APRI, Cairo.

Birds and diets

Two experiments were conducted to study the effect of supplemental xylanase, protease or their combination to corn-soybean meal broiler diets on growth performance and carcass quality. These experiments were conducted at the same concurrent time and place with previous experiments that were conducted to evaluate single supplementation of xylanase or protease in broiler diets and published by Selim., *et al* [17,26]. The first experiment in the current study was designed to evaluate the effect of xylanase and protease in single supplementation or their combination to standard broiler diets, on the nutrient's requirements of chicks at all phases. The second experiment examined supplementing the combination of X and P in different formulae, which were lower than the nutrients requirements of broiler chicks. In both experiments, one-day old Arbor Acres unsexed broiler chicks provided from commercial hatchery were used. Chicks were housed in open system broiler house and fed *ad libtium* on diets based on corn-soybean meal during starting (1 - 10d), growing (11 - 24d) and finishing (25 - 40d) periods. Chicks were provided with free access to water and received the same lighting and veterinary care program during the experimental periods. The enzymes preparations used in this study were commercial products whereas, xylanase source (Econase XT25[*]) was supplemented at a level of 100 mg/kg diet to provide 16000 U xylanase/kg diets, and protease source (CIBENZA® DP100[†**]) was supplemented at a level of 300000 U/kg of the diet based on label or product recommendations.

Experiment 1 (EXP1): One hundred and sixty broiler chicks were randomly allocated (one way design) to four treatments; each had four replicates (n = 10). Chicks from the control group were fed corn-soybean meal standard diets (STD), and chicks from the other treatments received STD supplemented with xylanase (X), protease (P), or xylanase + protease (XP). The STD diets were formulated to be 3000 kcal of ME/kg and 23% CP, 3100 kcal of ME/kg and 21% CP and 3200 kcal of ME/kg and 19% CP during starting, growing and finishing periods, respectively (STD in table 1).

Experiment 2 (EXP2): Four hundred one-day old Arbor Acres unsexed broiler chicks provided from commercial hatchery were used. Chicks were randomly allocated (2 x 5 factorial design) in ten treatments, each had four replicates (n = 10). Chicks were fed on five formulae of corn-soybean meal different in energy and protein levels without or with supplementation of both XP at the same rate in EXP1. The five formulae were: F1, (STD); F2, contained 10% reduced CP and 100 kcal less ME from values of CP and ME of STD diet of each feeding phase (10 - 100); F3, contained 10 % less CP and 150 kcal ME less from values of CP and ME of STD diet of each feeding phase (10 - 150); F4, contained 15 % less CP and 100 kcal less ME from values of CP and ME of STD diet of each feeding phase (15 - 100); F5, contained 15 % less CP and 150 kcal less ME from values of CP and ME of STD diet of each feeding phase (15 - 150). Diets were formulated to contain the same total lysine/CP and total SAA (methionine + cystine)/CP percentages of STD for each feeding phase (Table 1).

Measurements

Parameters of growth performance for the two experiments including individual body weight (BW) and feed intake/pen (FI) were recorded for each feeding phase. BW gains (BWG), and feed conversion ratio (FCR) for each feeding phase and overall experimental period were calculated. At the end of each experiment, cost of feeding for each treatment was calculated according to prices of feed ingredients and feed additives in Egyptian market in Egyptian pound (LE).

[*]Econase XT25: Produced by AB Vista Feed ingredients, UK.

†CIBENZA® DP100: Produced by NOVUS International, Inc., USA.

	Starter Diets (1-10 d)					Grower diets (11-24 d)					Finisher diets (25-40 d)				
Diet Ingredients	**F1# STD**	**F2 (10-100)**	**F3 (10-150)**	**F4 (15-100)**	**F5 15-150)**	**F1 STD**	**F2 (10-100)**	**F3 (10-150)**	**F4 (15-100)**	**F5 (15-150)**	**F1 STD**	**F2 (10-100)**	**F3 (10-150)**	**F4 (15-100)**	**F5 (15-150)**
Yellow corn	55.07	59.66	60.32	63.61	62.71	60.62	66.03	65.30	65.56	66.76	64.71	68.63	69.52	67.58	68.77
Soybean meal (44%)	31.00	32.94	32.31	28.48	32.33	23.84	24.13	26.12	26.0	25.8	19.25	21.51	22.73	24.26	24.06
Corn gluten meal (62%)	7.50	2.12	2.59	3.05	0.40	8.57	4.50	3.21	1.7	1.7	8.16	3.30	2.34	0.0	0.0
Soybean oil	1.80	0.73	0.20	0.2	0.0	2.27	0.72	0.84	2.29	1.29	3.28	2.11	1.0	3.66	2.66
Di-Ca-P	2.0	1.96	1.97	1.91	1.91	1.75	1.84	1.82	1.75	1.75	1.55	1.75	1.73	1.71	1.71
Limestone	1.15	1.33	1.32	1.47	1.42	1.46	1.43	1.42	1.50	1.50	1.58	1.40	1.39	1.60	1.60
Min. &Vit. mix*	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
NaCl	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Sodium bicarbonate	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
DL-Methio-nine	0.24	0.23	0.23	0.19	0.24	0.19	0.18	0.19	0.18	0.18	0.17	0.16	0.21	0.19	0.19
L-lysine (HCl)	0.34	0.14	0.16	0.19	0.09	0.40	0.27	0.20	0.12	0.12	0.40	0.24	0.18	0.10	0.10
Total	100.0	100.0	100.0	100.0	100.0	100	100.0	100.0	100.0	100.0	100	100.0	100.0	100.0	100.0
Calculated analysis															
Crude protein %	23	20.70	20.70	19.55	19.55	21	18.9	18.9	17.85	17.85	19	17.1	17.1	16.15	16.15
ME kcal/kg	3000	2900	2850	2900	2850	3093	3000	2950	3000	2950	3200	3100	3050	3100	3050
Calcium %	1.00	1.00	1.02	1.01	1.02	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Available P %	0.52	0.51	0.51	0.50	0.50	0.47	0.47	0.47	0.47	0.47	0.45	0.45	0.45	0.45	0.45
Sodium %	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
L. Lysine %	1.38	1.25	1.25	1.17	0.1.17	1.24	1.11	1.11	1.05	1.05	1.06	1.02	1.02	0.96	0.96
Methionine %	0.67	0.61	0.61	0.59	0.58	0.60	0.62	0.61	0.51	0.51	0.51	0.44	0.44	0.46	0.46
Met + Cys (SAA) %**	1.05	0.95	0.95	0.90	0.90	0.95	0.86	0.86	0.81	0.81	0.81	0.77	0.77	0.74	0.74
Lys./CP	6.0	6.0	6.0	6.0	6.0	5.90	5.90	5.90	5.90	5.90	5.95	5.96	5.96	5.96	5.96
Met./CP	4.56	4.58	4.58	4.60	4.60	4.52	4.55	4.55	4.53	4.53	4.54	4.56	4.56	4.55	4.55
C:P ratio	130.4	140.9	137.6	148.3	145.7	147.2	166.6	156.0	168	165.2	168.4	181.2	178.3	191.9	188.8
Cost/ ton at Egyptian Local Price (LE)***	4235	3868	3839	3746	3695	4113	3759	3743	3750	3667	4032	3696	3602	3712	3629

Table 1: *Composition and calculated values of experimental diets.*

** Vitamins and minerals premix will provide each kg of diet with: vit. A, 11000 iu; vit. D_3, 5000 iu; vit. E, 50 mg; vit k_3, 3mg; vit. B_1, 2 mg; vit. B_2 6mg; b_6 3 mg; b_{12}, 14 mcg; nicotinicacid 60 mg; folic acid 1.75 mg, pantothenic acid 13 mg; and biotine 120 mcg; choline 600 mg; copper 16 mg; iron 40 mg; manganese 120 mg; zinc 100 mg; iodine 1.25 mg; and selenium 0.3 mg; ** SAA = methionine + cystine; ***cost of xylanase supplemented diets increased 21 le/ton feed of each formula and cost of protease supplemented diets increased 70 le/ton feed of each formula; # f= formula. Std = standard requirements.*

Slaughtering and Carcass measurements

For both EXP1 and EXP2, at 40 days of age, and right after completing the final weighing of birds and calculating the average BW/ pen, 1 bird per pen (4/treatment) was chosen for slaughtering. A total number of 16 birds/EXP1 and 40 birds/EXP2 slaughtered after 8h of feed withdrawal. After slaughtering, bleeding, and feather pecking, gut, heart, spleen and abdominal fat pad were removed and weighed. The whole carcasses weighed, washed with cold water and left for 1h then complete cutting was performed. Breast quarter and leg quarter from each carcass were separated, and then weights of breast meat and its skin, thigh and its skin, and drumstick and its skin were recorded. Samples of breast fillet, thigh and drumstick without skin placed in Ziploc bags, and kept for 24h at 4°C to complete the measurements of meat quality.

Meat quality

Samples of breast fillet and thigh were used to evaluate the effect of experimental treatments of both experiments on some physical and chemical parameters of broiler meat. After chilling samples at 4°C for 24h, samples were lightly blotted using filter paper before reweighing. Drip loss % was calculated as the percentage of the difference between weights before and after chilling for 24h and divided by the first weight as described by Saenmahayak., *et al.* [29]. Ultimate pH (pH_u) was measured using pH meter, provided by a temperature control system, by probe method. The minimum depth to adopt was 1 cm after incision of the muscles as described by Selim., *et al* [30]. Samples of thigh and drumstick deboned and mixed with breast fillet of the same bird were then stored at -20°C for 4 days before chemical measurements. Low density lipoprotein (LDL) and high-density lipoprotein (HDL), malondialdehyde (MDA) contents, and total protein (TP) were determined by colorimetric methods using analytical kits[‡].

Statistical analyses

The obtained data of EXP1 were statistically analyzed using SAS [31] one-way ANOVA (the general linear model's procedure) to determine the effect of experimental supplementation X, P or XP) to STD diet on growth performance and qualities of carcass and broiler meat. Regarding EXP2, data of two levels of XP supplementation (with and without) and five feed formulae were subjected to two-way analysis of variance to detect the effects of XP and feed formula on growth performance and qualities of carcass and broiler meat. For both EXP1 and EXP2 variables showing a significant F-test ($P \leq 0.05$) were compared to each other's using Duncan's Multiple Range Test [32].

Results and Discussion

The recorded and calculated results of growth performance parameters in EXP1 are summarized in table 2. The obtained results clearly showed significant effects of X and P supplementation in corn soybean meal broiler diets in single or combined status on overall FCR, while FI and BWG were not affected. The highest effect was detected during the starter and grower periods in both FI and FCR while all performance measurements did not change during finishing period. The overall results showed significant ($P \leq 0.05$) improvement in FCR due to adding X, P or XP and the best value recorded by using XP (1.55) and P (1.58). These results of XP supplementation combined with 6.18% significant ($P \leq 0.05$) increase of dressing % and 3.84% numerical increase of breast fillet (relative to live BW), and numerical

‡Produced by Biodiagnostic Co., Egypt.

reduction of edible parts, abdominal fat and skin of thigh and drumsticks (DS) compared with other treatments (Table 3). According to drip loss determined values, reductions were recorded in breast fillet samples by using XP (P ≤ 0.0747) and thigh samples by using X (P ≤ 0.0775) supplementation (Table 4). Furthermore, XP supplementation enhanced broiler meat quality through reduced concentrations of MDA and LDL significantly (P ≤ 0.05) compared with other treatments. Following the same trend, XP supplementation increased concentrations of both TP (P ≤ 0.0780) and HDL (P ≤ 0.0689) significantly compared with STD treatment.

Treatments	Starter Diets (1-10 d)			Grower diets (11-24 d)			Finisher diets (25-40 d)			Overall period (1-40d)			
	BWG (g)	FI (g)	FCR	BWG (g)	FI* (g)	FCR	BWG (g)	FI (g)	FCR	BW (g)	BWG (g)	FI (g)	FCR
STD	128.33	206[a]	1.61[a]	615.00	1030 [a]	1.69 [a]	1126.67	1951	1.74	1910.00	1870.00	3187	1.71 [a]
STD+X	120.67	177[c]	1.47[ab]	602.00	945 [ab]	1.57[ab]	1187.33	1983	1.67	1950.00	1910.00	3105	1.63 [b]
STD+P	132.33	191[b]	1.44 [b]	614.00	898 [b]	1.46 [b]	1210.33	2010	1.66	1996.67	1956.67	3099	1.58[bc]
STD+XP	125.00	173[c]	1.38 [b]	646.73	910 [b]	1.41 [b]	1188.27	1964	1.65	2000.00	1960.00	3047	1.55 [c]
Mean SE	±3.65	±3.55	±0.04	±30.75	±31.47	±0.05	±31.54	±45.92	±0.04	±40.16	±40.16	±57.47	±0.02
Probability	0.2179	0.0007	0.0308	0.7655	0.0655	0.0154	0.3402	0.8174	0.4884	0.3928	0.3928	0.4340	0.0042

Table 2: *Effect of xylanase and protease enzymes supplementation on growth performance of broiler chickens during period of 1-40 days of age.*

a, b= Means in the same column with different superscripts, differ significantly (P≤ 0.05).

** Duncan test showed different superscripts at P value 0.0655.*

STD= Standard diet; X = Xylanase enzyme; P=protease enzyme; XP = Xylanase+ protease enzymes.

Treatment	Live weight	Carcass			Deboned Breast quarter		Leg quarter				
		Dressing %	Edible Parts	Abdominal fat	Breast Fillet	Skin	Total	Thigh		Drums stick (DS)	
								Weight	Skin	weight	Skin
STD	1893.33	69.85 [b]	5.61	1.11	13.55	1.00	12.68	7.52	0.63	5.16	0.54[a]
STD+X	1956.67	72.32[ab]	5.30	1.25	12.52	0.80	12.62	7.54	0.55	5.09	0.54[a]
STD+P	1983.33	70.85[b]	5.93	1.21	12.43	0.70	12.34	7.15	0.51	5.19	0.54[a]
STD+XP	2006.67	74.17[a]	4.98	0.93	14.07	0.72	12.70	7.59	0.53	5.11	0.37[b]
Mean SE	±36.01	±0.86	±0.28	±0.21	±0.83	±0.16	±0.40	±0.44	±0.09	±0.17	±0.05
Probability	0.2172	0.0348	0.1745	0.7207	0.4710	0.5673	0.90	0.8873	0.8078	0.9735	0.0912

Table 3: *Effect of xylanase and protease enzymes supplementation on carcass characteristics (% from life body weight) of broiler chickens at 40 days of age.*

a, b= Means in the same column with different superscripts, differ significantly (P ≤ 0.05).

STD= Standard diet, X = Xylanase enzyme, P=protease enzyme, and XP = Xylanase+ protease enzymes.

Treatment	Breast Fillet		Thigh		MDA (nmol/100g meat)	TP (mg/100g meat)	LDL (mg/100g meat)	HDL (mg/100g meat)
	Drip Loss (%)	pH_u	Drip Loss (%)	pH_u				
STD	4.22[a]	6.09	4.56[ab]	6.32	459.86[ab]	46.54[b]	1382.86[a]	666.98[ab]
STD+X	2.64[ab]	6.86	2.79[b]	6.34	471.13[a]	53.60[ab]	1304.29[a]	638.17[b]
STD+P	2.85[ab]	6.42	5.29[a]	6.46	487.00[a]	52.06[ab]	1327.86[a]	651.27[ab]
STD+XP	1.78[b]	6.26	3.38[ab]	6.44	419.51[b]	55.59[a]	1178.57[b]	685.32[a]
Mean SE	±0.55	±0.58	±0.62	±0.13	±14.53	±2.13	±36.08	±10.85
Probability	0.0747	0.8078	0.0775	0.8124	0.0540	0.0780	0.0216	0.0689

Table 4: *Effect of xylanase and protease enzymes supplementation to broiler diets on quality of broiler meat.*

a, b= Means in the same column with different superscripts, differ significantly (P≤ 0.05).

STD= Standard diet; X = Xylanase enzyme; P=protease enzyme; XP = Xylanase+ protease enzymes; MDA= Malondialdehyde;

TP= Total protein; LDL= Low density lipoprotein; HDL= High density lipoprotein.

These results confirmed those reported by previous investigators wherein growth performance of broiler chicks fed corn soybean meal diets was improved by xylanase supplementation [7,16,17], protease supplementation [9,21,26,33].

Similar to our obtained results, some reports showed improved FCR of broilers fed xylanase supplemented diets without change of consumed feed [8] and were explained by improved feed efficiency [34] through the ability of xylanase to hydrolyze non starch polysaccharides (NSP) compounds and increased the available energy for growth [12]. Selim., *et al.* [17] showed improved overall FCR, BW and BWG accompanied with no significant effect on FI of broiler chicks fed on corn soybean meal diets supplemented with xylanase enzyme. Freitas., *et al.* [21] reported improved FCR when using supplemental protease to broiler diets because of decreased FI, while Ghazi., *et al.* [33] reported the same trend of FCR through enhancing digestibility of nitrogen and improving true ME with no effect of FI during the experiment.

In addition, some authors studied the effect of combination of supplemental X and P in broiler diets and reported positive effects on growth performance [27,35,36]. Olukosi., *et al.* [24] compared the effect of protease supplementation at two levels 5000 and 10,000 PU/kg diet as single additive, and a combination containing X, amylase (A), and P (XAP) on digestibility of protein and NSP from corn-soybean meal based diets fed to broiler chickens. They applied fixed ratio 10:1:25 of XAP in the mixture. The results showed better effect of XAP than P alone on the utilization of protein and NSP components and that improvement decreased with increasing age of chicks. These results supported our finding, whereas the recorded significant reduction of FI and improvement of FCR by X, P or XP supplementation disappeared at the finisher phase of feeding. In contrary, Kalmendal and Tauson [27] compared the effect of X, P, and XP in broiler diets and reported improvement of feed utilization with X and P, but their combination was not superior to when supplied separately. The current recorded results confirmed those reported in previous study [26] as protease supplementation could not enhance dressing %. While results of Selim., *et al.* [17] showed significant increase in dressing % by adding X to corn soybean meal broiler diets. The superior effect of XP supplementation on dressing % might be due to better utilization of both energy [37] and protein [33].

Based on these results the EXP2 was designed as an attempt to increase benefits of using XP supplementation by examining the supplementation in different low-cost feed formulae.

The obtained results of EXP2 are presented in table 5-9. Results of growth performance measurements (Table 5) showed significant improvement in growth measurements during all feeding phases due to XP supplementation as a main effect. Values of FCR (1.64, 1.72, 1.82, and 1.77) improved by 12.7, 14.4, 12.9 and 13.2 % during starter, grower, finisher and overall period by adding XP enzymes to different formulae broiler diets. These improvements in FCR were combined with both increased BWG and decreased FI in all feeding phases. In addition, the main effect of feed formula caused significant differences in growth performance values. Generally, results of using STD formula (F1) gained the best performance followed by 10 - 100 formula (F2). As nutrients contents of the diets were reduced, a clear corresponding reduction of BW, BWG and increase in FI values were reported when using formulae from F1 to F5. Values of overall FCR for different feed formulae as a main effect (1.73, 1.92, 2.0, and 2.23) were depressed by 5.5, 17.1, 21.9, and 36% for F2, F3, F4 and F5 as compared with F1 (STD) value (1.64), respectively. Interactions between XP supplementation and feed formulas showed a significant effect on growth performance. The recorded values of all experimental treatments (Table 6) showed on the one hand better performance of birds on treatments XPSTD, STD and XP10 - 100 than others, while an accepted BW of treatments XP10 - 150 and XP15 - 100 accompanied with higher FI and FCR values. On the other hand, chicks that received 15 - 150 formula (F5) showed the worst growth performance during all experimental periods. Supplementation of XP to F5 (15 - 150) could not compensate all the lost BW due to the great reduction of CP and ME for this formula which might have caused an imbalance in nutrients concentration.

	Starter Diets (1-10 d)			Grower diets (11-24 d)			Finisher diets (25-40 d)			Overall period (1-40d)			
	BWG (g)	FI (g)	FCR	BWG (g)	FI (g)	FCR	BWG (g)	FI (g)	FCR	BW (g)	BWG (g)	FI (g)	FCR
Main Effect													
XP Supplementation (16000 Xylanase U +300 000 Protease U/kg diet)													
Without	122.53^{b}	229^{a}	1.88^{a}	561.10^{b}	1109^{a}	2.01^{a}	1032.06^{b}	2124^{a}	2.09^{a}	1755.68^{b}	1715.68^{b}	3461^{a}	2.04^{a}
With	133.03^{a}	217^{b}	1.64^{b}	600.62^{a}	1026^{b}	1.72^{b}	1131.30^{a}	2046^{b}	1.82^{b}	1904.95^{a}	1864.95^{a}	3289^{b}	1.77^{b}
Mean of SE	±1.51	±2.32	±0.03	±7.21	±10.31	±0.02	±10.50	±11.85	±0.01	±10.14	±10.14	±13.60	±0.01
Feed Formula (F)													
F1 (STD)	133.37^{a}	199^{d}	1.50^{d}	617.38^{a}	973^{d}	1.58^{d}	1189.25^{a}	2013^{b}	1.69^{e}	1980.00^{a}	1940.00^{a}	3185^{c}	1.64^{e}
F2 (10-100)	129.50^{ab}	212^{c}	1.65^{c}	618.38^{a}	1049^{c}	1.70^{c}	1135.39^{b}	1993^{b}	1.76^{d}	1923.26^{b}	1883.26^{b}	3255^{b}	1.73^{d}
F3 (10-150)	127.86^{ab}	227^{b}	1.78^{bc}	586.79^{a}	1104^{ab}	1.89^{b}	1092.50^{bc}	2120^{a}	1.96^{c}	1847.14^{c}	1807.14^{c}	3451^{a}	1.92^{c}
F4 (15-100)	125.00^{b}	227^{b}	1.82^{b}	583.58^{a}	1135^{a}	1.96^{b}	1046.68^{c}	2124^{a}	2.05^{b}	1795.26^{d}	1755.26^{d}	3486^{a}	2.00^{b}
F5(15-150)	123.83^{b}	247^{a}	2.01^{a}	501.38^{b}	1075^{bc}	2.17^{a}	952.15^{d}	2174^{a}	2.30^{a}	1617.36^{e}	1577.36^{e}	3497^{a}	2.23^{a}
Mean of SE	±2.36	±3.60	±0.05	±11.22	±16.05	±0.04	±16.41	±18.44	±0.02	±15.78	±15.78	±21.15	±0.02
Probability													
XP	0.0001	0.0011	0.0001	0.0005	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Formula	0.0572	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
XP * Formula	0.0020	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Mean of SE	±3.34	±5.10	±0.07	±15.86	±22.69	±0.05	±23.21	±26.08	±0.03	±23.31	±22.31	±29.92	±0.02

Table 5: *Effect of adding both xylanase + protease to different feed formula on growth performance of broiler chickens during period of 1 - 40 days of age.*

a, b= Means in the same column with different superscripts, differ significantly (P ≤ 0.05).

XP = Xylanase+ protease enzymes. F1 (STD)= Standard diet; F2 (10-100) = lower 10% from CP of STD and lower 100 kcal from STD.

F3 (10-150) = lower 10% from CP of STD and lower 150 kcal from STD; F4 (15-100) = lower 15% from CP of STD and lower 1 00 kcal from STD F5 (15-150) = lower 15% from CP of STD and lower 150 kcal from STD.

	Starter Diets (1-10 d)			Grower diets (11-24 d)			Finisher diets (25-40 d)			Overall period (1-40d)			
Treat-ment	BWG (g)	FI (g)	FCR	BWG (g)	FI (g)	FCR	BWG (g)	FI (g)	FCR	BW (g)	BWG (g)	FI (g)	FCR
1 STD	130.50^{abc}	209^{ed}	1.60^{def}	619.25^{a}	1045.75^{cd}	1.69^{ef}	1185.25^{ab}	2035.50^{de}	1.72^{e}	1975.00^{a}	1935.00^{a}	3290.25^{e}	1.70^{f}
2 XPSTD	136.25^{a}	190^{f}	1.40^{f}	615.50^{ab}	900.25^{e}	1.47^{g}	1193.25^{a}	1990.50^{ef}	1.67^{e}	1985.00^{a}	1945.00^{a}	3080.78^{f}	1.58^{g}
3 10-100	124.75^{bcd}	220.00^{cde}	1.77^{bcd}	608.75^{ab}	1088.89^{bc}	1.79^{de}	1114.28^{b}	2041.17^{de}	1.83^{d}	1887.78^{b}	1847.78^{b}	3350.06^{de}	1.81^{e}
4 XP10-100	134.25^{ab}	205.00^{ef}	1.53^{ef}	628.00^{a}	1010.00^{d}	1.61^{fg}	1156.50^{ab}	1945.25^{f}	1.68^{e}	1958.75^{ab}	1918.75^{ab}	3160.25^{f}	1.65^{fg}
5 10-150	121.00^{cd}	230.00^{bc}	1.90^{bc}	566.50^{bcd}	1150.00^{ab}	2.04^{bc}	982.50^{c}	2100.00^{bcd}	2.14^{c}	1710.00^{c}	1670.00^{c}	3480.00^{bc}	2.09^{c}
6 XP10-150	133.00^{ab}	225.00^{bcd}	1.70^{cde}	602.00^{abc}	1070.00^{cd}	1.78^{de}	1175.00^{ab}	2135.00^{bc}	1.82^{d}	1950.00^{ab}	1910.00^{ab}	3430.00^{cd}	1.80^{e}
7 15-100	118.33^{d}	229.75^{bc}	1.94^{b}	553.34^{cd}	1170.25^{a}	2.13^{b}	960.33^{cd}	2150.50^{b}	2.24^{b}	1672.00^{c}	1632.00^{c}	3550.50^{b}	2.18^{b}
8 XP15-100	131.67^{abc}	224.00^{bcd}	1.71^{cde}	613.83^{ab}	1100.00^{abc}	1.79^{de}	1133.02^{ab}	2096.94^{bcd}	1.85^{d}	1918.52^{ab}	1878.52^{ab}	3420.94^{cd}	1.82^{e}
9 15-150	117.67^{d}	255.00^{a}	2.17^{a}	459.00^{e}	1100.00^{abc}	2.40^{a}	905.56^{d}	2286.50^{a}	2.52^{a}	1522.22^{d}	1482.22^{d}	3641.50^{a}	2.45^{a}
10 XP15-150	130.00^{abc}	240.00^{b}	1.85^{bc}	543.75^{d}	1050.00^{cd}	1.93^{cd}	998.75^{c}	2062.00^{cde}	2.07^{c}	1712.50^{c}	1672.50^{c}	3352.00^{de}	2.00^{d}
Mean of SE	±3.34	±5.10	±0.07	±15.86	±22.69	±0.05	±23.21	±26.08	±0.03	±23.31	±22.31	±29.92	±0.02
Probability	0.0020	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Table 6: *Effect of experimental treatments of EXP2 on growth performance of broiler chicks during period of 1-40 days of age.*

a, b = Means in the same column with different superscripts, differ significantly (P≤ 0.05)

XP = Xylanase+ protease enzymes. STD= Standard diet; 10-100 = lower 10% from CP of STD and lower 100 kcal from STD

10-150 = lower 10% from CP of STD and lower 150 kcal from STD; 15-100 = lower 15% from CP of STD and lower 100 kcal from STD

15-150 = lower 15% from CP of STD and lower 150 kcal from STD.

These results are in agreement with those reported previously [17,26] where decreasing dietary ME of broiler diets more than 100 kcal/kg or decreasing dietary protein more than 10% from the requirement of each feeding phase resulted in depression of growth performance that could not be compensated even by applying X or P supplementation, respectively. Following the same trend of the current results, Kamran., *et al.* [38] evaluated the effect of feeding broilers on corn soybean diets at decreased levels of CP and ME during the three phases of feeding to same constant calorie: protein (C:P) ratio. They recorded significant decrease in BWG and increased FI and poorer FCR with the reduction of CP and ME for grower and finisher phases. Although FI was increased by decreasing CP and ME of diet, chicks that received low levels of CP and ME (20, 19 and 17 CP, and 2640, 2717, and 2635 kcal ME for starter, grower and finisher phases, respectively) could not compensate for normal growth (had lighter BW) and this might be due to deficiency of one or more essential amino acids. Amerah., *et al.* [18] examined the effect of X, A and P in combination on growth performance of broilers fed low ME and CP corn/soybean meal-based diets. Their results showed superior effect of XAP in improving FCR compared to single enzyme supplementation, and suggested a synergistic effect between X, A and P on broiler performance and nutrient digestibility. In addition, results of Olukosi., *et al.* [24] supported the synergistic effect between X, A and P on broiler performance and nutrient digestibility. The improvement of growth performance of chicks in XP10 - 100 group indicated that chicks could compensate for reduced 10% of CP and 100kcal ME in these diets by the synergistic effect between X and P on increasing nutrient utilization of feed.

Results of carcass traits (Table 7) measurements showed significant (P ≤ 0.05) increases in dressing % due to XP supplementation as a main effect, while all carcass parts were not affected significantly (P > 0.05). A numerical increase (3.8%) in breast fillet % from final live BW was obtained at P value of 0.07 by using XP supplementation. Applying different feed formulas from F1 to F5 did not change carcass traits significantly (P > 0.05). In addition, the interaction between XP and feed formula was not effective (P > 0.05). Results of XP10 - 100 treatment showed the highest numerical values of dressing %, breast fillet, and leg quarter % (72.92%, 13.05%, and 13.44%, respectively). Compared with STD records (71.02, 12.27, and 12.41%, respectively), applying XP10 - 100 treatment could numerically increase % of dressing %, breast fillet, and leg quarter by 6.36, 2.67, and 8.30 %, respectively (Figures 1 and 2).

Treatment	Live weight (g)	Carcass			Brest quarter		Leg quarter				
		Dressing %	Edible Parts	Abdominal fat	Breast fillet	Skin	Total weight	Thigh		Drumstick (DS)	
								Weight	Skin	Weight	Skin
Main effect											
XP Supplementation (16000 Xylanase U +300 000 Protease U/kg diet)											
Without	1768.00^{b}	69.24^{b}	5.78	1.12	12.29	0.70	12.64	7.36	0.49	5.27	0.49
With	1917.00^{a}	71.88^{a}	5.40	1.06	12.99	0.83	12.98	7.74	0.52	5.24	0.49
Mean of SE	±48.01	±0.57	±0.17	±0.08	±0.26	±0.05	±0.22	±0.20	±0.04	±0.14	±0.04
Feed Formula											
F1 (STD)	1977.50^{a}	71.94	5.43	1.20	12.47	0.83	12.39	7.23	0.45	5.16	0.51
F2 (10-100)	1928.30^{a}	71.13	5.25	1.20	12.44	0.80	13.18	8.15	0.60	5.02	0.50
F3 (10-150)	1834.20^{ab}	70.06	5.54	1.01	12.55	0.69	12.44	7.08	0.44	5.35	0.45
F4 (15-100)	1793.30^{ab}	70.53	5.64	0.92	12.95	0.67	13.16	7.88	0.50	5.27	0.50
F5(15-150)	1679.20^{b}	69.13	6.09	0.13	12.79	0.80	12.89	7.40	0.53	5.48	0.49
Mean of SE	±75.92	±0.90	±0.27	±0.13	±0.42	±0.08	±0.34	±0.32	±0.06	±0.22	±0.06
Probability											
XP	0.0402	0.0038	0.1287	0.5989	0.0720	0.0775	0.2786	0.2002	0.5779	0.8563	0.9695
Formula	0.0866	0.2739	0.2775	0.4832	0.8901	0.5169	0.3298	0.1345	0.3124	0.6507	0.9709
XP * Formula	0.1439	0.1178	0.1814	0.4592	0.6983	0.3344	0.6269	0.2547	0.1921	0.7445	0.9359
Mean of SE	±107.36	±1.28	±0.38	±0.18	±0.59	±0.11	±0.49	±0.45	±0.08	±0.32	±0.08

Table 7: *Effect of adding both xylanase + protease to different feed formula on carcass characteristics as % from life body weight of broiler chickens during period of 1-40 days of age.*

a, b = Means in the same column with different superscripts, differ significantly (P ≤ 0.05).

XP = Xylanase+ protease enzymes. F1 (STD)= Standard diet; F2 (10-100) = lower 10% from CP of STD and lower 100 kcal from STD. F3 (10-150) = lower 10% from CP of STD and lower 150 kcal from STD; F4 (15-100) = lower 15% from CP of STD and lower 100 kcal from STD F5 (15-150) = lower 15% from CP of STD and lower 150 kcal from STD.

Results of broiler meat quality measurements are summarized in table 8. There were significant increases in thigh pH_u and meat HDL content and significant reductions in meat LDL concentration as a main effect of XP supplementation. On the one hand, and among five feed formulas, drip loss percentage of both breast fillet and thigh, TP and HDL values increased significantly (P ≤ 0.05) by applying any of the examined formulas compared with STD values. On the other hand, concentrations of MDA and LDL showed greater significant (P ≤ 0.05) reduction by applying any of the examined formulas compared with STD values. The interaction XP*Feed formula was significantly effective on chemical measurements of broiler meat while drip loss and pH_u of both breast fillet and thigh were not affected due to that interactions.

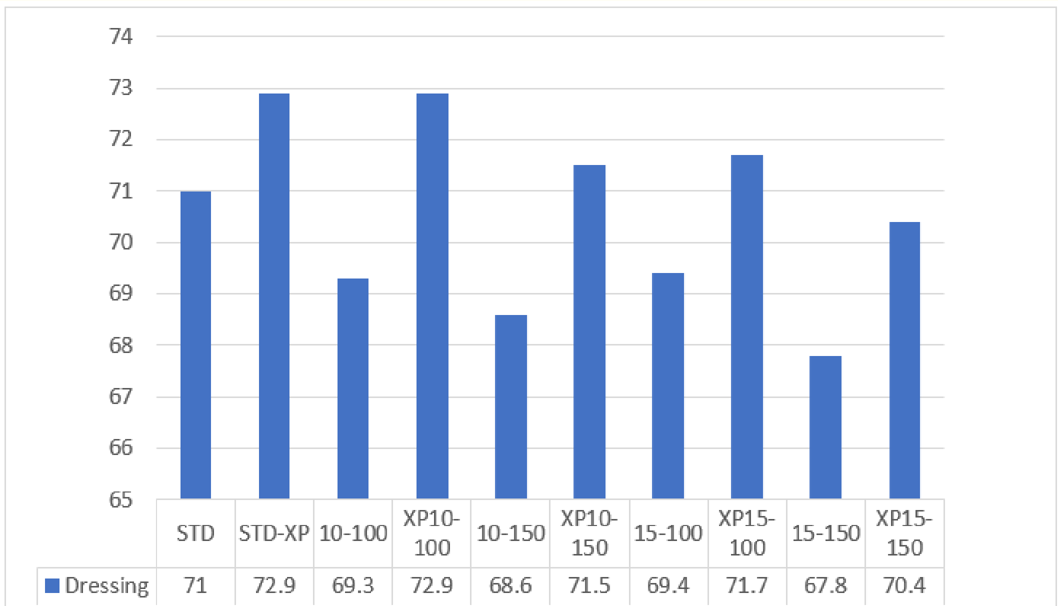

Figure 1: *Dressing %.*

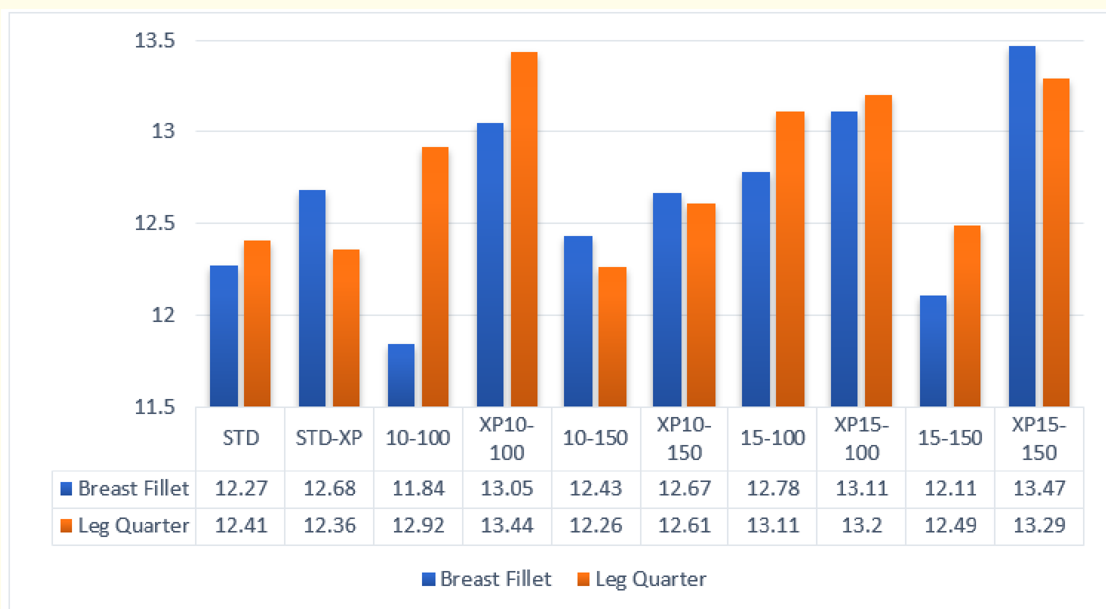

Figure 2: *Carcass Cuts.*

	Breast		Thigh		MDA (nmol/100g meat)	TP (mg/100g eat)	LDL (mg/100g eat)	HDL (mg/100g meat)
	Drip Loss (%)	pH_u	Drip Loss (%)	pH_u				
Main effect								
XP Supplementation (16000 Xylanase U + 300 000 Protease U/kg diet)								
Without	3.51	6.06	4.08	6.32^{b}	267.89	72.96	987.90^{a}	701.10^{b}
With	3.89	5.97	3.98	6.44^{a}	259.88	72.02	886.29^{b}	739.56^{a}
Mean of SE	±0.29	±0.18	±0.25	±0.03	±10.09	±1.10	±22.58	±11.41
Feed Formula								
F1 (STD)	2.40^{b}	6.49	2.69^{b}	6.34	452.51^{a}	53.48^{b}	1285.95^{a}	671.06^{c}
F2 (10-100)	3.68^{ab}	6.12	4.91^{a}	6.31	264.08^{b}	78.65^{a}	864.29^{b}	741.28^{ab}
F3 (10-150)	3.96^{a}	5.65	4.58^{a}	6.42	211.50^{c}	77.41^{a}	835.48^{b}	727.53^{ab}
F4 (15-100)	4.43^{a}	6.07	4.28^{a}	6.40	208.45^{c}	74.98^{a}	882.62^{b}	769.08^{a}
F5(15-150)	4.01^{a}	5.76	3.69^{ab}	6.42	182.87^{c}	77.94^{a}	817.14^{b}	692.69^{bc}
Mean of SE	±0.45	±0.29	±0.40	±0.05	±15.95	±1.73	±35.70	±18.04
Probability								
XP	0.3603	0.7149	0.7710	0.0176	0.5806	0.5510	0.0047	0.0272
Formula	0.0488	0.2978	0.0080	0.4297	0.0001	0.0001	0.0001	0.0081
XP * Formula	0.2116	0.5877	0.0781	0.2955	0.0001	0.0001	0.0001	0.0108
Mean of SE	±0.64	±0.41	±0.57	±0.08	±22.55	±2.45	±50.49	±25.51

Table 8: *Effect of adding both xylanase + protease to different feed formula on quality of broiler meat.*

a, b= Means in the same column with different superscripts, differ significantly (P≤ 0.05)

MDA= Malondialdehyde; TP= Total protein; LDL= Low density lipoprotein; HDL= High density lipoprotein.

XP = Xylanase+ protease enzymes. F1 (STD)= Standard diet; F2 (10-100) = lower 10% from CP of STD and lower 100 kcal from STD. F3 (10-150) = lower 10% from CP of STD and lower 150 kcal from STD; F4 (15-100) = lower 15% from CP of STD and lower 100 kcal from STD F5 (15-150) = lower 15% from CP of STD and lower 150 kcal from STD.

The current results showed significant (P ≤ 0.05) increases in dressing % due to XP supplementation while the rest of carcass traits were not affected. This finding is in agreement with those of [17] wherein single supplementation of broiler diets with X enzyme increased dressing % significantly. However in the report from [17], adding P enzyme to broiler diets with varying decreased levels of protein did not change dressing and carcass traits significantly. The significant increase in dressing % and numerically increased breast fillet % by XP supplementation in this study supports the effect of XP on increasing protein and energy utilization reported by [33,37]. Regarding feed formula, the current results parallel the same trend of those reported by Hidalgo., *et al.* [39] and Kamran., *et al.* [38] who showed no significant effect of using low CP and low ME broiler diets with constant C:P ratio on yield of carcass, breast, thigh and abdominal fat. They suggested that carcass traits were not affected by decreased CP and ME values because of adequate levels of lysine (Lys) and methionine (Met), which are exclusively used for protein accretion in the body [40,41]. The formulated diets in our study contained supplemental Lys and Met to provide chicks with the same Lys/CP and Met/CP ratio in STD diets of each feeding phase. These constant ratios of Lys/CP and Met/CP might have resulted in significant equal dressing % when feeding different feed formulae (F1 to F5).

The significant reduction of MDA and the increase of TP concentrations in broiler meat samples by using formulae with low CP and ME values are in agreement with previous reports when X and P were supplemented as single additives to low CP or ME diets, respectively [17,26]. However, some reports showed no significant effect adding carbohydrate enzymes preparations [42,43] or protease enzyme [19,26] to broiler diets on physical or chemical quality of broiler meat. Furthermore, Rada., *et al.* [44] examined the effect of adding mono-component serine protease expressed in *Bacillus licheniformis* (75000 Prot/g to save 15000 Prot/g feed) to low protein (16.7%) corn-soybean broiler diets during the period from 10 to 35 days of age and did not record any significant differences in carcass weight and carcass yield % between treatments.

Based on prices of feed ingredients in Egyptian market at the time of starting the experiment, table 9 shows the effect of experimental treatments on cost of feed ingredients to produce 1 kg of live BW and the relative change in the cost compared with STD (as an unsupplemented control diet) value. The calculated cost/kg of live BW showed the lowest cost for XP10 - 100 treatment (6.16 LE/kg) and highest cost value for 15 - 150 treatment (8.72 LE/kg). Compared with feed ingredients cost/kg live BW of STD group, the obtained values for XPSTD, 10 - 100, XP10 - 100 and XP10 - 150 were improved (lower cost than STD value) by 4.80, 2.4, 9.09, and 2.62 %, respectively. However, values of 10 - 150, 15 - 100, XP15 - 100, 15 - 150, and XP15 - 150 showed an increased cost/kg live BW relative to STD calculated value by 13.33, 16.70, 0.42, 28.63, and 7.92%, respectively. In addition, XP supplementation showed an ability to reduce cost and improve the benefits of production, but that ability did not present lower cost than STD value when feed formula was 15 - 100 or 15 - 150. These results indicate that broiler producers have the opportunity to reduce the cost of feed ingredients/kg live BW by 9.1% compared with the current traditional status (unsupplemented control diet) by adding both xylanase and protease to corn soybean meal diets at 10% lower protein and 100 kcal/Kg diet lower ME from the requirement of each feeding phase. If feed costs are 70% of total production cost (according to prices of Egyptian market), our reported results means that the total production cost of kg live BW can be reduced more than 6% by applying the recommended supplementation.

Treatment	Final BW (g)	Total FCR	Feeding cost/bird (L.E)	Feeding cost/kg of BW (L.E)	Relative improvement
STD	1975	1.7	13.39	6.78	
STD-XP	1985	1.58	12.81	6.45	4.80
10-100	1887	1.81	12.48	6.62	2.40
XP10-100	1958	1.65	12.07	6.16	9.09
10-150	1710	2.09	13.14	7.68	-13.33
XP10-150	1950	1.8	12.87	6.60	2.62
15-100	1672	2.18	13.23	7.91	-16.70
XP15-100	1918	1.82	13.06	6.81	-0.42
15-150	1522	2.45	13.27	8.72	-28.63
XP15-150	1712	2	12.52	7.32	-7.92

Table 9: *The effect of xylanase and protease enzymes supplementation on cost of feeding.*

STD= Standard diet XP = Xylanase+ protease enzymes. LE= Egyptian pound.

**Relative improvement = 100 x (Feeding cost/kg of BW of each treatment - Feeding cost/kg of BW of STD group)/ Feeding cost/kg of BW of STD group).*

Conclusion

According to the overall results of this study, growth performance, quality of carcass and meat, and cost of feeding could be enhanced by using XP supplementation to standard or lower 10% CP and 100 kcal ME broiler corn/soybean meal diets.

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