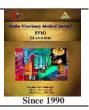
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Original Paper

Effect of dietary protease enzyme (Cibenza®) supplementation on growth performance and carcass quality of broiler chicks

Gomaa M. E. E, Nasser E. Khedr, Tahia E. Ahmed

Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Benha University, Egypt

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ABSTRACT

Keywords The present trial aimed to throw the light on the effect of dietary supplementation of protease enzyme on growth performance parameters and carcass quality of broiler chicks. A total Broiler chicks number of 360 Cobb broiler chicks of mixed sex (one day old) where collected from a local Carcass quality Egyptian private hatchery and classified into 4 groups of three replicates (30 chicks/replicate). The 1st group was fed diet containing soya bean meal 44% (G1), the 2nd Performance group was fed diet containing soya bean meal 44%+ protease enzymes (G2), the 3rd group **Received** 22/05/2021 was fed diet containing soyabean meal 46% (G3) while the 4th group was fed diet containing Accepted 15/06/2021 soyabean meal 46%+ protease (G4). Water and feed were offered ad-libitum. All birds were Available On-Line systematically vaccinated against Newcastle, IB and Gumbro and other needed prophylactic 01/10/2021 measures. Results showed that G4 showed an increase in the final BW by (8.1%, 7.58%, 1.97%), BWG by (8.3% , 7.7% , 1.97%) , FI by (1.88% , 0.88%, 0.06%) and FCR by (6.66%, 6.66% , 1.75%) than G1, G2 and G3, respectively. Carcass yields were improved in protease supplemented groups where G4 showed an increase in carcass relative weight than G3 by 1.07% and G2 showed an increase in carcass relative weight than G1 by 2.71%. Also, G4 showed an increase in breast relative weight by (1.8%, 5.1%, 0.05%) and liver relative weight (12.56%, 5.9%, 3.36%) than G1, G2 and G3, respectively.

1. INTRODUCTION

A new challenge in the poultry industry is to take advantage of the use of specific dietary supplements to achieve better poultry performance and feed conversion. Corn-soybean meal poultry feed is considered to be favorable because of its high nutritional value but soybean meal contains oligosaccharides that have been shown to decrease bird health and growth (Iji and Tivey, 1998). The cost of poultry feeding is increasing day by day due to continuous rising of soybean prices throughout the whole world, therefore, the trend of different strategies in broiler production feed is used to compensate these high feed prices with no compromise on the growth index. (Jabbar et al., 2021). The increased demand on livestock industry to phase out the use of prophylactic dosages of antibacterial growth promoters (AGP) in the European Union due to microbial resistance in animals and human and the prospective to do the same in other parts of world has stimulated increased interest in alternative natural growth promoters (Ján et al., 2012; Amerah et al., 2017). One such non-therapeutic alternative was the use of organic acids as feed additives in the animal production, (Adil et al., 2010). Considerable Progress has been made during the last decade in the manufacture, activity, quality, ability and specificity of supplemental enzymes for use in poultry diets.(Acamovic, 2001). Protease is an enzyme, which is responsible for proteolysis and subsequently improved crude protein digestibility in broilers (Freitas et al., 2011; Cowieson et al., 2017). Adding Protease enzyme to feed aimed to increase dietary protein hydrolysis and enable maximum nitrogen utilization. When animals utilize

nitrogen better, there is a possibility to decrease the diet protein content and in turn also reduce the content of nitrogen in manure. (Oxenboll et al., 2011).

This Trial was aimed to throw the light on the effect of dietary supplementation of protease enzyme on growth performance parameters and carcass quality of broiler chicks.

2. MATERIAL AND METHODS

The Trial was conducted at the Department of Nutrition and Clinical Nutrition, Faculty of Veterinary medicine, Benha University to study the effect of dietary supplementation of protease enzyme when mixed with a diet contain 46 % and 44 % SBM on growth performance, carcass quality and economic efficiency of broiler chicks.

2.1.Birds, housing, and management:

A total number of 360 one day old cobb broiler chicks of mixed sex were used in this study. The broiler chicks were randomly classified into 4 groups of three replicates (30 chicks per replicate). Water and feed were offered ad-libitum. All birds were systematically vaccinated against Newcastle, IB and Gumbro and other needed prophylactic measures.

2.2. Feeding program

Yellow corn, soyabean meal (44% and 46%), corn gluten, vegetable oil, limestone, salt, amino acids, and minerals & vitamins premix were used to formulate the basal diet and to achieve the optimal nutrient requirements according to (NRC, 1994). Experimental rations were given to the birds

Correspondence to: dr.elmnshawy1991@gmail.com

for 6 successive weeks. The enzyme used was Cibenza $^{\oplus}$ and added by 0.05% according to the manufacture recommendations.

The applied experimental design is shown in table (1). The ingredient composition and calculated analysis of the used experimental rations are presented in tables (2-4) respectively.

Vitamins-minerals mixture produced by AGRI-VET[®] Company 10^{th} of Ramadan city A2, Egypt, and each 3 kg composed of: Vit. A 12000000 IU, vit. D₃ 2000000 IU, vit. E 10000 mg, vit. K₃ 2000 mg, vit B₁1000 mg, vit. B₂ 5000 mg, vit B₆ 1500 mg, vit. B₁₂ 10 mg, Biotin 50 mg,

Table 2 The composition of used basal diets ingredients

pantothenic acid 10000 mg, Nicotinic acid 30000 mg, Folic acid 1000 mg, Manganese 60000 mg, Zinc 50000 mg, Copper 10000 mg, Iron 30000 mg, Selenium 100 mg, Iodine 1000 mg, Cobalt 100 mg, carrier (CaCo₃) add to 3 kg.

Table 1 The applied	experimental design
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Group	Soyabe	an meal	Protease
	44%	46%	
1	+	-	-
2	+	-	+
3	-	+	-
4	-	+	+

La cardinat (0)		Sta	rter			Gro	wer			Fir	nisher	
Ingredient (%)	G 1	G 2	G3	G4	G1	G2	G3	G4	G1	G2	G3	G4
Yellow corn	48.91	51.368	51.125	53.07	54.464	55.105	56.035	57.37	57.38	60.31	59.69	60.275
Soyabean meal 44%	36.5	36.5	0	0	32.4	34.7	0	0	33.3	31.3	0	0
Soyabean meal 46%	0	0	36	36			32.3	32.3	0	0	31.8	31.3
Corn gluten meal	6.3	4.5	5.3	3.6	4.5	1.6	3.5	2.1	0	0		0
Vegetable oil	3.5	2.8	2.8	2.5	4.2	4.2	3.75	3.8	5.4	4.4	4.6	4.5
Di calcium phosphate	1.625	1.65	1.75	1.75	1.4	1.4	1.5	1.5	1.25	1.275	1.35	1.35
Limestone	1.5	1.5	1.45	1.45	1.55	1.55	1.5	1.5	1.25	1.25	1.2	1.2
L -Lysine	0.4	0.37	0.31	0.315	0.28	0.185	0.19	0.15	0.145	0.165	0.095	0.1
Sodium chloride	0.325	0.325	0.315	0.315	0.31	0.31	0.3	0.3	0.315	0.31	0.3	0.3
DL -Methionine	0.28	0.285	0.26	0.275	0.235	0.25	0.22	0.225	0.285	0.275	0.26	0.26
Sodium bicarbonate	0.12	0.122	0.18	0.18	0.115	0.115	0.17	0.17	0.115	0.12	0.17	0.17
Ant-coccidia	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Ant-clostridia	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Ant-mycotoxin	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Energy enzyme	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
L -Threonine	0.03	0.02	0	0	0.011	0	0	0	0.025	0.01	0	0
Phytase	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Cibenza®	0	0.05	0	0.05	0	0.05	0	0.05	0	0.05	0	0.05
Emulsifier	0	0	0	0	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Vitamin-mineral mixture	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Total	100	100	100	100	100	100	100	100	100	100	100	100

Table 3 Calculated Chemical analysis of feed ingredients diet containing soybean meal 44 %

Parameters	Starter	Grower	Finisher
СР	23.01%	21%	19.01%
ME (K Cal/Kg)	3049	3179	3224
Crude fat	5.39%	6.23%	7%
Crud fiber	3.56%	3.2%	3.2%
Lysine	1.35%	1.2%	1.09%
Lysine Digestible	1.25%	1.1%	1.01%
Methionine	0.69%	0.55%	0.53%
Methionine digestible	0.65%	0.51%	0.5%
Methionine +cysteine	1.07%	0.91%	0.86%
Methionine +cysteine Digestible	0.96%	0.8%	0.77%
Threonine	0.96%	0.79%	0.76%
Threonine digestible	0.83%	0.67%	0.65%
Calcium	1.05%	0.95%	0.86%
Avi Phosphorous	1.05%	0.45%	0.42%
Chloride	0.23%	0.22%	0.22%
Sodium	0.18%	0.18%	0.16%
Potassium	0.88%	0.77%	0.75%

Table 4 Calculated Chemical analysis of feed ingredients of diet containing soyabean meal 46 %

Parameters	Starter	Grower	Finisher
CP	22.98%	21.09	18.99%
ME (K Cal/Kg)	3052.6	3173	3226
Crude fat	5.02%	6	7.03%
Crud fiber	2.21%	2.16	2.2%
Lysine	1.35%	1.21	1.09%
Lysine Digestible	1.21%	1.08	0.97%
Methionine	0.69%	0.56	0.54%
Methionine digestible	0.65%	0.52	0.51%
Methionine +cysteine	1.07%	0.91	0.86%
Methionine +cysteine Digestible	0.95%	0.80	0.76%
Threonine	0.98%	0.82	0.77%
Threonine digestible	0.83%	0.68	0.65%
Calcium	1.05%	0.94	0.85%
Avi Phosphorous	0.5%	0.45	0.42%
Chloride	0.23%	0.22	0.22%
Sodium	0.18%	0.18	0.16%
Potassium	0.85%	0.78	0.79%

2.3. Measurements

2.3.1. Performance:

Weight gain (expressed in grams) was calculated as the difference between two successive live body weights. Relative growth rate (RGR) and Feed conversion (FCR) were calculated where:

 $RGR=100 (W_{2}-W_{1}) / \frac{1}{2} (W_{2}+W_{1})$

Where: W_{1} = Body weight at the beginning of week or period.

 W_2 = Body weight at the end of week or period. Crampton and Lioyd (1959).

The Consumption of Feed was weekly estimated for each treatment. Live body weight was measured in grams for all birds at the start of the experiment and Every week .

FCR = Feed intake (g. bird / week) /Body weight gain (g). bird/week. Lambert et al. (1936).

2.3.2. Evaluation of carcass quality

At the end of trial (The day 39), 3 birds from each group were randomly chosen, fastened for 12 hours then weighted and slaughtered to complete their bleeding and weighted to determine the following:

2.3.2.1. Dressing percentage:

The birds from each group were eviscerated, weighted without feather and head and the dressing percentage was calculated according to the following formula: Dressing% = (Dressed carcass weight / Live weight) $\times 100$

2.3.2.2. Breast muscle, edible parts (heart and gizzard) and weight of immune organs: (Liver, spleen, bursa of fabrics and thymus) were weighted and recorded as relative weight proportion to live.

2.3.3. Statistical analysis

Results Obtained From this Trial were statistically analyzed for variance ANOVA with confidence limits set at 95 % (Significance at $P \le 0.05$ probability level) and critical difference as described by (Duncan, SPSS Ver.

10.0.7, June 2000). The results were reported as the mean \pm standard error (SE).

3. RESULTS

3.1. Performance

3.1.1. Live body weight development and weight gain

Data concerning live body weight development during the trial period for the different Trial groups of broiler is shown in table (5) fig. (1D). From the obtained data it was noticed that the inclusion of protease enzyme + SBM 46 % had a positive effect on final body weight changes comparing with other groups where it showed increased in Final BW by 8.1%, 7.58% and 1.97% than G1, G2 and G3 respectively. Data concerning BWG at the end of trial are shown in table (5) and fig. (1A), where it was noticed that there was a significant difference (P \leq 0.05) among different experimental groups where G4 showed significant increase by 8.3%, 7.7% and 1.97% than G1,G2 and G3 respectively.

3.1.2. Relative growth rate (RGR)

Our results detailed in table (5) and fig. (1F), cleared that, there is a significant difference (P < 0.05) among different

Table 5 Effects of protease enzyme on broiler chicks growth performance (n= 30)

treatment groups where the higher RGR observed at group 4 followed by group (3) followed by group (2) and group (1) where G4 showed increased RGR by 0.4%, 0.33% and 0.09% than G1, G2 and G3 respectively.

3.1.3. Feed intake

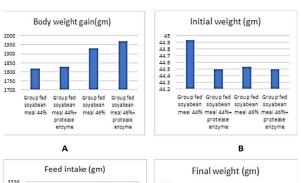
Our results cleared that, the means of average feed intake and the total feed consumption of soybean meal containing diets with or without protease enzyme showed in table (5) fig. (1C). There was a significant difference between experimental groups where groups 3 & 4 showed significant increase than the other experimental groups where G3 showed increased FI by 1.2% and 0.8% compared with G1 and G2. Also, G4 showed increased FI by 1.88% and 0.88% than G1 and G2.

3.1.4. Feed conversion ratio (FCR)

The effect of dietary addition of different levels of SBM with or without protease on FCR showed in table (5) and Fig (1E). The obtained data clarify that the group (4) showed significant improved in FCR by 6.66%, 6.66% and 1.75% than G1, G2, and G3 respectively.

Items		Mean ± Sta	andard error	
—	G 1	G2	G3	G4
Initial weight (gm)	44.93±0.60 ª	44.50±0.42 a	44.53±0.45 a	44.50±0.44 ª
Final weight (gm)	1862.00 ± 23.82^{d}	1871.67±45.07 °	1974.82±43.55 ^b	2013.28±24.96 a
Body weight gain(gm)	1817.07±7.78 ^d	1827.17±4.87 °	1930.29±10.90 ^b	1968.78±6.88 a
Relative growth rate	190.58±9.12 ^b	190.71±9.12 ^b	191.18±19.12 ^a	191.35±19.12 °
Feed intake (gm)	3276.28±26.77 °	3289.51±29.77 ^b	3316.77±28.77 °	3318.3±33.19 ª
FCR	1.80±0.15ª	1.80 ± 0.18^{a}	1.71±0.16 ^b	1.68±0.14 ^b

Values are (means ± standard errors) with different letters at the same raw differ significantly at (P≤0.05)



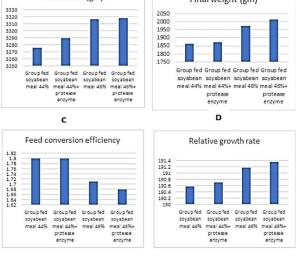


Figure 1 Effects of protease enzyme on broiler chicks growth performance

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3.1.5. Carcass quality

3.1.5.1. Carcass yield

The present data in the table (6), cleared that there was a significant difference in live weight, carcass weight and dressed carcass weight and their relative weight as the higher level observed in the group 4, followed by group 3. While the lower level observed in the group 1 where G4 showed increased in carcass relative weight than G3 by 1.07% and G2 showed increased in carcass relative weight than G1 by 2.71%.

Regarding relative breast weight, G4 showed increased in breast relative weight by 1.8%, 5.1% and 0.05% than G1, G2 and G3, respectively. Heart weight left lung weight, right lung weight, fat weight, head weight, leg weight and Gizzard weight, showed a higher level in the group 4, followed by group 3. While, the lower level observed in the group 2 followed by group 1. The abdominal fat showed significant increase in protease supplemented groups while the immune organs including the bursa and spleen showed non-significant differences among the experimental groups.

3.1.5.2. Relative immune organs weights proportion to live body weight of broiler chicks.

The effect of different dietary SBM with or without protease enzyme supplementation on organ relative weight is summarized in table (6). The result showed that the most obvious items in organ relative rate as carcass relative weight, net relative weight, breast muscle, liver, both lungs, bursa, proventriculus and intestinal relative weights showed non-significant differences between all experimental groups.

3.2. Intestinal length and width of broiler chicks

The effect of different dietary SBM with or without protease enzyme supplementation on intestinal length and width of broiler chicks is summarized in table (7). The

present results showed that the intestinal length, intestinal width, and intestinal opening width and their relative observed in the group (4), followed by group (3). While the

lower level observed in the group (2) followed by the group (1).

Table 6 The effect of different dietary SBM levels with or without pr	protease enzyme supplementation on organ relative rate (n= 3)
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Items		Mean ± St	andard error	
	G1	G 2	G 3	G4
Carcass relative weight	90.68±1.23 ^a	93.14±0.22 ª	91.07±0.35 °	92.05±0.39 °
Net relative weight	77.94±0.26 ª	78.00±0.45 ª	78.71±0.75 ª	78.14±0.96 a
Breast relative weight	18.86±1.55 ^a	18.26±0.73 ^a	19.19±0.71 ^a	19.20±0.67 ^a
Liver relative weight	1.91±0.03 °	2.03±0.08 ^a	2.08±0.08 a	2.15±0.05 ^a
Spleen relative weight	0.11 ±0.00 ^a	0.12±0.04 ª	0.14±0.03 a	0.09±0.02b
Heart relative weight	0.64±0.06 ª	0.59±0.00 ^a	0.48±0.03b	0.56±0.04°
Lung relative weight (Left)	0.25 ±0.05ª	0.27±0.03ª	0.29±0.02 ^a	0.31±0.04 ^a
Lung relative weight (Right)	0.28±0.01ª	0.27±0.03ª	0.26±0.03 a	0.31 ±0.04ª
Bursa relative weight	0.19±0.01ª	0.16 ± 0.00^{a}	0.16±0.03 ^a	0.16±0.03 ^a
Proventriculus relative weight	0.48±0.06 ^a	0.59±0.08 ^a	0.45±0.04 ^a	0.47 ± 0.06^{a}
Gizzard relative weight	2.00±0.06 ^b	2.14±0.02 ª	2.06±0.06 ^b	1.93±0.12°
Intestinal relative weight	10.53±0.66 a	9.70±0.56 °	10.59±0.30 ª	9.74 ±0.21 ^a
Fat relative weight	1.54±0.35 ^b	2.01±0.21 a	1.04±0.29°	1.86±0.29 ^b
Head relative weight	2.40±0.14 °	2.42±0.19 a	2.48 ±0.11 ^a	2.40±0.15 ª
Leg relative weight	3.97±0.12 °	3.53±0.27 ^d	3.79±0.23°	3.89±0.05 ^b

Values are (means ± standard errors) with different letters at the same raw differ significantly at (P≤0.05)

Table 7 The effect of different dietary SBM levels with or without protease enzyme supplementation on intestinal length and width (n= 3)

Items		Mean ± Standard error				
	1	2	3	4		
Intestine length	184.67±6.49 ^b	181.67±10.93 ^b	204.00±7.21 ª	200.67±10.59 ^a		
Intestinal width	1.00±0.00 ^a	1.07±0.07 ^a	1.00 ± 0.00^{a}	1.00 ± 0.00^{a}		
Intestinal opening width	1.83±0.17 ^b	2.00±0.00 ^a	2.00±0.00 ª	2.00±0.00 ^a		
Webser and (many a standard second se	1.66.	(D < 0.05)				

Values are (means \pm standard errors) with different letters at the same raw differ significantly at (P ≤ 0.05)

4. DISCUSSION

From the obtained data in table (5), it was noticed that the inclusion of protease enzyme + SBM 46 % had a positive role in improving final body weight changes comparing with other treatments. These results agreed with those of more recent studies, which showed that exogenous protease can provide a variety of production advantages, especially in feed that contain reduced levels of protein (Cowieson and Adeola, 2005). Also, result agreed with (Alagawany et al., 2017), who observed that, the activities of digestive enzymes (protease and amylase) were significantly (P < 0.05) influenced and enhanced by SFM and enzyme addition in diets, respectively. The activities of protease and amylase enhanced with SFM diet supplemented were with 0.1 g/kg enzyme in comparison with those with the none supplemented diet.

Concerning the weight gain our results agreed with those of (Kocher et al., 2015), who observed that the supplementation of protease enzyme to broiler diet improved the daily weight gain due to improve the digestion and utilization of the feed and protein intake. This result attributed to the protease enzyme improved the feed conversion and its utilization that will improve the body weight at the different stages of broiler production. Our results agreed with those of (Angel et al., 2011), who observed that the protease enzymes improved the apparent amino acids digestibility in broiler chickens, with improving the digestibility and body weight gain that, will improve the relative growth rate of broilers than the groups not fed on protease in the ration.

Results concerned to feed intake agreed with those of (Cowieson and Ravindran, 2008) where they recorded that, addition of protease enzymes to broiler diet facilitated the feed digestion and utilization with improvement of the amount of feed intake. This improvement may be attributed to the higher final body weight. This result agreed with those of Kocher et al. (2015) observed that the FCR was improved in birds fed protease at 20,000 HUT/kg compared with the control (-3.0%, 1.97 vs. 2.03 respectively; P < 0.05).

Furthermore, birds that fed the lowest protease dose (5,000 HUT/kg) gained more weight (+2.5%, 2,084 vs. 2,034 g respectively; P < 0.07) and exhibited a better FCR (-2.5%, 1.98 vs. 2.03 respectively; P < 0.09) compared with birds fed the control. No interaction occurred between dietary treatment and sex occurred during any of the phases (P > 0.05).

Data obtained from table (6) showed that the improvements in carcass quality and yield attributed to the improvement of protein and amino acids digestibility, that improve the growth, performance, and quality of the broiler carcass. The digestibility improvements were not reflected in improved growth performance. Our results are agreed with (Ajayi 2015) who reported that there is improvement in dressing% with protease inclusion in broilers diet, also agreed with (Abudabus, 2017) and (Law et al, 2017) who reported that protease enzyme improved carcass yield. On the other hand, our results don't agree with (Sumanasekara et al., 2020), (Freitas et al. 2011) and (Yadav and Sah, 2005), who reported that protease enzyme had non-significant effect on dressing% and carcass weight.

Concerning liver weight, the present results are agreed with (Nadzigaraye, 2019), who reported that the protease had no effect on the liver weight at the end of the experiment.

Data obtained from table (7) concerning to intestinal width and length agreed with (Law et al, 2017) that reported that protease supplementation significantly increased duodenal and jejunal absorptive surface area and jejunal villus height/crypt depth ratios and ileal villus height. Also, these findings agree with (Cowieson et al, 2016), (Ding et al., 2016) and (Xu et al., 2017)that reported that supplementation of exogenous protease enzyme increased villous height and crypt depth ratio.

5. CONCLUSION

From the obtained results, it was concluded that inclusion of protease enzyme with SBM 46 % in broiler diets from zero day till slaughtering age had a positive role in improvements in live body weight, body weight gain, feed intake feed conversion index and carcass yield than other groups.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data

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