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Expression of Genes Related to Growth Hormone of Broiler Chicken using Phytase Enzyme

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ABSTRACT

Background: The goal of this study was to investigate the gene expression that correlated to growth hormone in two groups of broiler chickens that fed of phytase in diets.

Methods: In this experiment the broiler chickens divided into two groups: Control (basal diet) and treated group, (addition of phytase enzyme, 900 U/kg. Arbor Acres chicks were fed experimental diets from 14 to 28 day (N=500 chick, 250 chick each group). Gene expressions (GH, IGF-I) related to growth hormones were evaluated using the ACTB gene as a reference. At 28 days of age, blood samples were obtained to measure the gene expression of the growth hormone.

Result: In comparison to the other group (control), the best results were obtained when baseline feed containing 900 U/kg of phytase enzyme was used to increase the weight of the chicken. Findings of this study may be used to guide future efforts to increase broiler weight while using less feed.

Key words: Arbor acres broiler, Gene expression, GH, IGF-I genes, Phytase enzyme.

INTRODUCTION

Phytase enzymes are mostly used in poultry feeds to release phosphorus from phytic acid in materials produced from plants (Ravindran *et al*., 1995). Several phytase enzymes have been commercialized in recent years and they can be obtained from a variety of sources (Liu *et al*., 1998). Exogenous enzymes have been shown to boost nutrient utilization and, as a result, broiler chicken growth performance when added to diets (Bedford, 2018). Nevertheless, the way that various enzymes react is not always consistent, however, the reactions of different enzymes are not always the same and can vary based on the type of food ingested (Bedford, 2018). Feed enzymes have been utilized extensively to enhance animal development and nutrient absorption. But according to recent research has shown that, feed enzymes-more especially, phytase and xylanase may improve the intestinal health and microbiota of broiler hens (Moita, 2022). Phosphorus is released from the phytate compound by the exogenous digestive phytase enzyme. Since phytic acid and its salts contain the majority of the phosphorus stored in cereal grains, a significant portion of the phosphorus found in poultry feed is in the form of phytate Phosphorus, which is not readily absorbed by the digestive tracts of several poultry strains (Selle and Ravindran, 2007). Exogenous phytase enzyme supplementation has, however, significantly improved the consumption of phytate-bound Phosphorus and decreased Phosphorus excretion; furthermore, additional data indicates that phytase also enhances protein and energy utilization (Marchal *et al*., 2021). Arbor Acres broilers were shown to have superior meat quality features and higher dressing percentages when compared to other strains (Wang *et al*., 2020). Because these qualities can be important selling advantages in competitive marketplaces, this is especially advantageous for farmers who prioritize meat quality. Additionally, research has been done on dietary approaches that improve the overall quality of meat and muscle properties. It has been demonstrated that Arbor Acres broilers have genetic predispositions that enhance the quality of their meat, highlighting the necessity of carefully adjusting the diet to optimize these qualities (Qiu *et al*., 2021).

Growth hormone (GH) and the IGF-I gene have a major impact on animal growth rates (Paswan *et al*., 2016). Kansaku *et al*. (2008) state that the chicken cGH contains 4,101 base pairs, consisting of four introns and five exons. The pituitary gland produces and secretes cGH, a polypeptide hormone that influences several physiological processes related to growth performance (Apa *et al*., 1994). According to several papers, the growth hormone gene is one of the best important genes influence the performance

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traits of chickens. It affects metabolism and growth rates (Vasilatos-Younken *et al*., 2000). Similarly, IGFs are produced in various tissues and circulate in the bloodstream, where they can also bind to receptors on target cells. Upon binding, IGFs can activate gene expression programs that promote cell growth, differentiation and survival. The interaction between growth hormones and IGFs is complex, as they often work together to regulate growth and metabolism. The emphasis is on circulating hormones, local growth factors andgene transcription factors which regulate growth and differentiation of skeletal muscle, bone andadipose tissue (Hossner, 2005). Growth rates are significantly influenced by growth hormone (GH) and insulin-like growth factor I (IGF-I). In slow-growing hens at various ages, we sought to examine the impact of GH and IGF-I genotypes on body weight (BW), dominance and gene expression. In order to enhance chicken production performance, selection molecular markers can be used as a technique to speed up the genetic response of desirable features (Rayan and Rezk, 2025). The purpose of this study was to investigate how the genotypes of GH and IGF-I affected the expression of genes and body weight in chickens given a higher dosage of the phytase enzyme in broiler feed.

MATERIALS AND METHODS

Broiler management

This research was carried out at Poultry Breeding Research Farm, Poultry Production Department, Faculty of Agriculture, Ain Shams University. This experiment was conducted during the winter season from November to December 2024. A total of Five hundred one-day-old Arbor acres broilers chicks were reared from one to twenty-eight days. The experimental was divided into two groups: Control as a basal diet and treatment (250 birds in each group).

Nutrition and feeding program

The chickens were fed ad libitum pelleted feed in standard conditions. Throughout their starter and grower periods (Table 1). The trial period was in between 14 to 28 days. The chicks were divided in two groups, the experimental groups were control group which was basal feed (commercial diet) and the treatment which was the basal feed with extra phytase enzyme addition (900 U/kg). Feed and water for all birds were provided ad libitum. Routine vaccination, medication and management practices were followed.

Productive traits

Growth performance

Body weight and body weight gain

The difference between two consecutive records was used to determine body weight increase and the formula for body weight gain (g) = Final body weight at finish period-Initial body weight at start was used to calculate the cumulative average body weight gain (BWG) for each replicate per group. At 1, 7, 14, 21 and 28 days of age, the body weight was recorded.

Feed conversion ratio and feed consumption

Cumulative feed consumption (FC) was calculated for each group (calculated as the difference between the amounts of feed supplied to the birds and the amount of feed that residue at the end of feeding period) according to the following formula:

$$FC\ (g/bird) = \frac{\text{Offered feed - Residual feed}}{\text{Two groups}}$$

Feed conversion ratio (FCR) was calculated and recorded by the total cumulated feed consumed per bird by cumulated weight gain according to the following formula:

$$FCR = \frac{\text{Sum of the birds' feed intake (g)}}{\text{Their live weight growth (g)}}$$

Molecular genetics

Gene expression (GH, IGF-I) correlated to growth hormones were evaluated using the β-actin ACTB gene as a reference. Blood samples were taken on twenty-eight day of age for Growth Hormones genes expression as in (Table 2).

Material used for cDNA synthesis

The Maxima® First Strand cDNA Synthesis Kit for RT-qPCR (#K1641, Thermo Scientific) is specifically designed for two-step real-time quantitative RT-PCR applications. The kit employs Maxima Reverse Transcriptase, an engineered variant of M-MuLV RT with enhanced robustness, thermost-ability and catalytic efficiency compared with the wild-type enzyme. This allows for highly efficient and reproducible cDNA synthesis over a wide range of RNA inputs (1 pg-5 µg) and at elevated reaction temperatures (50-65°C). The reverse transcription process is rapid, typically requiring only 15-30 minutes.

Material used for RT-PCR

Using the Maxima SYBR Green/ROX qPCR Master Mix after reverse transcription procedures (RT-qPCR) with varying starting RNA transcript dosages (5 ng to 0.5 fg), Thermo Scientific's Maxima SYBR Green qPCR Master Mix (2X) kit number K0251 was amplified. The correlation coefficient is higher than 0.99, the slope varies from -3.09 to -3.58 and the reaction efficiency ranges from 90 to 110 percent. The genotypes of GH and IGF-I were ascertained by the polymerase chain reaction (PCR) employing restriction fragment length polymorphism (RFLP). Table 2 lists the forward and reverse primers, enzymes and annealing temperatures for the GH and IGF-I gene PCR-RFLP.

Statistical analysis

The general linear model (GLM) was used to perform a one-way analysis of variance on the data of (SAS, 2006). The model generated was fitted for the effect of treatment as a main effect:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where,

µ= Overall mean.

T_i= Treatment effect.
e_{ik}= Experimental error.

RESULTS AND DISCUSSION

Productive traits

Body weight and body weight gain

The present results in (Fig 1 and 2) show significant variances (P<0.05) in cumulative body weight gain and body weight between the treatment and control groups of broiler chicken. The treatment group recorded the heaviest body weight at two and four weeks of age in comparison the other group. Likewise, the highest body weight gain was recorded by treatment group from 15 to 28 day of age (P≤0.05). As is well known, a lot of emphasis has been paid recently to the use of microbial phytase to release phytate-bound phosphorous and rise its overall availability in chicken diets. According to certain published research, broiler performance can be enhanced during the whole growth period by supplementing with phytase (Selle and Ravindran, 2007). The showed increase in body gain and feed conversion in phytase treatment across the entire raising period in the current investigation validated the anticipated effects of varying phytase doses. According to the current study, phytase enzymes improved the amounts and availability of phosphorus in the grower and finisher stages of broiler chickens. These findings concurred with those of Tazi *et al*. (2009), who found that adding phytase to broiler diets dramatically increased body WG and FCR. When broilers are fed more phytase, their growth performance may improve due to improvements in metabolizable energy and essential amino acids (Ravindran *et al*., 2000). Protein, energy, digesting enzymes and amino acids that were chelated with phytic acid may be released by diets that contain phytase. These factors would undoubtedly enhance broiler growth performance (Lelis *et al*, 2012). A wide range of phytase enzymes are now commercially available. Furthermore, it has been demonstrated that adding exogenous enzymes to diets enhances nutrient utilization, which benefits broiler chicken development performance (Bedford, 2018). Nevertheless, the way that various enzymes react is not always consistent and it may change depending on the kinds of foods that are consumed (Bedford, 2018).

Feed consumption and feed conversion ratio

The Results of feed intake and feed conversion ratio are showed in (Fig 3 and 4). The significant variances (P<0.05) in both cumulative feed consumption and feed con-version ratio among the two groups of broiler chicken were observed. The treatment group consumed less feed compared to control one from 15 to 28 days of age. Likewise, the treatment group had a better FCR comparison to the control group throughout the trial period. Enzymes can have a major impact on FI throughout the finisher phase due to the larger amounts of enzymes in older broiler intestines and their greater efficacy in using phytate phosphorus. These findings concur with the Nelson *et al*. (1968) research.

Gene expression of GH gene and IGF-I gene

The findings in Table 3 demonstrated that GH and IGF-I have an impact on the growth of broiler chicks. Because the homozygous genotype had the maximum body weight for the treatment group, GH and IGF-I can be employed as marker genes in growth selection systems. In comparison to the control group, it seemed that the GH and IGF-I genes were significant (P<0.01) in the treatment group. Though

Table 1: Composition and quantitative evaluation of the foundational diets for starters and growers.

Ingredients (%)	Starter 0: 14 days	Grower 15: 28 days
Yellow corn	52.96	55.96
Soybean meal	33.00	32.10
Corn gluten meal	7.00	5.00
Soybean oil	2.40	2.80
Monocalcium phosphate	0.96	2.18
Limestone	2.50	0.88
HCL-Lysine	0.28	0.21
D-L Methionine	0.30	0.27
Salt	0.30	0.30
Premix*	0.30	0.30
Total	100.00	100.00
Price $ /(ton)***	43	41
Calculated composition		
Metabolizable energy (Kcal/kg)	3017.48	3050.29
Crude protein (%)	23.07	21.57
Crude fiber (%)	2.53	2.53
Calcium (%)	0.97	0.87
Available phosphors (%)	0.49	0.44
Lysine (%)	1.40	1.29
Methionine (%)	0.666	0.61
Methionine + cysteine (%)	1.09	0.99

The experimental diets were calculated and analyzed in accordance with (Rostagno *et al*., 2017). *** Cost in Data 2024.

Table 2: Primer sequences and their applications.

Gene	Primer (forward/reverse)	AT (°C)	PCR product (bp)	Enzyme
cGH	5′-TCCCAGGCTGCGTTTTGTTACTC-3′	65	429	*Eco*RV
	5′-ACGGGGGTGAGCCAGGACTG-3′			
IGF-I	5′- TCAAGAGAAGCCCTTCAAGC-3′	60	813	*Hinf*I
	5′-CATTGCGCAGGCTCTATCTG-3′			

The genes for chicken growth hormone (GH), insulin-like growth factor (IGF-I) and polymerase chain reaction (PCR) (Nguyen *et al*., 2015).

growth hormones (GH) and IGF-I are the primary hormones needed to sustain proper growth, a number of genes are involved in growth traits (Kita *et al*., 2005). GH is involved in numerous physiological processes, including as growth, body composition, egg production, aging and reproduction (Su *et al*., 2014) and it plays a critical role in growth and metabolic rates (Anh *et al*., 2015). The expression of GHR gene was more than 3-fold higher in dwarf chickens,

Table 3: The mRNA abundance of the IGF-I gene and cGH of broiler.

Item	Treatment	Control	Significance
Fold change of cGH	2.38^{A}+0.09	1^{B}+0.002	*
Fold change of IGF-1	1.30^{A}+0.04	1^{B}+0.003	*

A, B * Significant at P<0.05; NS = Not significant; over-all within a row with distinct superscripts are significantly different. Sample size = 6\treatment\gene= 24 sample.

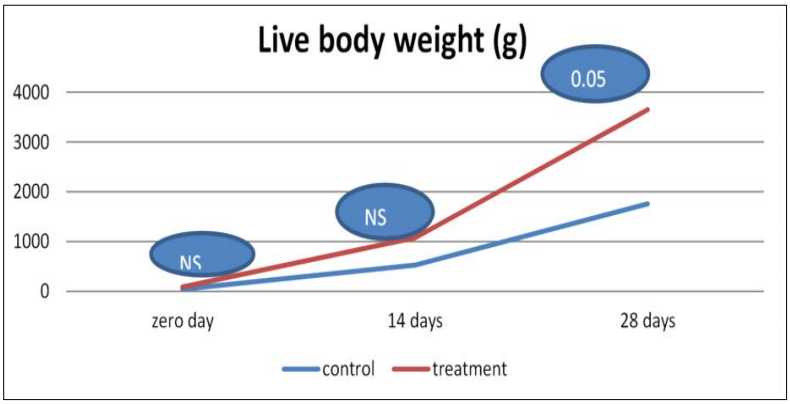


Fig 1: Live body weight of the studied two groups (from 0 to 28 d of age).

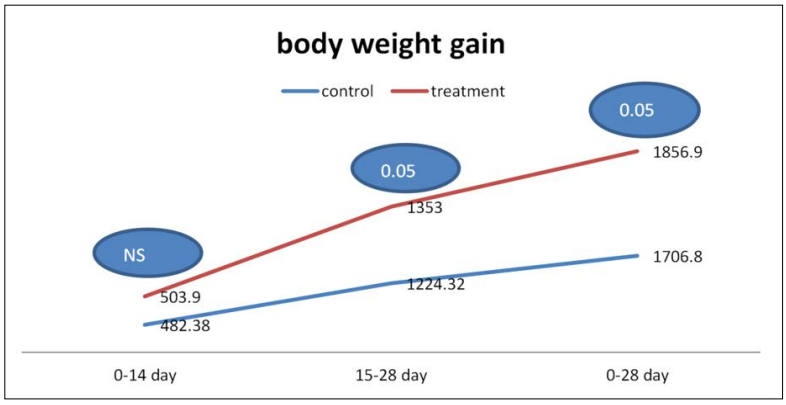


Fig 2: Body weight gain of the studied two groups (from 0 to 28 d of age).

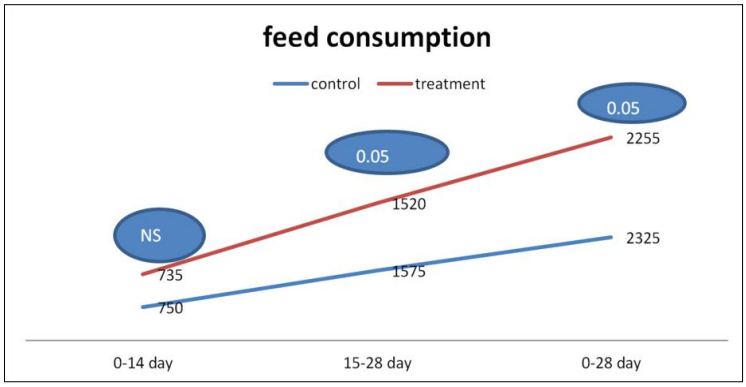


Fig 3: Feed consumption of the studied two groups (from 0 to 28 d of age).

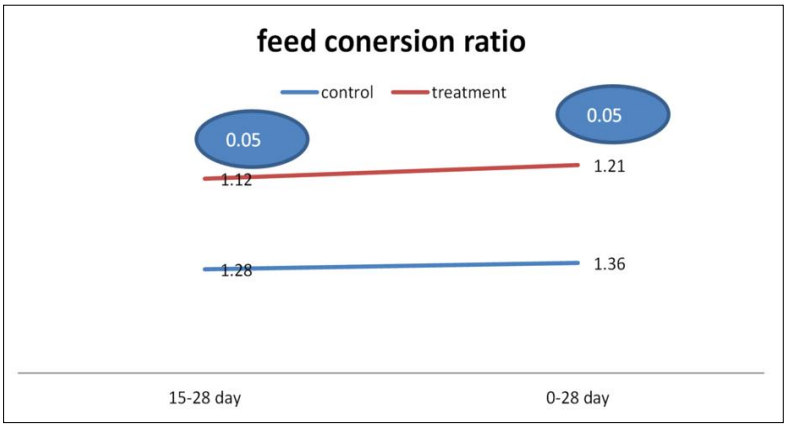


Fig 4: Feed conversion ratio of the studied two groups (from 0 to 28 d of age).

compared with that of the normal. However, the transcription level of IGF-1 decreased dramatically in the sex-linked dwarf. These observations suggest that the dwarf phenotype occurs independent of GH action, but is due to a dysfunctional GHR. A 1.7 kb deletion has been reported in exon 10 and the 3'untranslated region (UTR) of the GHR gene in dwarf chickens. This transcript could be translated into a protein that does not retain its normal function, which would ac-count for the absence of GH-binding activity in liver membranes of the dwarf chickens (Gui-Qin *et al*., 2007). IGF-I is a hormone with multiple metabolic and anabolic functions that shares structural similarities with insulin. For chickens to grow normally and generate bone and fat tissue, this hormone is essential (Boschiero *et al*., 2013).

CONCLUSION

In conclusion, the current results indicate that the inclusion of additional phytase enzyme in the diet, without increasing feed costs, was associated with some improvements in broiler performance. The gene expression findings provide supportive evidence that may help explain these effects in the treated group.

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Ethics statement

The researchers followed all national regulations governing animal care as well as the guidelines established by the relevant ethics committee. This experiment was conducted at the Poultry Breeding Farm, Faculty of Agriculture, Ain Shams University.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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