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SEVALUATION OF USING PROTEASE ENZYME AT DIFFERENT LEVELS OF PROTEIN IN CORN-SOYBEAN MEAL BROILER DIETS.

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ABSTRACT: The aim of this study was to evaluate the effect of adding protease enzyme to low protein corn-soybean meal broiler diets to reduce feeding cost and improve the quality of meat. Two hundred and forty 1-d old unsexed Arbor Acres broiler chicks were distributed to six treatments (2*3 factorial design) of 4 replicates each. Two levels of protease activity (without and with 300 000 U/kg of the diet), and three levels of dietary crude protein were examined (Standard, STD; less 10% or less 15% from STD protein recommendation of each feeding phase, L10 and L15, respectively). The dietary protein levels were 23, 20.7and 19.5% at the starter phase; 21, 18.9 and 17.8% at the grower phase; and 19, 17.1 and 16.1% at the finisher phase. Diets were formulated to save the strain requirements of all other nutrients. All chicks were housed in open system broiler house and received the same managerial conditions and veterinary program during experimental period (1-40 d of age). Parameters of growth performance, carcass characteristics, physical and chemical evaluation of broiler meat were carried out and feeding cost was calculated. The recorded results showed that using protease improved final body weight (4.1%) and feed conversion ratio (6.1%) and reduced skin% of drum stick significantly ($P<0.05$). Decreasing protein of diets to (L15) increased consumed feed, drip loss of thigh, and back quarter weight (thigh and drumstick). Also, skin and pH of breast and concentrations of low density lipoprotein and malondialdhyde (MDA) in broiler meat were depressed significantly ($p<0.0001$). Among all treatments results showed that adding protease to L10 diets recorded the same marketing weight (2015 g), made broiler meat healthier (MDA decreased by 36%), and depressed the feeding cost/kg of life body weight by 3.94% relative to value calculated for STD group. Generally it could be concluded that adding 300,000 U protease enzyme/kg to low protein broiler diets could help producers to achieve acceptable marketing weight, get better meat quality, and reduce feeding cost according to Egyptian prices of feed ingredients.

Key words: Broiler, protease, protein level, performance and meat quality.

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INTRODUCTION

Protein is very important and expensive component in broiler diets (**Kamran *et al*., 2004**). Therefore, reducing dietary crude protein without deleterious effects on broiler performance is a great challenge for broiler nutritionist. Not only reduced protein regimes diets in poultry nutrition is considered an alternative application to reduce feeding costs, but also to reduce the environmental pollution **(Kobayashi *et al*., 2013**).

The gastrointestinal tract syntheses different endogenous proteases enzymes and releases them in amounts that are accounted to be sufficient to optimize feed protein utilization (**Le Heurou-Luron *et al.*, 1993; Nir *et al.*, 1993).** However, regular increasing growth rate of broiler chicks in the last 20 years may demand an external support for higher protein utilization.

In the last decades, many poultry nutritions investigated the effect of using exogenous enzymes, either in single or mixture enzyme products, on feed utilization and most of them recorded beneficial effects as keeping the gut healthy, or improving the utilization of nutrients **(Campbell and Bedford, 1992; Seskevicience *et al*., 1999; and Leeson and Summers, 2005; Selim *et al*., 2010 and Ferket, 2011).** Many commercial products of enzyme mixtures were available during last decade, while single protease enzyme products appeared in poultry nutrition field only few years ago.

In recent years, proteases have grown in profile, there are currently several stand-alone proteases available, and new mechanisms of action have been proposed (**Adeola and Cowieson, 2011**). Some researchers could improve crude protein digestibility **(Angel *et al*., 2011; and Freitas *et al*., 2011)** or reduced nitrogen excretion, a major pollutant worldwide, **(Doskovic' et al., 2013; and Leinonen and Williams, 2015)** by using poultry diets supplemented with protease enzyme. On the other side the recent report of **Yuan *et al*., (2015)** showed negative effect of supplemental protease enzyme at level from 160 mg/kg diet (contained 8000 U protease activity /kg diet) on synthesis and excretion of pancreatic trypsin.

The relationship between dietary protein and carcass characteristics have been studied by many researchers. Some of them concluded the response of carcass yield to dietary crude protein (**Rezaei *et al*., 2004; and Sterling, *et al*., 2006)**, while other researchers reported an effect of dietary protein on either chemical or physical characteristics of chicken meat (**Furlan *et al*., 2004; Berri, *et al*., 2008; and Yalcin *et al*., 2010)**. On the other side, some researchers failed to detect any significant difference in quantitative or qualitative broiler carcass characteristics as a response of varying dietary protein level or amino acids profile (**Jamroz *et al.*, 1981; Jamroz *et al*., 1984; Daszkiewicz *et al*., 1998; Gardzielewska *et al*., 2005; and Guardia, *et al*., 2014**). As a result of development of meat processing, meat products, and consumer needs, researchers in poultry nutrition are interested in studying the relationship between poultry feeding and the quality of produced meat and its processing ability (**Berri, *et al*., 2008; and Guardia, *et al*., 2014).**

This study was aimed to evaluate the effect of adding protease enzyme to low protein corn-soybean meal broiler diets to reduce feeding cost and improve the quality of meat. The evaluation included broiler performance, carcass characteristics, physical and chemical parameters of broiler meat and comparison of the feeding cost of experimental treatments.

MATERIALS AND METHODS

Experimental diets and birds:

Two hundred and forty 1-d old unsexed Arbor Acres broiler chicks were distributed to six treatments (2*3 factorial design) of 4 replicates each. Two levels of protease activity (without and with 300 000 U/kg of diet; CIBENZA® DP100), and three levels of dietary crude protein were examined (Standard, STD; less 10% or less 15% of STD protein recommendation of each feeding phase, L10 and L15; respectively). The dietary crude protein (CP) levels were 23, 20.7and 19.5% at the starter phase; 21, 18.9 and 17.8% at the grower phase; and 19, 17.1 and 16.1% at the finisher phase. Both L10 and L15 diets were formulated to contain the same total lysine/CP and total SAA (methionine + cystine)/CP percentages of STD of certain feeding phase. The examined protease product (CIBENZA® DP100) is produced throw fermentation of *Bacillus licheniformis* and defined as a heat stable enzyme by the producer company. Diets were formulated to provide the strain requirements of the rest nutrients. Composition and calculated analysis of experimental diets are shown in Table (1) and the experiment design is presented in Table (2). Birds were fed *ad libitum* during feeding phases which were starter (1-10 d of age), grower (11-24 d of age) and finisher (25-40 d of age). All chicks were housed in open system broiler house and received the same managerial conditions and veterinary program during the experimental period (1-40 d of age). Parameters of growth performance including body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were recorded and feeding cost was calculated during the experimental period.

Slaughter and carcass characteristics:

Four birds around the average live body weight of each treatment were slaughtered at the end of the experiment (40 days of age), then carcass characteristics including weights of carcass, abdominal fat, giblets (liver, gizzard and heart), as percentages of live body weight were recorded. Weights of breast muscles, drumstick and thighs of samples were recorded as percentage of carcass weight. Then skin of every part was removed, weighed and calculated as percentage of total weight of the same part. After that, all breast fillets, drumstick and thigh samples without skin (24 samples of each cut) were weighed, placed in Ziploc bags, and kept for 24 h at 4°C to complete the physical and chemical analysis of broiler meat.

Meat quality measurements.

Physical measurements of broiler meat:

Drip loss: A total of 72 samples (24 breast fillets, 24 drumstick and 24 thigh samples) were used for drip loss analysis. After keeping samples at 4°C for 24 h, 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 24 h. and divided by the first weight as described by **Saenmahayak *et al*. (2012).**

Ultimate pH (pHu): After 24 h of chilling 72 samples of breast fillets, drumsticks and thighs on 4°C, ultimate pH (pHu) 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.* (2013).**

Chemical measurements of broiler meat:

Mixture of meat without skin of the three carcass parts (breast fillet, drumstick and thigh) were stored on -20°C for 4 days before chemical measurements, low density lipoprotein (LDL) and high density lipoprotein (HDL), and malondialdehyde (MDA) contents were determined by colorimetric methods using analytical kits produced by Biodiagnostic Company, Egypt.

Statistical analyses: Data of two levels of protease (with and without) and three levels of crude protein (L) were subjected to two way analysis of variance to detect the effects of protease and levels of dietary crude protein as the following model:

$Y_{ijk} = \mu + P_i + L_j + (PL)_{ij} + e_{ijk}$.

Where:

Y_{ijk} = Trait measured

μ = Overall mean

P_i = Protease supplementation
L_j = Crude protein level
$(PL)_{ij}$ = Interaction between protease and levels of crude protein
e_{ijk} = Experimental error
Data of all experimental treatments were subjected to one way analysis of variance to detect the differences between all treatments as the following model:
$Y_{ij} = \mu+T_i+e_{ij}$
Where:
μ = overall mean of Y_{ij},
T_i = effect of treatment, i = (1,…,6)
e_{ij} = Experimental error
Variables showing a significant F-test (p = 0.05) were compared to each other’s using Duncan's Multiple Range Test (**Duncan, 1955**). The statistical procedures were computed using **SAS (2001)**.

RESULTS AND DISCUSSION

Growth performance: The recorded results of growth performance parameters (Table 3) showed significant improvements of recorded final body weight (4.1%), overall body weight gain (3.8%) and feed conversion ratio (6.1%) during the experimental period due to addition of protease enzyme to broiler diets, while feed consumption did not significantly affected. On the other side reducing dietary crude protein level to L15 level resulted in increasing feed consumption and increased feed conversion ratio up to 1.84 compared with 1.68 for STD group. Using L10 level of dietary protein had no significant effect on all growth performance parameters during the experimental period compared with STD recorded values. Among all treatments, results showed the worst feed conversion ratio (1.90) recorded by decreasing dietary protein to L15 level while STD+P group recorded the best feed conversion value (1.63) followed by L10+P group (1.67). These results showed that feed conversion ratio could be improved by 5.2% or 2.9% by adding protease enzyme to STD diets or L10 diets, respectively without depression in final body weights.

These results confirmed those reported previously by **Berri *et al.* (2008)** when fed Ross broiler chicks on diets contained different levels of true digestible lysine (0.83,0.93,1.03 or 1.13%). They recorded improvement of feed conversion and growth rate when broilers fed on diets contained true digestible lysine at levels 0.83 or 0.93 %. Similarly, **Fru-Nji *et al.* (2011)** detected insignificant, but partially improvements in FCR and higher BWG in Low CP diets (200 and 190 g CP per kg feed in the starter and grower phases, respectively) supplemented with exogenous protease compared with normal CP level diet (211 and 200 g CP per kg feed in the starter and grower phases respectively). Also, **Freitas *et al.* (2011)** used protease and reported improvement of FCR as a result of general decrease in FI of birds fed protease supplemented diets, while BWG was not affected. The obtained results of growth performance in this study confirmed that reported by **Angel *et al.* (2011)** who found that reducing dietary crude protein level of broiler chicks resulted in worst FCR while adding protease enzyme prepared from *Bacillus licheniformis* improved the situation by increasing amino acids digestibility by 6.1%. They showed that the effects of exogenous proteases added to poultry diets on live performance are frequently inconsistent due to many factors like the type of tested protease and the nutrients of the control diet.

In previous studies of **Ghazi *et al.(*2003) and Walk *et al.* (2011),** proteases extracted from *Bacillus subtilis* failed to enhance growth performance parameters of broilers**,** while protease produced from *Aspergillus niger* resulted in higher FI and BWG of broilers through increasing digestibility of nitrogen and improving true metabolizable energy (**Ghazi *et al.*, 2003**).

The presented results of this study showed significant increase of FI by reducing dietary protein level to L15 level and this reflected on FCR values. While protease supplementation improved FCR throw raising final body weight of broilers without significant change of FI. These results are supported by **Yadav and Sah (2005)** who indicated that reduction of crude protein increased the feed intake of broilers aged 28 and 42 days. Generally, feed consumption was decreased in broilers fed a reduced crude protein plus protease diet. They suggested that the increased feed intake in broilers fed low crude protein diets could be due to their tendency to consume more feed in order to ingest a required level from a low- protein diet. Conversely, the reduced intake of protease-supplemented low-protein feed may be due to significant increase in protein digestibility. Recently, **Ajayi (2015)** found that protease supplementation enhanced performance of birds fed on the diets with 17.5% CP for starter phase and 14.4 % CP for finisher phase which making them comparable to birds fed the control diets (23% CP for starter phase and 20% CP for finisher phase). The author concluded that CP in broiler chicken diets can be reduced by about 5.6% without deleterious effect on performance. The same trend of improving FCR by adding protease to corn-soy poultry diets was detected by **Vieira *et al*. (2013)** when Nicholas were fed turkey low protein and amino acid diets during 1 to 26 days of age. They obtained better growth performance of birds in protease supplemented groups and explained by increasing amino acids digestibility.

The overall detected growth performance in this study showed no significant deference in final life body weight while FI and FCR were increased by reducing dietary protein. This confirmed the previous reports about effect of protease on increasing protein digestibility of low protein diet to get the optimum protein for growth.

Carcass traits: The recorded values of carcass characteristics measurements (Table 4) show that neither protease supplementation to broiler diets nor dietary crude protein level could make any significant (P>0.05) difference in dressing %, edible parts % (liver, gizzard and heart), or abdominal fat % of broilers at 40 days of age. Beside that values of carcass cuts, front quarter (FQ) and back quarter (BQ) including thigh and drum stick (DS), and skin % of each cut showed significant (P>0.05) increase of skin % of both thigh and DS due to adding protease to broiler diets. It is important to mention that the insignificant values of the percentage of total FQ and BQ weights were combined with numerical increase of dressing %, and the latter was increased significantly by adding protease enzyme (Table 4). On the other side reducing dietary protein from STD level to L15 level led to significant (p>0.05) reduction of FQ skin % (from 8.75 to 4.79%), significant increase of thigh weights on *P* value 0.06, and significant reduction of thigh skin % on *P* value 0.08 (from 8.83 to 6.70 %). The obtained results overall treatments showed significant reduction of skin% of FQ of L15 (4.5%) and L15+P (5.08%) compared with STD and STD+P (8.88 and 8.63%, respectively) at *P* value 0.08 and significant (P<0.05) increase of BQ weight of L15 samples compared with STD and L10 samples. Furthermore samples of L15 thigh showed the lowest thigh skin % (5.01%). However the rest of carcass measurements did not affected significantly due to the examined treatments, it was observed that broilers fed on L10 and L15 showed numerical increase of dressing % and abdominal fat % by protease supplementation.

Several previous studies had been interested with the effects of dietary protein on carcass traits of broilers **(Rezaei *et al*., 2004; Sterling *et al*., 2006; and Yalcin *et al*., 2010)** and some reports- in agreement with the present study- failed to detect significant change in dressing % **(Berri *et al*., 2008),** abdominal fat **(Kobayashi *et al*., 2013; and Guardia *et al*., 2014),** breast meat yield % **(Guardia *et al*., 2014)** or thigh yield % **(Berri *et al*., 2008)**. Non-significant effect of protease supplementation to low protein broiler diets which recorded in the current study confirmed the results of **Freitas *et al*. (2011)** who reported insignificant effect of adding graded levels (from 100 ppm to 1600 ppm) of protease (75000 U/g) to low protein (1%) and low energy (up to 150 kcal/kg diet) diet on % of carcass yield, abdominal fat, breast meat, thigh and drumsticks. Furthermore, **Rada *et al*. (2013)** 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 Corn-Soybean broiler diet (16.7%) during the period from 10 to 35 days of age and could not record any significant differences in carcass weight and carcass yield% between treatments. Concerning to skin% of FQ and thigh the reported reduction by decreasing dietary protein is on the same trend reported by **Shawangizaw *et al.* (2011).** They recorded better skin yield of Rhode Island Red (RIR) chicks reared on lower protein diets compared to those fed on higher dietary protein.

Meat quality: The relationship between either protease supplementation, dietary protein level, or among all treatments and some physical and chemical parameters of broiler meat are presented in Table (5). The physical parameters of broiler meat included determination of drip loss and pH of breast meat, thigh, and DS. Adding protease enzyme to broiler diets did not cause any significant change in determined values of drip loss and pHu of all examined broiler cuts. While reducing dietary crude protein from STD level to L15 level reduced pHu of breast meat from 6.63 to 5.95, and increased drip loss of thigh from 2.72 to 5.35 %, significantly (P<0.05). Among experimental treatments, drip loss % of both breast meat and DS were not affected significantly, while birds received L15 or L15+P diets had significantly higher values for drip loss of thigh when compared to STD group. The recorded values of pHu of breast meat showed reduction trend for low protein treatments while L15+P showed the highest pHu value of thigh. Generally, the obtained overall results showed that the best physical characteristics recorded for L10+P group.

The reduced pHu and increased drip loss of some broiler cuts due to decreasing dietary protein in this study confirmed the published results of **(Allen *et al.*, 1997; Ferguson *et al*., 1998; and Lilly *et al*., 2011). Berri *et al*. (2008)** who found that feeding Ross broilers between 21 and 42 d of age on diets containing graded levels of digestible lysine (0.83,0.93, 1.03 or 1.13%) could change pH and drip loss of breast meat at 42 d. The significant increase of final breast meat pH recorded at level from 0.83 to 1.03% digestible lysine in the diet (6.02 vs. 5.91 and drip loss correlatively decreased (0.85 vs. 1.10). Also, **Guardia *et al.* (2014)** found that, it was possible to alter the pH of breast meat by changing amino acids (AA) profile over a short period before slaughter (3 days). Decreasing pH values were observed in broilers fed lysine deficient diets containing a high amount of other AA (Lys−/AA+) than in broilers fed diets containing low or adequate AA amounts.

There was a relation between the pH of breast meat and its content from glycogen after slaughter, as the lowest pH value recorded with the highest glycogen concentration of broiler breast meat **(Le Bihan-Duval *et al*., 2008)**. In case of protein or amino acids deficiency, a probable consequence limited protein synthesis, while a proportion of nutrients used to energy storage causing increase of glycogen and decreased pH **(Tesseraud *et al*., 2001)**. As a result of this situation the water holding capacity and tenderness of meat will decrease (**Barbut, 1993**). These overall traits decreased the processing yield of meat **(Zhang and Savage, 2010; and Guardia *et al* 2014)**. On the same way, additional benefits reported for decreasing pH value of broiler meat as increase shelf-life of muscles (**Allen *et al*., 1997**), whereas acidic conditions are important to limit rate of microbial spoilage (**Rey *et al*., 1976**).

The obtained results show different response of broiler cuts as affected by the experimental main factors. However the physical measurements of breast meat and thigh were different, both values of drip loss and pHu of DS were not changed due to protease supplementation or decreasing dietary protein (Table 5).

The presented results of some chemical characteristics (Table 5) show that although supplementation of protease enzyme to broiler diets did not cause any significant change of MDA, LDL, or HDL of broiler meat samples, the reduction of dietary crude protein from STD level to L10 and L15 resulted in decreased concentrations of MDA and LDL of meat. The results among all treatments showed a significant decrease in MDA and LDL of broiler meat of birds fed any experimental diet compared to both values in samples of STD and STD+P treatments. The lowest MDA concentration (335.68 n mol/100 g meat) recorded for birds fed L10+P diet (36% lower than STD), while the lowest LDL value recorded for birds fed L15 diet (1026.67 mg/100g meat). These results in general show positive effect of reducing dietary crude protein on the chemical quality of produced meat as the concentrations of MDA and LDL were decreased. The same trend was reported previously by **Lilly *et al*. (2011)** who studied the effect of diets with either deficient, low, high, or excessive dietary amino acid density during the period from 28 to 42 day of age on meat quality. They reported that feeding deficient amino acid diet yielded thigh meat with less protein and more fat while feeding high and excessive amino acid yielded more susceptible to oxidation thigh meat.

The overall results of broiler meat quality show that among the experimental treatments, chicks fed L10+P recorded general good meat quality compared to other treatments, whereas recorded acceptable values of drip loss, pH, MDA and LDL. These criteria of meat increase its shelf life and processing ability.

Feeding cost: Results of feeding cost for chicks fed the experimental diets are summarized in Table 6. There are considerable saving with using protease enzyme and low protein level as compared to the control group. Differences in feeding cost showed that diets supplementation with protease decreased the feeding cost/kg of life body weight. Birds of L10+P group recorded the best value (3.94% saving) relative to STD group, followed by STD+P, then L15+P which saved 2.74 and 1.37%, respectively compared to STD. This saving in feeding cost was due to the decreasing cost of low protein diets and to the improvement in the feed conversion by adding protease. Oppositely, using low protein diet without protease supplementation (L10 and L15) resulted in increasing feeding cost /kg body weight 0.34 and 3.78 % relative to STD group. These results show that CP in broiler diets can be reduced by about 10 or 15 % without deleterious effect on performance and feeding cost if the protease supplementation is applied.

CONCLUSION

The overall obtained results proved that protease supplementation to low protein broiler diets based on corn/soybean meal could enhance performance ,carcass traits, lead to get better meat quality and reduce feeding cost of the low protein diets (10-15 % CP) making them comparable to birds fed the control diets and help producers to achieve acceptable marketing weight.

According to the obtained results, the best overall growth performance, carcass traits, meat quality and feeding cost could be achieved by using L10+P feeding protocol to broiler chicks.

Table (1): Compositions and calculated analysis of the experimental diets.

Ingredients	Starter (1-10 days)			Grower (11-22 days)			Finisher (23-35 days)		
	STD	**Lower 10%**	**Lower 15%**	**STD**	**Lower 10%**	**Lower 15%**	**STD**	**Lower 10%**	**Lower 15%**
Yellow corn	55.10	62.04	62.40	61.65	67.09	63.06	64.74	64.02	65.95
Soybean meal (44%)	31.00	24.94	28.20	23.84	19.00	26.86	19.25	23.87	24.78
Corn gluten meal(60%)	7.50	7.20	3.40	8.57	8.00	1.70	8.16	2.70	--
Soybean oil (SO)	1.80	0.89	1.46	2.27	1.25	3.90	3.28	5.00	5.00
Di-Ca-P	2.00	1.95	1.92	1.75	1.82	1.75	1.55	1.60	1.55
Limestone	1.15	1.54	1.35	1.46	1.42	1.55	1.58	1.60	1.60
NaCl	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24
Vit. And Min. pre-mix*	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Sodium bicarbonate	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
DL Methionine	0.24	0.19	0.20	0.19	0.15	0.20	0.17	0.17	0.15
L-Lysine HCl	0.34	0.38	0.20	0.40	0.40	0.11	0.40	0.17	0.10
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated analysis									
Crude protein%	23	20.7	19.55	21	18.90	17.85	19	17.10	16.15
ME (kcal/kg diet)	3000	3000	3000	3093	3094	3106	3200	3200	3205
Crude fiber%	3.68	3.41	3.60	3.32	3.10	3.46	3.06	3.27	3.92
Ether extract%	4.52	3.48	3.91	5.05	4.26	6.74	6.21	7.75	7.07
Calcium %	0.98	0.98	1.01	1.00	1.00	1.02	1.00	1.02	1.02
Available P %	0.52	0.50	0.50	0.47	0.47	0.45	0.42	0.43	0.44
Sodium %	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Lysine %	1.38	1.25	1.18	1.24	1.12	1.06	1.11	1.00	0.95
Methionine %	0.67	0.59	0.56	0.60	0.53	0.51	0.55	0.50	0.45
SAA %**	1.05	0.94	0.89	0.95	0.86	0.81	0.87	0.78	0.74
Lys. / CP	6.00	6.03	6.03	5.90	5.92	5.94	5.84	5.85	5.88
SAA / CP	4.56	4.54	4.55	4.52	4.55	4.54	4.58	4.56	4.58
Cost/ ton at Egyptian Local Price (LE)***	3550	3323	3225	3443	3234	3250	3372	3297	3158

* Vitamins and minerals premix will provide each kg of diet with: Vit. A, 11000 IU; Vit. D3, 5000 IU; Vit. E, 50 mg; Vit K3, 3mg; Vit. B1, 2mg; Vit. B2 6mg; B6 3 mg; B12, 14 mcg; Nicotinic acid 60 mg; Folic acid 1.75 mg, Pantothenic acid 13mg; and Biotine 120 mcg ; Choline 600 mg; Copper 16mg; Iron 40mg; Manganese 120 mg; Zinc 100mg; Idoine 1.25mg; and Selenium 0.3 mg; ** SAA = Methionine + Cystine ; *** Cost of protease supplemented diets increased 70 LE/Ton feed of each formula. STD = Standard requirements

Table (2): Experimental Design**.**

Treatments	STD	STD+P	L10	L10+P	L15	L15+P
Protease Supplementation[1]						
Without	--		--		--	
With (P)		300 000 U/kg diet		300 000 U/kg diet		300 000 U/kg diet
Dietary Protein level[2]						
Standard (STD)	23, 21, and 19 %	23, 21, and 19 %				
Lower 10% from CP of Std.(L10)			20.7, 18.9, and 17.1%	20.7, 18.9, and 17.1%		
Lower 15% from CP of Std. (L15)					19.55, 17.85, and 16.15 %	19.55, 17.85, and 16.15 %

1: CIBENZA® DP100, Novus International, Inc.. The enzyme was supplemented at rate of 500 g/ Ton diet to supply 300 000 U protease activity /kg diet.
2: Dietary protein level was calculated to be standard, lower 10% or 15% from crude protein of standard for starter, grower and finisher stages.

Table (3): Effect of protease supplementation and dietary crude protein level on growth performance parameters of broiler chicks at 40 days of age

Treatments	Final weight (g)	Feed intake (g)	Body weight gain (g)	Feed conversion ratio
Main effect				
- Protease Supplementation				
Without	1922.42b	3398.69	1889.05b	1.80a
With (300000 U/kg, P)	2000.05a	3315.40	1960.10a	1.69b
Mean of SE	± 22.84	± 45.17	± 23.26	± 0.02
Probability	0.03	N.S	0.04	0.002
- Dietary Protein level				
STD	1976.6	3250.5b	1941.7	1.68b
L10	1958.6	3326.8b	1923.5	1.73b
L15	1948.5	3493.8a	1908.5	1.84a
Mean of SE	±27.98	±55.32	±28.49	±0.03
Probability	N.S	0.02	N.S	0.002
Treatments				
STD	1991.00	3345.30ab	1951.25	1.72bc
STD+P	1972.22	3155.70b	1932.22	1.63c
L10	1912.50	3347.70ab	1872.15	1.78b
L10+P	2014.67	3306.00ab	1974.81	1.67bc
L15	1883.75	3503.20a	1843.75	1.90a
L15+P	2013.25	3484.50a	1973.25	1.77b
Mean of SE	±39.57	±78.23	±40.29	±0.04
Probability	N.S	0.058	N.S	0.001

a,b,...= Means in the same column with different superscripts, differ significantly ($P<0.05$); N.S = Not Significant ($P>0.05$).
STD = Standard requirements; P= protease enzyme; L10= lower 10% from CP of STD; and L15= lower 15% from CP of STD.

Table (4): Effect of protease supplementation and dietary crude protein level on some carcass parameters of broiler chicks at 40 days of age.

Treatments	Carcass			Front quarter (FQ)		Back quarter (BQ)				
							Thigh		Drums stick (DS)	
	Dressing (% from live weight)	Edible Parts (% from carcass)	Abdominal fat (% from carcass)	Breast weight (% from carcass)	Skin (% from Breast)	Total weight (% from carcass)	Weight (% from BQ)	Skin (% from Thigh)	weight (% from BQ)	Skin (% from DS)
Main effect										
- Protease Supplementation										
Without	71.41	5.73	1.08	18.30	6.74	18.09	61.74	6.93b	38.26	8.12b
With (300000 U/kg, P)	72.20	5.81	1.27	17.28	6.64	18.39	62.13	8.50a	37.87	10.45a
Mean of SE	±0.73	±0.22	±0.07	±0.52	±0.65	±0.38	±1.27	±0.49	±1.27	±0.61
Probability	N.S	N.S	N.S	N.S	N.S	N.S	N.S	0.04	N.S	0.02
- Dietary Protein level										
STD	70.26	6.11	1.26	17.13	8.75a	17.99ab	61.63	8.83a	38.37	9.88
L10	72.93	5.59	1.23	18.09	6.52ab	17.50b	60.86	7.62ab	39.14	8.46
L15	72.21	5.60	1.03	18.15	4.79b	19.22a	63.32	6.70b	36.69	9.51
Mean of SE	±0.90	±0.27	±0.09	±0.63	±0.79	±0.47	±1.55	±0.60	±1.55	±0.75
Probability	N.S	N.S	N.S	N.S	0.01	0.06	N.S	0.08	N.S	N.S
Treatments										
STD	70.89	5.55	1.20	18.33	8.88a	17.40b	60.78	8.90a	39.22	9.54
STD+P	69.63	6.68	1.32	15.92	8.63ab	18.59ab	62.48	8.76a	37.52	10.21
L10	72.75	5.95	1.11	18.64	6.84abc	16.65b	59.68	6.88ab	40.32	7.02
L10+P	73.11	5.23	1.35	17.54	6.20abc	18.34ab	62.04	8.35a	37.96	9.89
L15	70.56	5.69	0.92	17.93	4.50c	20.21a	64.76	5.01b	35.24	7.79
L15+P	73.86	5.51	1.14	18.38	5.08bc	18.24ab	61.87	8.40a	38.13	11.24
Mean of SE	1.27±	0.38±	0.12±	±0.90	±1.12	±0.66	±2.20	±0.85	±2.20	±1.05
Probability	NS	NS	NS	N.S	0.08	0.04	N.S	0.05	N.S	N.S

a,b,...= Means in the same column with different superscripts, differ significantly ($P<0.05$); N.S = Not Significant ($P>0.05$). STD = Standard requirements; P= protease enzyme; L10= lower 10% from CP of STD; and L15= lower 15% from CP of STD. Front quarter (FQ): Breast quarter without wing Back quarter (BQ): Leg quarter including thigh and drum stick

Table(5): Effect of protease supplementation and dietary crude protein level on some physical and chemical parameters of broiler chicks at 40 days of age.

Treatments	Physical measurements						Chemical measurements		
	Breast		Thigh		Drum Stick				
	Drip Loss	pH_u	Drip Loss	pH_u	Drip Loss	pH_u	MDA (nmol/100g meat)	LDL (mg/100g meat)	HDL (mg/100g meat)
Main effect									
- Protease Supplementation									
Without	3.25	6.31	3.73	6.29	1.08	6.43	411.58	1175.08	698.14
With (300000 U/kg, P)	2.84	6.08	4.08	6.36	1.33	6.44	411.11	1194.29	658.71
Mean of SE	±0.26	±0.26	±0.38	±0.05	±0.18	±0.04	±13.83	±21.28	±16.92
Probability	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
- Dietary Protein level									
STD	3.38	6.63a	2.72b	6.24	1.00	6.37	523.24a	1372.38a	683.86
L10	3.09	5.56b	3.65b	6.33	1.50	6.48	353.99b	1118.33b	667.57
L15	3.65	5.95b	5.35a	6.40	1.11	6.45	356.81b	1063.33b	683.85
Mean of SE	±0.32	±0.31	±0.46	±0.06	±0.22	±0.06	±16.94	±26.06	±20.72
Probability	N.S	0.01	0.005	N.S	N.S	N.S	0.0001	0.0001	N.S
Treatments									
STD	3.13	6.78a	2.11b	6.34ab	0.68	6.42	524.88a	1382.86a	711.80
STD+P	3.65	6.48ab	3.33ab	6.14b	1.32	6.31	521.60a	1361.91a	655.93
L10	3.98	5.63b	3.67ab	6.26b	1.40	6.47	372.30b	1115.71b	679.20
L10+P	2.21	5.49b	3.62ab	6.40ab	1.59	6.50	335.68b	1120.95b	655.93
L15	2.65	6.17ab	5.41a	6.26b	1.15	6.39	337.56b	1026.67b	703.42
L15+P	2.66	5.73b	5.28a	6.54a	1.07	6.51	376.06b	1100.00b	664.28
Mean of SE	±0.45	±0.44	±0.65	±0.08	±0.31	±0.08	±23.95	±36.85	±29.30
Probability	N.S	0.07	0.03	0.05	N.S	N.S	0.0001	0.0001	N.S

a,b,...= Means in the same column with different superscripts, differ significantly ($P<0.05$); N.S = Not Significant ($P>0.05$).

STD = Standard requirements; P= protease enzyme; L10= lower 10% from CP of STD; and L15= lower 15% from CP of STD.

Table (6): Effect of experimental treatments on feeding cost of broiler chicks during 40 days of age**.**

Treatments	Final BW (g)	Total BWG (g)	FCR	Feeding cost/bird (LE)	Feeding cost/kg of BW (LE)	Relative difference of feeding cost from STD* %
STD	1991	1951	1.72	11.40	5.84	--
STD+P	1972	1932	1.63	10.97	5.68	2.74 (Saving cost)
L10	1913	1872	1.78	10.98	5.86	0.34 (Increasing cost)
L10+P	2015	1974	1.67	11.08	5.61	3.94 (Saving cost)
L15	1884	1844	1.9	11.18	6.06	3.78 (Increasing cost)
L15+P	2013	1973	1.77	11.37	5.76	1.37 (Saving cost)

*** Relative difference of feeding cost from STD** = 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).

STD = Standard requirements; **P**= protease enzyme; **L10**= lower 10% from CP of STD; and **L15**= lower 15% from CP of STD.

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الملخص العربى

تقيم استخدام انزيم البروتييز عند مستويات مختلفه من البروتين فى علائق الاذره وكسب فول الصويا لكتاكيت التسمين

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الهدف من هذه الدراسه تقيم تأثير اضافة انزيم البروتييز لعلائق الاذره وكسب فول الصويا المنخفضه فى البروتين لكتاكيت التسمين على خفض تكاليف التغذيه وتحسين جودة اللحم . تم توزيع مائتين واربعون كتكوت تسمين غير مجنس سلالة اربر ايكرز عمر يوم الى ستة معاملات (تصميم عاملى ٢*٣) كل معامله تحتوى على اربع مكررات. تم اختبار مستويين من انزيم البروتييز(بدون و ٣٠٠٠٠٠وحدة انزيم /كيلو جرام علف) وثلاث مستويات من البروتين الخام (االقياسي وأقل ١٠% و أقل ١٥% من البروتين الخام الموصى به وذلك لكل مرحله تغذيه (L10 و L15 على التوالى). و كانت مستويات البروتين الخام بالعلائق ٢٣ ، ٢٠,٧ ، ١٩,٥ % فى مرحلة البادى و ٢١، ١٨,٩ ، ١٧,٨ % فى مرحلة النامى و ١٩، ١٧,١ ، ١٦,١ % فى مرحلة الناهى. تم تكوين العلائق لتوفيراحتياجات السلاله من بقية المواد الغذائيه. تم تسكين الكتاكيت فى نظام العنابر المفتوحة و خضعت لنفس برنامج الرعاية و البيطرة خلال فترة التجربة (من ١-٤٠ يوم). تم تسجيل مقاييس الاداء الانتاجى وصفات الذبيحه وتقيم الصفات الطبيعيه والكيماويه للحم و تم حساب تكاليف التغذيه . اظهرت النتائج ان استخدام البروتييز أدى الى تحسن الوزن النهائى للجسم بنسبة ٤,١ % و معدل التحويل الغذائى بنسبة ٦,١ % وخفض نسبة الجلد فى الدبوس بمعنويه (P<0.05). كما أدى خفض البروتين فى العليقه بمجموعة (L15) الى زيادة العلف المستهلك وفقد الماء فى عينات لحم الفخذ ووزن الربع الخلفى. كما ادى خفض البروتين الي خفض نسبة الجلد و pH فى لحم الصدر ونقص تركيز الليبوبروتينات منخفضة الكثافة LDL و مركب المالون داى الدهيد MDA فى عينات لحم كتاكيت التسمين بمعنوية (P<0.0001). و اظهرت نتائج المعاملات ككل ان اضافة انزيم البروتييز الى العلائق المنخفضه فى البروتين بنسبة ١٠% أدت الى نفس وزن الجسم الحى عند التسويق (٢٠١٥جم) و تحسين جودة اللحوم (من خلال تقليل MDA بنسبة٣٦ % عند مستوى معنوية P<0.0001) وخفض تكاليف التغذية / كيلو جرام وزن حى بنسبة ٣,٩٤ % بالمقارنة بالمجموعة القياسية.

بوجه عام نستخلص من النتائج ان اضافة انزيم البروتييز بمعدل٣٠٠٠٠٠ وحده / كيلوجرام علف الى علائق كتاكيت التسمين المنخفضه فى البروتين يمكنها المساعده فى انتاج وزن جسم تسويقى مقبول مع تحسين جودة لحم أفضل بالإضافة الى خفض تكاليف التغذيه وفقا لاسعارمكونات الأعلاف بالأسواق المصريه.