

Study of genetic correlation coefficient of gene expression levels OF growth hormone receptor gene in broiler Ross-308

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Abstract :

This experiment conducted in poultry farm of the Animal Production Department / College of Agriculture – University of Kuf , from 9/23/2024 to 10/27/2024, for a period of 35 days. broiler chicks of 240 unsexed broiler Ross - 308 breed were divided into four treatments and three replicates per treatment, chicks were fed on standard leeches to which different proportions of nanomagnesium added the following proportions T1(0 mg/kg /diet) T2 (1 mg/kg /diet) T3(1.5 mg/kg /diet) T4(2 mg/kg /diet), open feeding with water and lighting available for 24 hours, RNA extracted from samples mashing (100 mg) taken from chicken chest area after slaughter at marketing, GAPDH initiator used to compare cycle thresholds (CT) between samples and estimate gene expression levels of growth hormone receptor gene, results of statistical analysis according to livak and schmittgen method showed, since individuals of T1 treatment (no addition of nanomagnesium) showed a significant superiority of $P \leq 0.05$ in the average values gene expression over all individuals of experimental transactions T2 and T3 and T4 (addition treatments of 1, 1.5 and 2 mg/ kg magnesium nanoparticles), while addition coefficients among them not show any significant differences in the average values gene expression a negatively affected due to those additives targeted to improves gene expression of growth hormone receptor gene in muscle mass of chest area of Ross-308 broiler. presence of a negative correlation coefficient between the values of regression and both gene expression in the trait of live body weight and average total weight gain (-63.446, -63.575) over 35-day breeding period. The values of determination coefficient (square of correlation coefficient) are the values of correlation coefficient (the values of constancy coefficient) ranging from questionable to weak and unacceptable. According to results, feed additives of nanomagnesium particular contributed to changes in genetic functioning of herd members.

Key words; Gene expression, Growth hormone receptor gene ,Magnesium Nano particular, Genetic correction coefficient,Epigenetic Effect.

Introduction:

Regulatory genes carry out gene expression of synthetic genes (their function is to encode synthetic proteins, enzymes and receptors that contribute to metabolic processes inside cell), and with encoded genetic information carry, contribute to an effective role in regulating gene expression (repeating gene expression, stopping it at a certain stage or changing its course to another product of essential proteins)

throughout life of cell (1), and functional role as service genes is supplemented with luxury or smart genes (luxury Genes) in differentiation of gene expression final product of essential proteins in cells of some niche (2). Is a functional integration of army growth hormone when it relates to one of its receptors, which stimulates the release of a wealthy collaborators mothers (Triiodothyronine-T3, Thyroxine-T4) and

growth factors like insulin to achieve expected growth in Chicks (3), and in particular to growth hormone with receptors mainly affects direct impact on growth and development, metabolic processes, and any change in genotype does lead to a change in level of gene expression and therefore the characteristics of growth and development in these individuals (4) chicks significantly outperformed average breast mass weight of fast-growing genotype individuals compared to slow-growing individuals. (5), depending on type of goods and their genetic and multiple environmental conditions have a (6,7), as identified varying levels of gene expression that have an impact on growth performance in the safety of chicken meat (8), Studied correlation coefficient between those authorities and the members of authority itself, as some of hormones responsible for growth in chickens, particularly the performance of gene growth hormone and its receptors (expressing gene) show correlation coefficient hereditary positive with the rate of body weight in Chicks Chicken Meat (9), and a constant exchange of gene expression returns to various factors, including the programmes and election long-term characteristics of the target page in election program (10), And also enhances mineral elements rare gene expression for its contribution in many biological functions important in maintaining growth performance and resistance to free radicals and support level of organization (11,12), so not allow the ideal environment only to express its potential genetic, noting (13) existence of significant variation in levels gene expression (performance and production) to members of Froggy meat-commercial use under study (roses, hearts, and Ariane) jam under similar conditions in Iran. Nanoparticles are interest in production of farm animals, including

poultry, due to their chemical and physical properties, and are used in various applications in nutrition. an improvement in poultry performance parameters has been observed after this modern technology recently integrated into poultry industry (14). addition, nanotechnology used in various aspects of livestock production to improve quantity of their products (15). Researchers have proposed their use in animal production and poultry feed additives to improve various aspects due to their antibacterial, antifungal and immunostimulating capabilities (16 ,17 ,18), metal oxide nanoparticles enjoying great interest due to their unusual properties compared to their pure counterparts for two reasons, subject of interest is phenomenon of surface effects and quantum effect (19), nanometallic oxides produced according to clean and environmentally friendly programs have received great attention and a wide range of vital applications (20), including breeding and improvement of broiler, nanotechnology is currently being used to improve antigens capable of targeting virulence factors without harming the components of living cells synthesis of metal nanoparticles represented a new alternative to chemical and physical methods, as they are used to meet need of body and as antioxidants (21).

The aim of the current study is to study the genetic correlation of gene expression levels of growth hormone receptor gene of individuals of Ross-308 broilers when presenting enhanced relatedness with different levels of the nanomagnesium particular .

Material and method:

This experiment carried out in poultry farm of the Animal Production Department, College of Agriculture, University of Kufa, for a period of 35 days, starting from September 23 to October 27, 2024. The experiment included

240 unsexed broiler chicks (Ross-308), one day old, with an average live body weight of 41 g. Were randomly distributed, into four treatments, with 60 birds per treatment, divided into three replicates (20 birds per replicate). Nano magnesium was added to the experimental feeds as proportion, T1 (0.0 mg/kg/ diet), T2 (1 mg/kg/ diet), T3 (1.5 mg/kg/ diet), T4 (2 mg/kg/ diet) respectively throughout of period 35 days. chicks Rose 308 strain raised in floor (cages) in a hall equipped

with a sufficient number of air vents, gas incubators, plastic feeders and troughs, in addition to lighting lamps and temperature and humidity measuring devices. Dry white sawdust used for the floor of hall to reduce risk of disease and 4 cm thick. A continuous lighting system (24 hours/day) and fed Ad libitum with three type's diets (starter 1-10 days, Grower 11-24 days and finisher 25-35 days).

Table 1 Composition of the feeds used in the experiment

Ingredient's ratio	Starter %	Grower %	Finisher %
Yellow corn ⁽¹⁾	60.92	64.82	65.78
Soybean Meal	34.01	30.82	29.92
Premix ⁽²⁾	0.30	0.30	0.30
Corn oil	0.71	1.00	1.44
Monophosphate calcium	0.81	0.42	0.11
Calcium carbonate ⁽³⁾	1.12	0.82	0.74
Amino Acid	1.85	1.54	1.43
Salt	0.28	0.28	0.28
Total	100	100	100
Chemical analysis			
Kcal/kg	2975	3050	3100
Crude protein	23.00	21.50	20.08
Total calcium	0.95	0.75	0.66
Phosphor availability	0.74	0.72	0.71
C/P ratio	129.3	141.8	154.05

(1) Soybean meal contains 44% protein and 2230 kcal/kg energy.

(2) Provimi3110 contains (2750 kcal/kg metabolizable energy 10% crude protein, 1.1% fat, 21% calcium, 11.0 phosphorus, 6.5% methionine, 6.5% methionine+cysteine, 4% lysine, 4.8% sodium, 5.4% chloride, (575,000 IU vitamin A, 201,250 IU vitamin D3, 1380 mg vitamin E, 138 mg K3, 138 mg vitamin B1, 345 mg vitamin B2, 1840 mg vitamin B3, 552 mg vitamin B5, 184 mg B6, 46 mg vitamin B9, 1000 mcg vitamin B12, 6900 mcg biotin, 14,000 mg choline chloride, 460

Copper amalgam, 2760 mg iron, 3680 mg manganese, 3680 mg zinc, 50 mg iodine, 9.2 mg selenium, 30000 mg phytase, 250 mg antioxidants, 250 mg lincomycin, 2400 mg salinomycin)/kg .

(3) Dicalcium phosphate containing: 22% inorganic calcium and 18% inorganic phosphorus .

(4) Nano magnesium was added at a rate of (1, 1.5, 2 mg per kg feed) for treatments (T2, T3, T4) respectively. The nutritional components of each material calculated using the NRC tables (1994) (22). and the

nutritional requirements according to the ROSS guide (2019).(

Experimental Animals and Tissue Samples: Samples were taken from chest area tissue of broiler in the amount of 100 mg by a sterile medical scalpel, and mashed

RNA extraction:

add 1 ml of GENEZOL to each sample (RNA extraction solution)

add 200 microliters of chloroform to samples and then mix them with substance for ten second put samples in a centrifuge for 15 minutes at a temperature of 4 centigrade to form granules of ribose lactic acid RNA extract leachate containing RNA and leachate consists of three layers * upper layer contains RNA take the leachate of container on RNA and precipitate by 500 microliters and add isopropanol in same amount and then the

samples are stirred until leachate mixes with substance isopropanol reacts with leaching except RNA is deposited.

gently discard the leachate to avoid RNA packet falling off , add to each sample 1 ml of ethanol alcohol at a concentration of 70% to purify RNA from suspended residues samples are then returned with a VORTEX machine to wash RNA packet samples are placed in a centrifuge for 5 minutes at a temperature of 4 Celsius, then gently discard leachate so RNA package does not fall, and then the abendrov is turned over on a sterile napkin until the sample dries from ethanol 20-50 microliters of RNase-Free Eater and incubate the samples after adding Water Bath for 1-10 minutes at a temperature of 55° . -60 °. After which RNA for applications

Table 2 One step CDNA and (RT-PCR)

Component	Volume
RNA Template	3 µl
Forward Prime(10µm)	0.1 µl
Reverse Prime(µm)	0.1 µl
2×perfect Start™Geen One- Step qPCRSuper Mix	10 µl
TransScript®Geenone-stepRT/RI Enzyme Mix	0.4 µl
RNase-Free Water	6.4 µl
Total volume	20 µl

Table 3. The primer sequences:

Gene	Forward	reverse
GHR	AGTCCGATCAAGACAACGTAC	CTAAGAACCAGGGAAACTCG
Gapd	TCCTCCACCTTTGATGCG	GTGCCTGGCTCACTCCTT

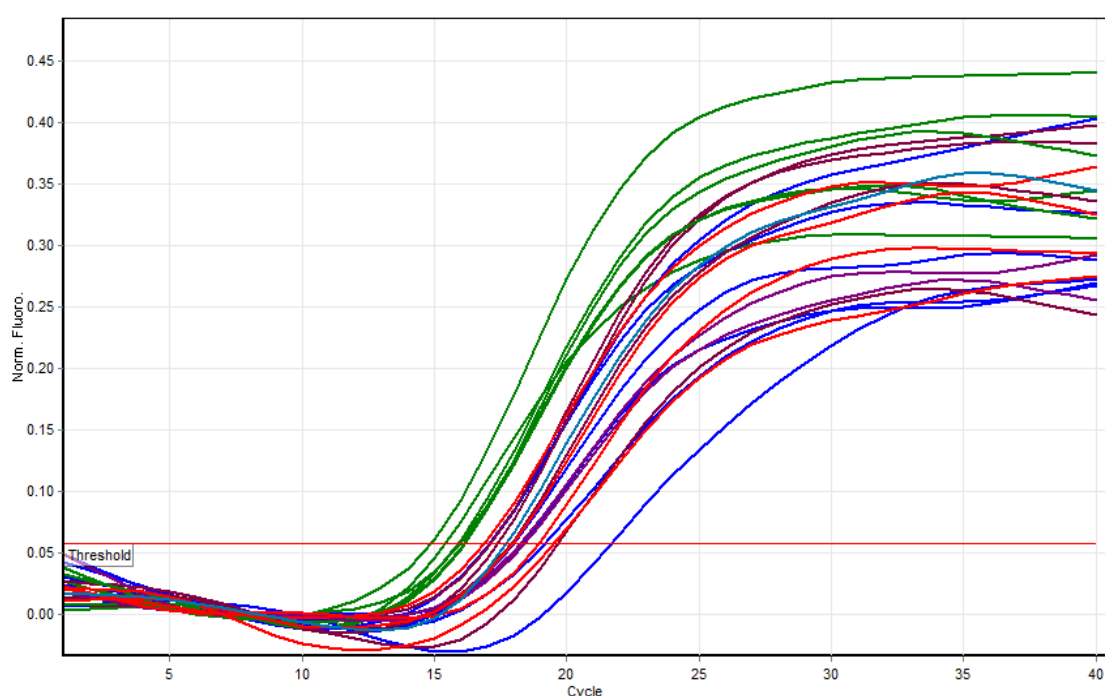
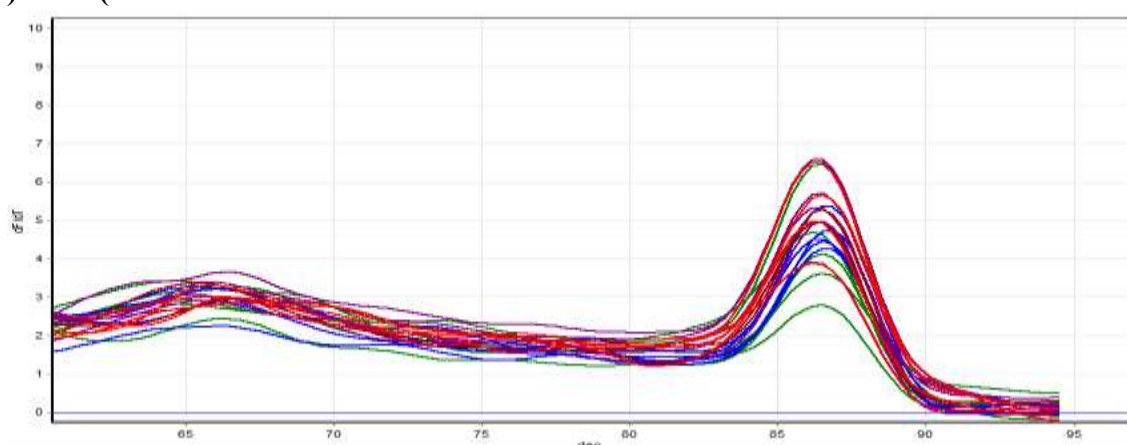


Figure:A Critical cycle (ct) curves for the target gene, growth hormone receptor gene) GHR(



FigureB : Growth hormone receptor gene expression estimation by rt-Q real time PCR, traces with green, blue, brown and red represent the experiment groups G1, G2 , G3 and G4 respectively. A amplification curves , B melting analysis curves .

Studied

-1 Productive traits

1- 1 gene expression of the growth hormone receptor gene

2-1 the effect of gene expression of the growth hormone receptor gene on some productive qualities of broiler ross308

Relative gene expression measurement:

characteristics;

As for the Cycle thresholds (CT) of the compared samples, GAPDH was used, according to the Livak method (2- $\Delta\Delta C_T$ and Schmittgen, 2001), and according to the following equation: $\Delta C_T \text{ reference} = \text{Thresholds } C_{\text{cycle of control}} - \text{Cycle thresholds of reference}$

$\Delta C_T \text{ sample} = \text{Thresholds Cycle of sample} - \text{Cycle thresholds of reference}$

$\Delta\Delta\text{CT} = \Delta\text{CT sample} - \Delta\text{CT reference}$

The final result is raised to the power of $(2^{\Delta\Delta\text{CT}})$.

Statistical analysis :

The data for the studied traits analyzed using a completely randomized design (CRD) to determine the effect of the different treatments. The significance of the differences between the treatments was tested using the (24)]. test at a probability level of 0.05 and using the statistical program (SAS The statistical analysis system, 2012) [13]. in the statistical analysis according to the following model:

$$Y_{ij} = \mu + t_i + e_i$$

Results and Discussion:

-1 - 1 gene expression of the growth hormone receptor gene

Table 4 shows the average gene expression values of growth hormone receptor gene for experimental coefficients T1, T2, T3 and T4 in the area of pectoral muscles of Ross - 308 broiler, where individuals of T1 treatment (no addition of the nanomagnesium element) showed a significant superiority of $P \leq 0.05$ in average gene expression values over all individuals of experimental coefficients T2, T3 and T4 (addition coefficients 1, 1.5 and 2 mg/ kg in addition, average gene expression values were negatively affected due to those additives targeted to enhance gene expression of growth hormone receptor gene in muscle mass of thoracic region of Ross - 308 broiler .

Table 4 average gene expression values of experimental coefficients when adding the nanomagnesium element to the Ross broiler Bush-308 $\pm$ standard error

Average gene expression values	Gene expression ranges between curves		Gene expression values for curves		Number of samples	Treatments
	maximum	minimum	maximum	minimum		
1.164 $\pm$ 0.675 a	2.22	0.44	1.870	0.450	12	T1
0.204 $\pm$ 0.210 b	0.54	0.06	0.383	0.024	12	T2
0.192 $\pm$ 0.950 b	0.30	0.05	0.314	0.230	12	T3
0.1924 $\pm$ 0.257b	0.20	0.14	0.225	0.170	12	T4

*

The vertically different small letters indicate significant differences between the mean values of gene expression of growth hormone receptor gene, at a significant level $P \leq 0.05$. N.S (No, significant) there are no significant differences. T1 (first treatment without addition), T2 (addition of nanomagnesium 1 mg/ kg / diet), T3 (addition of nanomagnesium 1.5 mg/ kg / diet) and T4 (addition of nanomagnesium 2 mg/ kg / diet) experimental transactions, respectively. data of Table 5 and Diagram 1 show that their results match and are similar to Table 4, that addition of

nanomagnesium (T2 1, T3 1.5 T4 2 mg/ kg) to Ross - 308 broiler and throughout breeding period, did not contribute to enhancing levels of gene expression of growth hormone receptor gene in building muscle mass of chest area, and these results may be due to the fact that nanomagnesium not one of factors involved in the mechanism of action of growth hormone receptor gene and is far from its hormonal path, or perhaps levels of addition of nanomagnesium were rather high, or it targeted gene that not studied in this experiment (insulin-like growth factors, myostatin,

another receptor for growth hormone gene, thyroid hormones), especially that duration of the addition (throughout the breeding period) may be inappropriate to enhance gene expression, or incompatibility in raising level of gene expression may be due to the (VIT.E, zinc) to make it more abundant in the metabolic processes supporting the gene

expression of the growth hormone receptor gene, or the nanomagnesium element may have to be present in a free Unbound form when added to the broiler meat, or the nanomagnesium element must be dissolved in drinking water or alcohol solution or be a mixture of saturated carboxylic - hydrogen bonds.

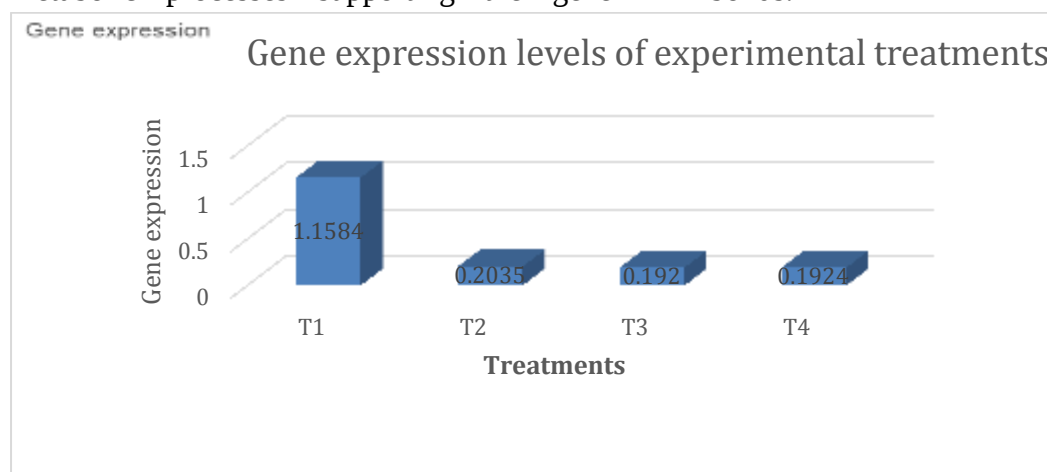


Diagram 1 gene expression levels of growth hormone receptor gene in chest area of Ross - 308 broiler when level of added Nano- magnesium to Bush.

Table 5: Effect of adding nano magnesium to Ross-308 broiler feed on expression levels of growth hormone receptor gene

Gene expression values for arrays		experimental error	Average variance ($M_1 - M_2$)	M ₁	M ₂
Maximum	Minimum				
1.38	0.532	0.203 ±	*0.955	1	2
1.40	0.543		*0.974		3
1.40	0.542		*0.970		4
-0.531	-1.38	0.203 ±	*- 0.955	2	1
0.435	-0.411		0.012		3
0.434	-0.412		0.011		4
-0.543	-1.40	0.203 ±	*-0.974	3	1
0.412	-0.434		-0.012		2
0.422	-0.432		-0.00		4
-0.542	-1.420	0.203 ±	*-0.970	4	1
0.412	-0.434		-0.011		2
0.423	-0.423		0.000		3

It indicates that there are significant differences between the mean values of the gene expression of the growth hormone receptor gene, at a significant level $P \leq 0.05$.

M1 and M2 are the matrix of empirical coefficients. T1 (treatment without addition), T2 (addition of nanomagnesium 1 mg/ kg/ diet), T3 (addition of nanomagnesium 1.5 mg/ kg diet) and T4 (addition of nanomagnesium 2 mg/ kg / diet) experimental transactions, respectively. These results are consistent with what (3) indicated that activity of growth hormone gene integrates with Binding of receptor gene to enhance role of insulin-like growth factor-1, which contributes to influencing the level of gene expression and functions of growth hormone gene and its receptor in metabolic and growth-building processes, role of triiodothyronine (T3) and thyroxine (T4), any of gene complementing the effect directly contribute to growth and building of expected muscle mass in chickens (4), and zinc from trace minerals that participate in many biological functions performed by growth hormone gene and its receptor in development of growth performance, immune system support and resistance to free radicals, as well regulating growth and development of skeleton and lipid metabolism (11,12), especially that vitamin E is a dietary supplement and a fatty component in cell membranes that resists oxidative radicals, it is a vital vitamin for growing chicks, and vitamin E is one of essential vitamins in poultry Nutrition (25), and that presence of vitamin in high dose in broiler meat contributed to raising level of gene expression of growth hormone receptor gene (26). Results of Table 4 and tables 5 and Diagram of 1, Think about moral and $P \leq 0.05$ in values of gene expression of members of first transaction (T1) compared with values of

gene expression of member of the transactions of pilot (T2 and T3 and T4), as well moral $P \leq 0.05$ personnel transactions pilot (T2 and T3 and T4) in average value of productive performance (increase weighting, the weight of the Vivo conversion factor flag) compared with individuals in treatment T1, always show table above is an inverse relationship between them (value of gene expression, performance and production) Then add nano magnesium 5, 2, magnesia, Nano-mg/ kg / diet) had contributed to the inhibition of gene expression and the army of future growth hormone, or contributed to the enhanced gene expression and army of other linked operations metabolism and representation for essential nutrients (carbohydrates, proteins and amino acids, fats, sugars, trace minerals, vitamins, and activity of digestive enzymes), and results correspond referred to (27) That existence of coefficient correlation is positive between the value of gene expression and average of broiler performance, well as to manifestations of hereditary multiple for a given gene may be responsible for varying levels of gene expression between strains (5), as effect of physiological factors and environmental conditions in levels of gene expression (6), as accompanied by enhanced levels of gene expression to stimulate growth of muscle mass and skeleton, and these molecular changes last case (28), Particularly to show environmental factors appropriate coefficient high correlation with levels of gene expression and loss of genetic-related performance of broiler (8), while explained (29), that level of gene expression and high loss of genetic responsible for the growth and development of the muscles of the chest embryos chicken meatballs safety of Cobb, and help in increasing value of average body weight at marketing, has been detected

differences in levels of gene expression and the extent of variation in average of productive performance (body weight, neighborhood, muscles, chest area) Some breeds of chicken meat (9 .(

2Effect of gene expression of growth hormone receptor gene on some productive qualities of Ross-308 broilers :

results of Table 5 indicated the presence of a negative correlation coefficient between

regression values and both gene expression in trait of live body weight and average total weight gain (-63.446, -63.575) for entire breeding period of 35 days, well as positive relationship shown by above table for trait of the food conversion coefficient with values of regression coefficient of gene expression (0.129), Table 5 also showed values of and unacceptable

Table 6 prediction equations of live body weight decline, weight gain and nutritional conversion coefficient end of experiment on gene expression of growth hormone receptor gene for muscle mass of the chest area for broiler Ross-308

coefficient of determination R^2	Significance	Equation of a straight line	regression coefficient	Descending qualities
0.44	*	$Y^{\wedge} = 2198.002 - 63.446X$	-63.446	Final live body weight/g
0.45	*	$Y^{\wedge} = 2155.871 - 63.446 X$	-63.575	Weight gain g/bird
0.66	**	$Y^{\wedge} = 1.425 + 0.129 X$	0.129	Feed conversion ratio kg feed/kg meat
		$(P \leq 0.05) *$, $(P \leq 0.01) **$		Significance level

The values of the stability coefficient are 0.90 excellent, 0.80 good, 0.70 acceptable, 0.60 doubtful, 0.50 weak and even less unacceptable .

negative values in the regression coefficient between descending traits (weight gain, live body weight, nutritional conversion factor) and gene expression of growth hormone receptor gene in muscle mass of the chest area may be due to fact that these gene were not affected by addition of different levels of nanomagnesium to Bush throughout experiment as dietary factors affecting genetic performance (epigenetic the possibility of changes in genetic performance of herd members. Explained (30), delegation of average body weight in the safety of ISA Vedette compared to the safety of my Arbor Acres and Hybro; it breeds of chicken meat Mutual in speed of growth from day old to 6

weeks, and results agree with what is referred to (31,32), That focus on genetic and molecular changes in gene expression genes growth and muscle building contributed to an increase in body weight of living members of the herd chickens nearly doubled in half compared to five decades ago, also showed heterogeneity in level of gene expression and loss of genetic contribution in growth and development of muscular structure of members of the Froggy meat to access significant differences in average body weight at age of three weeks and six weeks in the safety of chicken meat business (33), and the results of (34). To the vast discrepancy in average body weight, average daily increase can be attributed to differences in levels of gene expression unrefined of genetic represented by models, genetic, and contributed to genetic patterns chicken meat breed Cobb-500 in level of expression of

Jenny higher to average a higher body weight at age of one day compared to free frog meat Ross-308 at age of one day also (35), in particular, showed many of other studies the impact of levels of gene expression (genotype) average weight of the Vivo primary at age of day, and average body weight already, weight of final body, and turn power between different shops at different ages (36, 37), and exchange of level of gene expression of genes of IGF-1 and Jane meet contributing to growth and development of construction of muscular structure in safety of chicken meat business(Ross-308 and Hubbard and Cobb-500) and effect on some of characteristics and productive performance as average body weight of the neighborhood average increase weighting coefficient of conversion of food(38)And (26), indicated that relative expression of DNA raise messenger (mRNA) of genes of receptors for GH has been committed with addition of cornmeal to the

Conclusions: :

1.magnesium nanoparticle did not contribute to influencing mechanization of action growth hormone receptor gene.

2.magnesium nanoparticle targeted another gene responsible for development and growth that not studied in this experiment.

3.use of a synergistic mixture with Nano magnesium (vit E) to enhance cycle of contributor to metabolic processes, resistance to free radicals and fight oxidative stress.

4.general state of health of herd improved and sudden death syndrome not appeared.D.S (Sudden Death Syndrome.)

Recommendations:

diets chicken fattening because it contains a higher proportion of vitamin E and other elements such as metals zinc, because addition of some minerals are essential for natural activity of many enzymes and structural proteins, hormones, and is necessary for growth and development and regulate the gene expression and synthesis of proteins (39), while among (40). Vulnerability peace in the levels of gene expression gene growth hormone and its receptors and growth factors like insulin type of feed additives in herds safety of chicken meat of Cobb, as well as varying levels of gene expression depending on type of marker genetic (gene target), type safety, has contributed to these variations in levels of gene expression end of period of high growth performance and body weight have safety of Cobb, compared with Arian and Ross-308 (13 .(

-1use of magnesium nanoparticle with addition of other dietary supplements to stimulate the gene expression of growth hormone receptor gene .

-2study of other genes associated with growth, such as insulin-like growth factor (IGF-1) or myostatin .

-3using other breeds such as Hubbard or Cobb in genetic improvement programs with ross308 breed for comparison.

- 4study of density and concentration (colonial unit/ CFU) of the beneficial intestinal flora in the digestive tract of broilers when introducing magnesium nanoparticle with the diet.

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