Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

The effects of exogenous enzyme (kemzyme) supplementation to low-protein broiler diet on growth performance, blood parameters and cGH gene expression

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Received:9/1/2023. Accepted:9/3/2023. Published:1/6/2023

Abstract

The objective of this study was to assess the effects of a low protein content (low CP) level diet supplemented with a kemzyme on the growth-prompting effect, the biochemical effects of kemzyme with cGH gene expression, and other related effects. Three (3) groups with (90) birds in each group with (3) replicates, depending on the concentration of protein that met the nutritional requirements of the broiler nutritional composition of the starter, grower, and finisher, were used in our experiment with 270 broiler chicks strain (Ross 308) one day old birds to determine the effect of feeding programs with different CP levels (CP-3%) lower dietary CP content, using at a dose of 500-1000 gm/ton (con). Kemzyme enhanced weight of body and FCR, according to results ($p<0.05$). levels of serum total protein and albumin were significantly higher in the low CP and kemzyme-fed groups compared to the low protein level-only groups ($P<0.05$), while the liver and kidney enzymes were unaffected. Kemzyme supplementation slightly significant increased the expression of the cGH gene.
Key words: kemzyme, low crude protein, GH genes expression, broiler.

Introduction

One of the largest developmental difficulties facing the majority of emerging countries is how to efficiently feed their rapid population growth and the appropriate level of animal protein. One of the fastest ways to alleviate the pervasive protein shortfall issue in underdeveloped countries has been touted as the expansion of the chicken industry (1). In combination, antibiotics have been used as growth promoters for a very long time, it has been shown that antibiotics reduce mortality rates while increasing performance, feed conversion, and daily body gain in hens, and the problem with the creation of germs resistant to both human and animal sources was raised, though, as a result of the usage of antibiotics to promote growth (2). In order to promote productivity and bird growth, adding produced enzymes as nutrients additives to birds' diets is a well-known feeding method. (3 ; 4 ; 5 ; 6 and 7). (8) showed that the availability, digestion ability , and performance of broiler raised on maize and SBM were increased by enzymes addition to their diets. The multi-enzyme supplement kemzyme includes multiproteases, multiamylases, and enzymes that hydrolyze nonstarch polysaccharides (NSPs), it was developed specifically to release more energy and amino acids and boost nutrient availability in multi-ingredient broiler feeds like maize-SBM and wheat-SBM (9). Studies on low-crude-protein diets are being done to determine how to combine economic, environmental, and productive variables. However, when the diet's crude protein (CP) content is decreased, various factors, such as the reduction of significant amino acids, must be evaluated. Growth traits of broiler and blood measurements are significantly affected, However, threonine addition to a low crude protein diet boosts

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weight gain, feed intake, and serum profile even when CP content is reduced 2.5 % less than natural (10). Understanding chicken performance requires knowledge of both the chicken GH and GH receptor, and determining the outcomes of decisions due to their vital functions in growth, by examining the organic phenomenon patterns and growth characteristics of modern chicken with hereditary broilers that match those of meat-type birds before intensive selection, a unidirectional diagrammatical structure may be seen. Consequently, the primary purpose of this study was to determine how kemzyme-supplemented low-protein diets will affect the growth performance and biochemical parameters of broiler chickens (11).

Methodology

Three hundred broiler chicks (Ross 308) that were one day old were utilized in this experiment. They were purchased from a commercial hatchery in the Iraqi city of Al-Diwaniyah. The University of Qadisiyah's college of veterinary medicine's chicken farm served as the site for this experiment. The area was fumigated with a professional disinfectant and left for two days, windows were opened, and all ventilators were switched on before chicks arrived to totally remove any leftover dangerous fumes, before sanitizing the floor, walls, and ceiling with clean water and sodium hypochlorite. Feeders and waterers were cleaned and sterilized before being handed to the different groups. The floor of the hall was properly littered, and other elements, such as the lights and ventilation, were kept in good working order. Chicks were vaccinated against New Castle sickness, Gumboro diseases, and IB on days seven and twenty-two (at 7th and 22th day).

Table (1) vaccination program

Age of chicks / Day	Disease	Type of vaccine	Administration rout
7	ND+IB	Clone 30+IB Intervet (Netherland)	Dropping
14	Gumboro	Bio-Med (India)	Via drinking water
22	ND+IB	Clone 30+IB Intervet(Netherland)	Via drinking water

On a daily basis, the health of the chicks was observed. The experimental setup will last for 35 days, with the number of birds divided into (3) groups with (90) birds in each group and (3) replicates depending on the amount of protein that satisfies the nutritional needs of the broiler according to (12), and the nutritional makeup of the starter, grower, and finisher to ascertain the effect of feeding programs with different CP levels (CP-3%) lower dietary CP content (table 2). using kemzyme as directed by the manufacturer, at a dosage of 500–1000 gm/ton (Kemin technical literature).

Al-Qadisiyah Journal of Veterinary Medicine Sciences
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www.qu.edu.iq/journalvm

Table (2) Components of study nutrition

Components %	Starter	Grower (Control) CP	Grower CP-3%	Finisher Control CP	Finisher CP-3%
Yellow corn	25	18.8	36.5	20.5	40
Wheat	30.2	37.3	31	39.6	30.3
Soybean meal (48%)	30.1	27.3	17.5	25	14.6
Protein concentrated	10	10	9	10	9
Dicalcium phosphate	1.1	1.9	1.2	0.67	0.95
Salt(NaCl)	0.3	0.3	0.3	0.3	0.3
Oil	2.6	2.8	2.6	2.8	2.6
Vit.Mineral premix	0.2	1.21	1.1	0.76	1.47
Lysine	0.22	0.16	0.48	0.16	0.47
Methionine	0.28	0.23	0.32	0.21	0.31
Calculated chemical analysis					
Metabolize energy (Kcal/Kg)	2900	3000	3000	3025	3025
Crude protein (%)	21.6	20.8	17.8	19.8	16.8
Calcium	8.0	6.5	6.5	5.5	5.5
Phosphor	6.2	5.5	5.5	4.2	4.1
Lysine	11.5	10.5	10.5	9.9	9.9
Methionine	5.6	5.0	5.5	4.7	5.2

Productive parameters

At the starting, middle, and the end of the study, broiler body weights (BW) were determined to all birds (1 day, 21 days, and 35 days, respectively). Each meal's history was preserved. For each bird, the average of daily gain , average of daily feed intake , and feed to gain ratio were determined while accounting for mortality. Two broilers with body weights close to the means were chosen from each replicate at the end of the experiment, weighed, and then put to death by cervical disruption (13).

Biochemical Parameters

The serum biochemical markers were measured following the procedure, from the wing vein, blood samples were collected using a disposable syringe for spectrophotometer and ELISA testing. The serum was then immediately separated, chilled for an overnight period at 4°C (in the freezer), then centrifuged as instructed for 5 minutes at (3000 rpm) (14).

Gene expression of cGH:

While -actin mRNA transcript levels were present, this method was used in accordance with the comparative Ct technique (Ct), normalised to the level of the control group. This was done in accordance with the suggestions of (15). The cGH gene was amplified for this purpose using the forward primers 5' CACCACAGCTAGAGACCCACATC 3' and the reverse primer 5' CCCACCGGCTCAAACTGC 3'. A internal reference gene (-actin) was employed as the forward primer (5' ACCCCAAAGCCAACAGA 3') and the reverse primer (5' CCAGAGTCCATCACAATACC 3') for the assay normalization.

Quantitative Reverse Transcription Real-Time PCR (RT-qPCR):

Al-Qadisiyah Journal of Veterinary Medicine Sciences
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www.qu.edu.iq/journalvm

RNA extraction:
Extraction of RNA was done according to the protocol of kit from ADDBio (Korea).

cDNA synthesis

A total of RNA (about 1 µg) was reversed transcribed to cDNA using the kit from ADDBio (Korea) as following: H2O 3 µl, 10 µl of 2x add script cDNA, 2 µl of dNTPs, 1 µl of random oligos hexamer, and 4 µl of RNA. The thermal conditions were 25 C 10 min for priming followed by 50 C for 60 minutes for reverse transcriptase (RT) and finally 80 C for 5 minutes to RT inactivation.

Quantitative Reverse transcriptase PCR (RT-qPCR) Preparation:

RT-qPCR amplification:

Initially, the amplification was achieved using AddScript RT-PCR Syber master (AddBio, Korea). The reaction was including 7 µl of H2O, 10 µl of AddScript RT-PCR, forward and reverse primers (1 µl) (0.5 pmol/20 µl), and 1 µl of cDNA. This was carried out for the internal reference gene in a same components and thermal conditions as following: 50 C for 2 minutes followed by 95C for 10 minutes for initial denaturation then 40 cycles of 95 C for 15 seconds, 60 C for 60 seconds. Melting analysis was also achieved as 95 C for 15 seconds, 60 C for 1 minute then +0.3C of 95C for 15 seconds.

Ethical approval:

The researchers obtained ethical approval from the research Ethical Approval Committee of the College of Veterinary Medicine, University of Baghdad.

Statistical analysis

By using SAS's one-way ANOVA statistical analysis tool, the data for growth performance, carcass attributes, and biochemical evaluation were examined (16).

Results

Productive parameters

The data are displayed in table (3); during the first week, In terms of BW and FI, none of the groups' differences were statistically significant, but after 3 and 5 weeks, G3 and the other groups' differences were ($P< 0.05$). BWG and FCR were also improved in G3..

Table (3) Effect of kemzyme supplementation on productive parameters in broilers

Productive parameters	G1[1]	G2[2]	G3[3]	LSD
B.W (g)				
1st w	121.8 ± 2.81	112.5 ± 2.88	131.8 ± 2.28	0.075
3rd w	566.5 ± 12.3 ^{Ac}	481.3 ± 15.87 ^{Bc}	607.7 ± 11.26^{Ac}	
5th w	2004.2 ± 24.04^{Af}	1957.1 ± 30.53 ^{Ac}	2241.2 ± 27.81^{Cf}	
Feed intake (g)				
1st w	115.4 ± 1.69 ^{Aa}	112.8 ± 3.87 ^{Aa}	112.2 ± 4.70^{Aa}	0.05
3rd w	522.6 ± 3.61 ^{Ac}	526 ± 6.40 ^{Ac}	518.2 ± 3.18^{Ac}	
5th w	964.2 ± 10.1 ^{Ac}	935.4 ± 18.3 ^{AB}	901.6 ± 40.01^{Af}	
Weight gain (g)				
1st w	121.8 ± 2.81 ^{Aa}	112.5 ± 2.88 ^{Aa}	131.8 ± 2.28^{Aa}	0.05
3rd w	617.5 ± 18.13 ^{Ac}	495.3 ± 21.41 ^{Bc}	592.7 ± 13.42^{Ac}	
5th w	698.2 ± 18.16 ^{Ae}	600.2 ± 5.99 ^{Be}	740.8 ± 17.07 ^{Ce}	
FCR				
1st w	1.10 ± 0.009 ^{ABa}	1.15 ± 0.01 ^{Ab}	1.07 ± 0.006^{Ba}	0.075
3rd w	1.50 ± 0.04 ^{Ab}	1.60 ± 0.008 ^{Bc}	1.39 ± 0.02^{Cb}	
5th w	1.38 ± 0.04 ^{Ac}	1.38 ± 0.04 ^{Ac}	1.22 ± 0.02^{Cc}	

(1) **control**

Al-Qadisiyah Journal of Veterinary Medicine Sciences
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www.qu.edu.iq/journalvm

(2) (- 3%) protein
(3) (- 3%) protein + kemzyme supplementation

Biochemical Parameters

Total protein and albumin recoded significantly ($p<0.05$) higher values in G3 compared with G2, while there is no significant different with control group (Table 4).The activity of liver's enzymes (AST and ALT) enzymes no significantly values, and Kidney function parameters (Uric acid and Creatinine) show no significant between all groups (Table 4).

Table (4) the effects of Kemzyme supplementation's on broilers' biochemical traits

Biochemical parameters	G1[1]	G2[2]	G3[3]	LSD
Total protein g/dL	4.027±0.19 A	3.521± 0.10 B	4.032 ± 0.06 A	0.354
Albumin g/dL	2.10 ± 0.08 A	1.93 ± 0.08 A	2.21 ± 0.18 B	0.187
ALT U/mL	15.07 ± 1.8	15.57 ± 1.12	14.22 ± 0.75	3.63
AST U/mL	24.51 ± 1.42	25.95 ± 1.75	25.33 ± 0.58	3.49
Uric acid mg/dL	5.30 ± 0.43	5.41 ± 0.08A	4.87 ± 0.18	0.79
Creatinine mg/dL	1.39 ± 2.39	1.38 ± 2.82	1.20 ± 0.08	0.27

(1) control
(2) (- 3%) protein
(3) (- 3%) protein + kemzyme supplementation

cGH gene expression

The amplification of the assay

Results in figure (1) show successful amplification of both genes and indicating successful RNA extraction and cDNA synthesis.

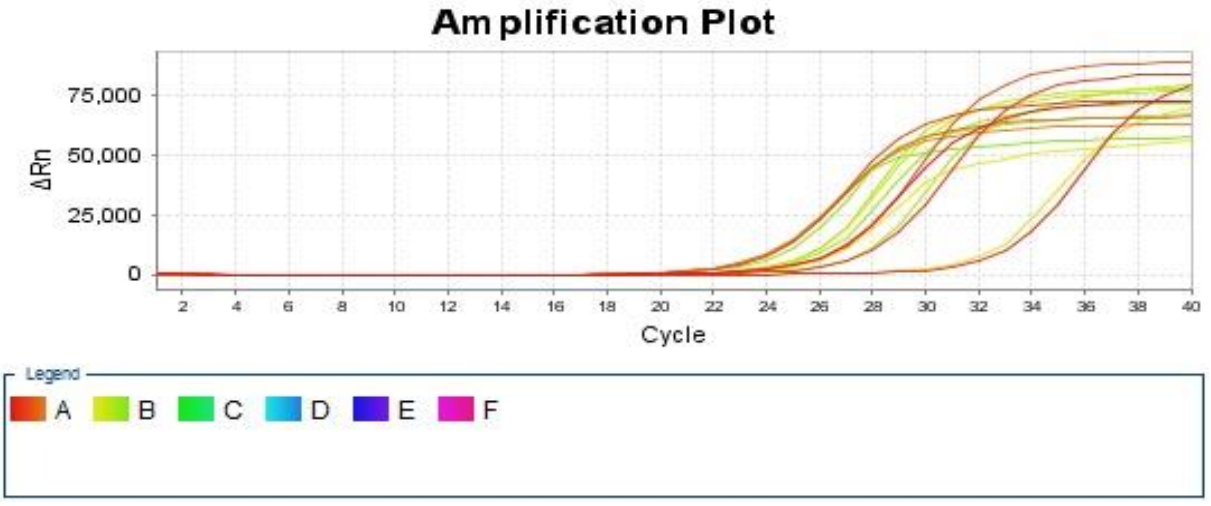

Figure (1) amplification curve of the tested samples represents the cGH gene of interest and β-actin as an internal reference gene

Al-Qadisiyah Journal of Veterinary Medicine Sciences
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www.qu.edu.iq/journalvm

while figure (2) show melting curve and analysis of the amplified products of both cGH gene of interest and β-actin as an internal reference gene. This shows an efficient without non-specific amplification.

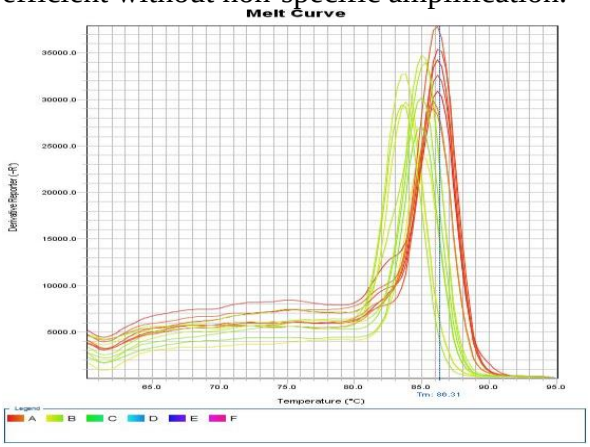

Figure (2) melting curve analysis of the amplified products of both cGH gene of interest and β-actin as an internal reference gene

Analysis of the qRT-PCR gene expression data

Comparison between the groups that shows a significant fold change of the tested groups in comparison with control.

Table (5) fold changes values of groups

Gropes	Fold changes
G1	17.87 ± 0.03 B
G2	17.38 ± 0.11 B
G3	19.44 ± 0.29 A
LSD	0.553

(1) **control**
(2) **(- 3%) protein**
(3) **(- 3%) protein + kemzyme supplementation**

Capital letters vertically refers to a significant differences (P<0.05) among groups

Discussion

Obviously from the study that in terms of BW, WG, and FCR, the group receiving kemzyme supplements outperformed the other groups. Numerous researchers have found that adding kemzyme to a diet increased the live body weight (17 and18). The insufficient enzyme activity and enzyme dosage utilised in the study by (19) can be blamed for the lack of impact that multi-enzyme addition had on live body weight. Multienzyme supplementation has been demonstrated in the past to raise BWG and FCR while lowering feed intake (20). According to (21), the higher BW and BWG brought on by The higher nutritional availability and absorption caused by the consumed foods' increased digestibility may be related to multienzyme supplementation (22 and 23). But the results of multienzyme depend on the type of enzyme and the composition of the food (24). The feed conversion ratio is raised by hydrolzation the Vegetable NSP components in foods by exogenous enzymes for simple stomach consumption (25). The most recent findings are reliable, (26), They found that adding the xylanase enzyme to broiler diets including de-oiled rice bran caused a noticeably ($P < 0.05$) higher increase in body weight compared to the control group. Adding kemzyme to broiler diets raised the feed conversion ratio across all trial times in a significant ($P < 0.05$) manner. table (4-6). The reverse of (27), who observed even with the addition of enzyme xylanase supplements, a decrease in energy and protein in the diet leads to reduced serum total protein, albumin, and globulin levels AST and ALT were unaffected by kemzyme supplementation and low energy protein diet; the decline in blood biochemical on the supplementation of enzymes in the current study perhaps caused by a low-energy protein levels. Our results are consistent with those of numerous investigations of (28 and 29) study showed a significant decline in UA was seen by broiler chickens fed low-CP diets. Growth hormone (GH) regulates the production of the insulin-like growth factor-I (IGF-I), then it is released into the

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

bloodstream. This is the main method by which GH, which regulates growth, exerts its effects (30). Thyrotropin-releasing hormone (Stimulatory), somatostatin, and development hormone-discharging factor (Stimulatory) (Inhibitory)are the three peptidergic discharging factors that influence to the release of GH in poultry (31). Lower plasma IGF-I levels have been associated with protein or energy deficiency, which slows growth (32). While food supplements containing arginine, cysteine and methionine boosted young chickens on a high-protein diet had higher plasma IGF-I levels and gained weight. (33), there was also a positive impact of select plants on the expression of the cGH genes (34).

Conclusions

The current study revealed significant differences in growth performance, biochemically enhance total serum and albumin, and no effect of liver and kidney functions enzymes, the supplementation with kemzyme has a positive effect and increase in folds changes of the cGH genes expression.

Conflict of Interest: there is no conflict of interest.

Acknowledgments

The study was reinforced by the university of Baghdad / College of Veterinary Medicine and with support for laboratories from the College of Veterinary Medicine, University of Al-Qadisiyah

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