EFFECT OF DIETARY *ERUCA SATIVA* SEEDS MEAL AND NANO-CHITOSAN ON PERFORMANCE AND RELATED GENE EXPRESSION TO GROWTH AND LIPID PROFILE IN BROILER CHICKS

Hayam M. A. Abou El-Maaty[1*], M. H. Abd El-Aziz[2], M. Z. El-Diasty[2] and Eman A. El-Said[3]

[1]*Poultry Production Department, Faculty of Agriculture, Mansoura University, Egypt.*

[2]*Genetics Department, Faculty of Agriculture, Mansoura University, Egypt.*

[3]*Poultry Production Department, Faculty of Agriculture, Damietta University, Egypt.*

*Corresponding author: Hayam M. A. Abo El- Maaty; Poultry Production Dept., Fac. Agric., Mansoura University,Mansoura 35516, Egypt. Tel: +201008961731 Fax: +20502245268

SUMMARY

This study was carried out to evaluate the impacts of inclusion of two plant proteins (soybean meal or rocket meal) in the starter and growing–finishing diets fed with/without nano-chitosan for Cobb500 one-day old broiler chicks on growth performance and serum parameters, as well as expression patterns of *PPARα*, *PPARγ* and *IGF*-1 genes in liver tissue. A total of 220 chicks were randomly distributed into four treatments in a floor pen under hygienic conditions. Results indicated that birds fed 7.5% rocket seed meal (RSM) exhibited the best means of FBW, BWG and FCR. While least feed intake with higher FBW and BWG was found in nano-chitosan fed birds. RSM fed chicks had a significant decrease in serum levels of TG, LDL, total lipids, AST and ALT with insignificant changes in serum albumin, total cholesterol and HDL compared with the control group. Significant increases in serum levels of IgG, IgM, IgA, CAT, TAC, SOD, T3, T4 and FSH were found in RSM-fed birds as compared to the control group. Insignificant differences were noted in gene expression among the different experimental groups of chicks regarding *IGF*-I and *PPARα* genes. While the *PPARγ* gene showed significant decreases in transcriptional activity in chickens fed on RSM with nano-chitosan. Our findings declared that RSM can be used in chicks diet up to 7.5 % singly or with nano-chitosan without any adverse effect on performance, blood parameters and related gene expression with a beneficial effect of RSM with nano-chitosan in reducing the lipogenesis in broiler chicks.

Keywords: Eruca sativa seed meal; nano-chitosan; broiler performance; blood parameters and gene expression.

INTRODUCTION

Basically, protein nutrition represents a principal challenge to poultry production mainly in the area where protein-rich feedstuffs are restricted (Beski *et al.,* 2015). Formulating broiler diets based on corn and soybean meal has augmented soybean meal demands and consequently raised the feeding cost. Two possible approaches can be employed to reduce the feed cost for broiler chicks. The first is the use of the alternative locally available protein sources. Rocket seed meal is predictable to increase in local market owing to its use as an aromatic plant. Furthermore, research work with RSM is finite. Additionally, El-Shafei *et al.* (2007) observed that RSM can fruitfully be used in growing Japanese quail diets as a partial substitute for SBM up to 32% without any unfavorable effects on their growth performance, carcass characteristics or blood constituents. They also, indicated that RSM can serve as immune-stimulant due to its antioxidant properties. Abo El-Maaty (2009) reported that hens fed the RSM-containing diets displayed no significant differences in DFI, FCR and BWG compared with the control group.

Chitosan nanoparticles (ChNPs) exhibit more superior activities than chitosan since nanonization possesses have many advantages, such as increasing compound solubility and improving its absorbability (Wen *et al.*, 2011). Not surprisingly, ChNPs have been showed to have an immune-enhancing effect, antimicrobial activity and anticancer properties (Iqbal *et al.*, 2003). It also has potential adjuvant properties, such as promoting endocytic uptake and elevating immune responses. Additionally, ChNPs possess lower cytotoxicity and more stability (Neimert-Andersson *et al.*, 2011). If we try to view some of this genetic research positively, we may open doors to drastically change the future of the poultry industry. The nutritional effects can be assessed by measuring of gene expression levels in liver and muscle tissues for nuclear hormone receptors such as Peroxisome Proliferator-Activated Receptors (*PPARs*) (Wahle *et al*., 1995 and 2003) and growth factors such as IGF-1 (insulin like growth factor-1) which depended on the nutrition status (Heck *et al.,* 2003 and Guernec *et al.,* 2004). Where, IGF-1 and *PPARs* mRNA contents in tissues could give valuable information for formulating an optimal feed for broiler chickens. Demonstrating that *PPARγ* the important metabolic effect of *PPARγ* in chicken is its task in formation of fat cells (adipogenesis) and tissues (Navidshad and Royan, 2015). On the other hand, Peroxisome Proliferator-Activated Receptor α (*PPARα*) increase the fatty acid oxidation through up-regulating the expression of acyl-coenzyme A oxidase and the carnitine palmitoyl transferase enzymes (Bell *et al.*, 1998 and Pineda *et al.*, 1999).

Therefore, the existing study was planned to assess the effects of Rocket seed meal (RSM) as a partial substitute for SBM and chitosan nanoparticles on productive performance, blood biochemicals and immunological responses and related gene expression to growth and lipid profile of broiler chicks.

MATERIALS AND METHODS

The present experiment was carried out at the Poultry Research Unit, Agricultural Research and Experimental Center, Faculty of Agriculture, Mansoura University, Egypt, under climates of autumn season in Egypt (October to November, 2018). The range of environmental temperature during this period was 33-38 °C. The main purpose of the study was to illustrate the possible beneficial effect of feeding an untraditional source of plant protein without or with nano-chitosan on subsequent growth performance, some physiological and immunological responses and gene expression traits in broiler chicks.

Preparation of chitosan nanoparticles:

The ionic gelation method was used for the preparation of nanoparticles of hydrophobic polymers**.** The preparation method was extremely mild and involved a mixture of two aqueous phases at room temperature. In the first phase, 0.2 g chitosan (Sigma-Aldrich, Egypt) was dissolved with 200 ml distilled water containing 1 ml acetic acid at 25°C for 30 min. The mixture pH was adjusted to be 7.20, while in the other phase 0.066 g polyanion sodium tripolyphosphate (TPP) was dropped slowly with stirring. Zeta sizer showed the size of nano-chitosan was between 30 ~ 40 nm (Fig. 1). The sub main factor was chitosan nanoparticles coating applications 10 ml/ Kg diet.

Experimental design, birds and diets:

The experimental design used was a completely randomized factorial design, with two sources of plant protein (soybean meal or rocket seed meal) in the starter and growing–finishing diets fed without or with nano-chitosan. Thus, there were 4 dietary treatments. Each diet was fed to 55 one d-old unsexed Cobb 500 broiler chicks divided equally among five replicates. Each replication of chicks was kept in floor pen (100x150 cm). The experimental chicks were distributed randomly among the experimental diets at day of hatch, with approximately similar initial live body weights among replicates and treatments. The diets were formulated based the nutrients composition of feedstuffs as reported by NRC (1994) except for rocket seed *(Eruca sativa*) meal (RSM) which was determined, and all feed formulations met nutrient requirements of broiler chicks (NRC, 1994). Feed and water were offered *ad libitum* throughout the experiment. Broiler chicks were kept under the same managerial and hygienic conditions and exposed to 23h light/d up to 42 d of age. Common management practice was used for brooding and rearing the birds. Vaccination and veterinary care were made according to common veterinary medicine practice for chicks. Experimental diets are shown in Table (1).

Criteria of response:

Performance traits:

Chicks were weighed at one and 42 d. At the end of the experimental period, body weight (BW), body weight gain (BWG) and feed intake (FI) of broilers were recorded. Feed conversion ratio (FCR) was

calculated by dividing FI (g) by body weight gain (g). The study was ended at the 6 weeks of the experiment.

Slaughter test and blood samples:

Six blood samples were collected during slaughtering in heparinized tubes. Serum was separated by centrifugation at 3000 rpm for 15 minutes and stored rapidly at -20ºC until analysis. Blood serum concentrations total protein (TP), albumin (Alb), triglycerides (TG), total cholesterol (Chol) and high-density lipoprotein-cholesterol (HDL-C) and were determined using commercial kits. Blood serum level of low-density lipoprotein-cholesterol (LDL-C) was also estimated by using the equation of Friedewald *et al.* (1972), as follows: LDL-C=Total cholesterol–(HDL-C+vLDL); where vLDL are very low-density lipoprotein which was calculated as concentration of serum triglycerides divided by 5. Globulin was calculated by differences between total protein and albumin. Also, total antioxidant capacity (TAC), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), immunoglobulins G (IgG, IgA, IgM), triiodothyronine (T3), thyroxin (T4) and follicle-stimulation hormone (FSH) and activity of liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and alkaline-phosphatase (ALP) were determined by commercial kits.

Table (1): Composition and chemical analyses of the tested diets fed to broiler chicks from 0 to 6 weeks of age.

Ingredient (%)	Starter diet		Growing and finisher diet	
	Control	RSM	Control	RSM
Ground yellow corn	61.50	58.23	67.20	64.70
Soybean meal (44%CP)	16.00	9.67	15.00	8.00
Corn gluten meal (60% CP)	16.50	18.3	12.00	14.00
Rocket (*Eruca sativa*) seed meal	-	7.50	-	7.50
Sunflower oil	1.00	1.30	1.00	1.00
Ground limestone	2.00	2.00	2.00	2.00
Dicalcium phosphate	1.80	1.80	1.80	1.80
Vitamins and minerals Premix[3]	0.30	0.30	0.30	0.30
Common salt (NaCl)	0.30	0.30	0.30	0.30
DL-Methionine	-	-	-	-
L-Lysine-HCl	0.60	0.60	0.40	0.40
Total	100	100	100	100
Calculated analysis (As fed basis: NRC, 1994)				
Metabolizable energy (ME), Kcal/kg	3149	3126	3143	3110
Crude protein (CP), %	23.06	23.08	20.13	20.05
Ether extract (EE), %	3.88	5.03	3.97	4.86
Crude fiber (CF), %	2.69	2.60	2.68	2.57
Calcium, %	1.20	1.18	1.20	1.18
Nonphytate P, %	0.45	0.45	0.45	0.44
Lysine, %	1.22	1.16	1.10	0.94
Methionine, %	0.46	0.47	0.39	0.41
Methionine + Cystine, %	0.85	0.89	0.74	0.78
Determined analysis (DM basis: A.O.A.C., 1990)				
Dry matter (DM), %	92.93	92.89	91.87	91.54
Crude protein (CP), %	24.81	24.85	21.91	21.90
Ether extract (EE), %	4.18	5.42	4.32	5.31
Crude fiber (CF), %	2.89	2.80	2.92	2.81
Ash, %	9.11	9.16	8.71	8.92
Nitrogen-free extract (NFE), %	59.01	57.77	62.14	61.06

[1]*Premix at 0.30%of the diet supplies the following /kg diet:*
Vit.A,1000IU;Vit.D₃,2000IU;Vit.E,10mg;Vit.K,1mg;Vit.B₁,5mg;Vit.B₂,5mg;Vit.B₆,1.5mg;Vit.B₁₂,0.01mg;Folic acid,0.35mg;Biotin,0.05mg;Pantothenic acid,10mg;Niacin,30mg;Choline chloride, 250mg;Fe,30mg;Zn, 50mg;Cu, 4mg and Se,0.1mg.

Gene expression analysis:

RNA isolation and cDNA synthesis:

Liver tissue samples (three birds from each treatment) were taken immediately after slaughter, instantly frozen in liquid nitrogen and stored (about two d.) at –80°C until use. From each sample, total RNA was isolated using Gene JET RNA Purification Kit (Thermo Scientific cat No. #Ko731) as stated by manufacturers instruction. Purity and concentration of isolated RNA were checked by Nanodrop spectrophotometer (Thermo Fisher Scientific Inc.) and by electrophoresis using ethidium bromide staining, 18S and 28S rRNA were appeared as sharp two bands. After that, DNase I, RNase-Free was used to removal of genomic DNA from RNA preparations.

In order to cDNA synthesis, total RNA (1μg) was reverse transcribed with Revert Aid™ First Strand cDNA Synthesis Kit (Thermo Scientific) was used following the protocol provided by the producer. The PCR amplification was performed for cDNA synthesis by Stepone (Applied Biosystems). Next, Ribolock RNase inhibitor were added, then terminate the reaction by heating at 70°Cfor 5 min and stored at −80◦C until use.

Primer design:

Specific primer pairs (forward and reverse) for target and reference genes of interest in this study were designed using Primer-BLAST and based on previous reports (Table 2). These primers were procured from Introgen (Thermo Fisher Scientific) and checked on cDNA samples using PCR then gel electrophoresis, where produced specific PCR product (Table 2).

Table (2): The specific primers for target and reference genes of used in quantitative real time PCR analysis.

	Genes	Name	Sequence (5′→ 3`)	length bp	GC %	Tm °C	NCBI Reference Sequence:	Sources
			Specific primer	**PCR Product**				**References**
Target	Peroxisome Proliferator-Activated Receptorα	*PPAR α F*	AGGCCAAGTTGAAAGCAGAA	217	37.79	77.67	XM_025150258.1	Konig *et al.* (2007)
		PPAR α R	GTCTTCTCTGCCATGCACAA					
	Peroxisome Proliferator-Activated Receptor γ	*PPAR γ F*	GACCTTAATTGTCGCATCCAT	237	44.73	81.85	NM_001001460.1	Zhang *et al.* (2006)
		PPARγ R	CGGGAAGGACTTTATGTATGA					
	Insulin-like growth factor 1	*IGF-1 F*	TGGCCTGTGTTTGCTTACCT	166	51.81	81.99	NM_001004384.2	Konig *et al.* (2007)
		IGF-1R	TCCCTTGTGGTGTAAGCGTC					
Reference	Beta-actin	*β-actin F*	ATGAAGCCCAGAGCAAAAGA	223	51.12	81.97	NM_205518.1	using Primer BLAST
		β-actin R	GGGGTGTTGAAGGTCTCAAA					
	Glyceraldehyde-3-Phosphate Dehydrogenase	*GAPDH F*	TCAAATGGGCAGATGCAGGT	291	51.55	83.4	NM_204305.1	using Primer BLAST
		GAPDH R	TGATGGCATGGACAGTGGTC					

Quantitative RT-PCR:

Quantitative 2-step RT-PCR was performed using a Maxima SYBR Green qPCR Master Mix (2X),ROX Solution provided protocol in Veriti® 96-well thermal Cycler (Applied Biosystems).The real time PCR program was as follows : initial denaturation at 95 °C for 10 min.; 40 cycles of denaturation at 95°C for 15 sec.; annealing/extension at 60 °C for 60 sec. Data acquisition performed during the annealing/extension step.

Primers specificity for target and reference genes were checked by evaluation of melting curves of RT- PCR products which were conducted from 55°C to 95 °C to confirm the amplification of single amplicon (Fig. 2).

Expression patterns of Target genes (*PPARα, PPARγ* and *IGF-1*) in liver tissue from sample of control and treatments were determined using $2^{-\Delta\Delta C_T}$ method according to Livak and Schmittgen (2001) and normalized by the expression of reference or housekeeping genes (β-*act and GAPDH*) as endogenous control. The output data were obtained as fold change (FC) relative to the control (fold relative expression). The relative expression values were automatically generated by entering C_T values from housekeeping and target genes into Microsoft Office Excel sheet contain equations of $2^{-\Delta\Delta C_T}$ method. The comparison of gene expression data was carried out using SAS 9 Software and the significance of difference was determined according to Duncan's Multiple Range Test at P ≤ 0.05.

A correlation coefficient and linear regression between normalized expression levels over to β-*act* and *GAPDH* as well as between relative gene expression for genes of interest and performance traits were calculated using Mintab17 statistical Software.

Statistical analysis:

Our data were subjected to statistical analysis as two factors-factorial analysis of variance using SAS Program (2004). Means were separated (P≤0.05) using Duncan's multiple range test (Duncan, 1955), for the comparison among means of the tested rations when the main effects were significant. The following statistical model was used:

$$x_{ijk} = \mu + L_i + P_j + (LP)_{ij} + e_{ijk}$$

Where:

x_{ijk} = an observation,
μ = overall mean,
L_i = effect of protein source (i = 1 and 2),
P_j = effect of level Chitosan nanoparticles (j = 1 and 2),
$(LP)_{ij}$ = effect of Interaction between LP (ij = 1,2,3 and 4) and
e_{ijk} = random error

RESULTS AND DISCUSSION

Productive performance:

Data on the impacts of feeding the experimental diets and nano-chitosan additives on the productive performance of broiler chicks, including, body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) are presented in Table (3). Fed nano-chitosan-included diets consumed the least amount of feed but had a higher FBW and BWG in comparison with the control chicks. Irrespective of the influence of dietary protein sources, nano-chitosan-supplemented diets significantly (P≤0.01) improved the FCR as compared to their control counterparts (the main effect of feeding the two plant protein sources, SBM and RSM) produced significant effects on FBW, BWG and FCR (P≤0.01) during the period from 0 to 42 d. of age. The best means of FBW, BWG and FCR were achieved by chicks fed the diet containing 7.5% rocket seed meal (RSM) as compared to their control birds. The improvements in FBW, BWG and FCR due to the impact of dietary RSM were estimated to be 8.88, 8.68 and 7.43 % for chicks fed the diet containing 7.5% RSM, respectively. The superiority of diets containing SBM and RSM indicated a complementary effect which might have increased availability of amino acid and/or eliminate the anti-nutritional substance or amino acid imbalance (Irish and Balnave, 1993 and Attia *et al.*, 2003). Therefore, the enhancement in growth performance in response to feeding RSM-containing diets could be attributed to the improved nutrient availability in the diets compared with their control counterparts. There were no significant differences in FI between groups of chicks fed the tested diets containing the two plant protein sources.

Osman *et al.* (2004) reported that feeding either radish or rocket meals up to 15% instead of a part of soybean meal in broiler diets had no effect on their live body weight. Abdo (2003) found that the best body weight gain of broilers was attained by the groups received rocket seed meal at levels of 10 and 25% in place of soybean protein. El-Tohamy and El-Kady (2007) found that using rocket meal at 50% a replacement of dietary soybean meal caused a significant increment of daily body gain, and daily feed intake, and improved feed conversion of rabbits. They suggested that such improvement may be

attributed to the properties of the tested material that could act as anti-bacteria, anti-protozoal, anti-fungal and antioxidants. Yasser *et al.* (2015) recommended that black cumin, mustard, sesame and rocket seed meals can safely be used as supplements at 3% level in broiler rabbit diets in order to get higher economic efficiency without any adverse effect on the rabbit performance.

Table (3): Growth performance (from 0 to 42-d.-old) of broiler chicks fed diets containing two protein sources without or with nano-chitosan addition.

Fact1	IBW	FBW	BWG	FI	FCR
	(g)	(g)	(g)	(g)	(g:g)
Protein sources (A)					
Soybean meal (A1)	44.8	2368.0[b]	2323.3[b]	4054.4	1.75[b]
Rocket seed meal (A2)	44.0	2573.5[a]	2529.5[a]	4074.5	1.62[a]
SEM	0.25	35.18	50.25	09.31	0.03
Sig.	NS	***	***	NS	**
Feed additives					
(0.0g ChNPs/kg diet) B1	44.15	2378.0[b]	2333.9[b]	4188.5[a]	1.79[b]
(1.0g ChNPs /kg diet) B2	44.60	2563.5[a]	2518.90[a]	3940.4[b]	1.57[a]
SEM	0.28	39.45	45.19	25.15	0.04
Sig.	NS	***	***	***	***
Interaction A*B					
A1B1	44.10	2306.0	2261.9	4166.0	1.85
A1B2	45.40	2430.0	2384.6	3942.8	1.65
A2B1	44.20	2450.0	2405.8	4211.0	1.75
A2B2	43.80	2697.0	2653.2	3938.0	1.49
SEM	0.24	47.18	40.25	36.45	0.03
Sig	NS	NS	NS	NS	NS

[a and b]*: Means in the same row having different superscripts differ significantly at P≤0.05. SEM: Standard error of the means.*
*NS: Not significant; *, ** and ***: Significant at P≤0.05, 0.01 and 0.001, respectively.*

There was no adverse effect of RSM on feed intake of broiler chicks and Japanese quail, as shown by Osman *et al*. (2004) and El-Shafei *et al*. (2007). Shakmak (2008) indicated that growth of chicks was significantly affected by dietary inclusion of 25% rocket meal of soybean meal.

El-Shafei *et al*. (2007) indicated that RSM could be used up to 15% in the diet for growing Japanese quail without any adverse effect on growth performance. While Fagbenro (2004) reviewed that RSM at 20% of total dietary protein did not affect growth of African catfish.

During the period from 0 to 42 d. of age, there were significant differences (P≤0.01) in FBW, BWG, FI and FCR of chicks in response to dietary nano-chitosan supplementation, regardless of dietary protein source compared to the control group (Table 3).

The reason why the present growth performance (FBW, BWG and FCR) was improved is thought to be related to increased nutrient absorption which might enhanced by dietary nano-chitosan. Nano-chitosan including diet had positive effects and can be used as a useful alternative to chitosan in broiler chickens' diets. Because chitosan had slow motility in the gastrointestinal lumen. It seems to stimulate the satiety center of the brain. Actually, in broiler chicks the capacity of the gastrointestinal tract is the limiting factor for feed intake (Leenstra, 1986 and McCarthy and Siegel, 1983). This suggests a possibility that the growth performance might be enhanced by feeding nano-chitosan-included diets but not the chitosan-supplemented diets.

Sayed *et al.* (2015) reported that Cobb 500 broiler chicks fed 10% buckwheat supplemented with 250 and 500 mg chitosan/kg displayed no significant differences in growth and feed intake. Because hazardous antibiotics are banned in poultry feed, including buckwheat with trace amounts of chitosan in broiler diets might be a useful alternative to antibiotics in the poultry industry.

A lot of animal studies have indicated that inclusion of chitosan at up to 50 g/kg in feed had no adverse effects on the growth and feed intake of the treated animals (Kobayashi *et al*., 2006; Han *et al*., 2007; Khambualai *et al*., 2009; Yan & Kim, 2011).

During the period from 0 to 6 weeks of age, there were no significant interaction between dietary protein source and feed additives on chicks' performance.

Blood parameters:

Data in Tables (4 and 5) revealed a significant (P<0.01) effect dietary of protein source on serum total protein and globulin concentrations. Birds fed rocket seed meal-containing diets had higher levels of serum total protein and globulin compared with the control one. Feeding the RSM-diets significantly reduced serum levels of TG, LDL, and total lipids, and activity of AST and ALT compared with the control group. The differences between the control group and the RSM-fed group were not significant in terms of serum concentrations of albumin, total Chol. and HDL.

Table (4): Biochemical constituents of blood serum of 42-d. old broiler chicks fed diets containing two protein sources without or with nano-chitosan addition.

Treatment	TP g/dl	Alb g/dl	Glob. g/dl	Chol mg/dl	Tg mg/dl	HDL mg/dl	LDL mg/dl	T. lipids mg/dl	AST U/dl	ALT U/dl
Protein source (A)										
Soybean meal (A1)	4.13[b]	2.26	1.88[b]	174.30	147.48[a]	50.61	94.19[a]	617.37[a]	74.97[a]	25.90[a]
Rocket seed meal (A2)	4.80[a]	2.46	2.34[a]	163.23	131.42[b]	59.53	77.42[b]	530.77[b]	61.57[b]	20.48[b]
SEM	0.14	0.09	0.08	4.10	4.23	4.22	4.77	18.22	2.79	1.74
Sig.	**	NS	**	NS	*	*	*	***	**	*
Nano-Chitosan level (B)										
(0.0 g ChNPs /kg diet) B1	4.27	2.25	2.01	171.35	136.83	50.62	93.36[a]	605.43[a]	73.14[a]	25.24
(1.0 g ChNPs /kg diet) B2	4.66	2.46	2.20	166.18	142.07	59.52	78.25[b]	542.70[b]	63.40[b]	21.14
SEM	0.19	0.09	0.12	4.65	5.43	4.22	5.04	22.59	3.46	1.91
Sig.	NS	NS	NS	NS	NS	*	*	**	**	NS
Interaction A*B										
A1B1	3.95	2.17	1.78	177.73	146.10	49.08	99.43	662.30	79.92	28.00
A1B2	4.31	2.34	1.97	170.87	148.87	52.14	88.95	572.43	70.02	23.80
A2B1	4.58	2.34	2.25	164.97	127.57	52.16	87.29	548.57	66.36	22.49
A2B2	5.01	2.57	2.43	161.50	135.27	66.89	67.55	512.97	56.78	18.47
SEM	0.18	0.11	0.10	6.19	6.37	5.51	5.06	15.68	2.74	2.32
Sig.	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a and b]: *Means in the same row having different superscripts differ significantly at P≤0.05. SEM: Standard error of the means. NS: Not significant; *, ** and ***: Significant at P≤0.05, 0.01 and 0.001, respectively.*

Results of our study also revealed that chicks fed RSM-diet exhibited significant reduction in serum levels of total Chol., TG & LDL while the level of HDL increased significantly. Reduced serum cholesterol level may be due to decreased absorption of dietary cholesterol from the intestinal lumen by plant sterols (Gupta *et al*., 1980). Blaževic and Mastelic (2008) found that Eugenol component in RSM inhibits peroxidation of lipids and causes hypocholestrolemia due to significant antioxidant action.

It was also reported that nutritional antioxidants increase HDL by activating sulfhydryl (SH) group of lecithin cholesterol acyl transferase (LCAT) enzyme (Wang and Ballatori, 1998). This enzyme incorporates free cholesterol from LDL into HDL and esterifies it (Eisenberg, 1984). The physiological action of T3 hormone is not only to stimulate the synthesis of cholesterol but also, its action in oxidation and biliary secretion. Hepatic uptake of cholesterol is increased by increasing expression of LDL receptors in liver. Therefore, the net effect is a significant decrease in plasma cholesterol and total lipids (Eisenberg, 1984 and Hubner *et al*., 1976).

The rats fed RSM-containing diet had reduced serum cholesterol levels (Al-Qudah and Amin 2017). Alam *et al*. (2006) reviewed that rats fed RSM-extract had potential renal protective activity, while Anac and Martin-Prevel (1999) and Bajilan and Al-naqeeb (2011) found that mice fed RSM-feeding extract-supplemented diet led to a weight loss in mice and may help in regulating cholesterol levels.

Results showed also that both AST and ALT activity levels, as indicators of liver function, were significantly reduced in chicks fed *Eruca sativa* containing diet as compared to those of control group. It is well known activity of that ALT is more specific indicator of liver function tests as compared to that of AST. Since, ALT enzyme was only produced in the liver, while the AST enzyme is also produced in the kidneys, muscles, skeleton, brain and heart in addition to the liver (Yuliani *et al.*, 2017). *Eruca sativa* was reported to have an antioxidant effect due to its free radicals scavenging activity *in vivo* (Villatoro-Pulido *et al.*, 2012) and *in vitro* (Abbasi *et al.*, 2013). Moreover, it has been reported to protect the hepatic injury through its potent antioxidant activity *in vivo* (Alqasoum *et al.*, 2010).

On the other hand, insignificant differences were observed in most serum parameters among treatments, except levels of serum LDL and total lipids, and activity of AST which were significantly lower (P<0.01) in broilers that were fed on the diets fortified with nano-chitosan. Although serum TP, albumin, globulin, total Chol, TG, HDL and ALT concentration were not statistically different from those of the control group, the dietary supplementation of nano-chitosan caused a reduction of LDL level and liver enzymes activity. Similar findings were reported by Razdan and Pettersson (1994), who found that broilers fed chitosan-enriched diet displayed a reduction in plasma triglyceride concentrations on 11 d. of age and plasma cholesterol concentrations on 11 and 19 d. of age and the addition of chitosan at a low level was not able to reduce the concentrations of serum cholesterol and triglyceride in broilers.

Also, chitosan statistically reduced levels of total cholesterol, low-density lipoprotein cholesterol in plasma and total triglyceride in rat liver (Xu *et al.*, 2007). Our results showed that the nano-chitosan-added diets had no effect on serum cholesterol and triglyceride of broilers compared with the control group. The results obtained agree with those reported by Nuengjamnong and Angkanaporn (2017), who found that feeding the broiler chicks on chitosan-supplemented diet had no effect on serum cholesterol and triglyceride.

Furthermore, the interaction between plant source of protein and feed additives had insignificant effect on all serum parameters presented in Tables (4 and 5).

Table (5): Biochemical constituents of blood serum of broiler chicks fed diets containing two protein sources with/ without nano-chitosan addition.

Treatment	ALP U/dl	IgG mg/dl	IgM mg/dl	IgA mg/dl	CAT U/ml/h	TAC nmol/ml	MDA nmol/ml	SOD U/ml/h	T4 ng/ml	T3 ng/ml	FSH ng/ml
Protein source (A)											
Soybean meal (A1)	63.68[a]	433.90[b]	178.47[b]	121.72[b]	53.87[b]	1.65[b]	28.90[a]	64.00[b]	20.12[b]	3.62[b]	1.99[b]
Rocket seed meal (A2)	47.45[b]	513.78[a]	221.85[a]	142.33[a]	71.32[a]	1.88[a]	19.06[b]	85.40[a]	23.22[a]	4.81[a]	4.61[a]
SEM	2.18	11.80	8.73	5.59	2.41	0.05	1.17	2.57	0.99	0.18	0.25
Sig	***	**	**	*	***	**	***	***	*	***	***
Nano-Chitosan level (B)											
(0.0 g ChNPs /kg diet) B1	58.42	463.00	191.83	129.32	58.30[b]	1.73	24.99	71.93	21.30	3.97[b]	3.02[b]
(1.0 g ChNPs /kg diet) B2	52.71	484.68	208.48	134.73	66.88[a]	1.80	22.97	77.47	22.04	4.46[a]	3.58[a]
SEM	4.04	20.85	12.51	7.15	4.17	0.07	2.45	5.29	1.19	0.30	0.63
Sig.	NS	NS	NS	NS	**	NS	NS	NS	NS	*	*
Interaction A*B											
A1 B1	65.73	431.20	178.43	116.20	48.77	1.65	29.22	62.00	18.58	3.24	2.13
A1B2	61.63	436.60	178.50	127.23	58.97	1.65	28.58	66.00	21.67	4.00	1.85
A2B1	51.11	494.80	205.23	142.43	67.83	1.81	20.77	81.87	24.02	4.69	3.91
A2B2	43.79	532.77	238.47	142.23	74.80	1.95	17.34	88.93	22.41	4.92	5.31
SEM	2.74	16.00	11.02	8.40	2.24	0.06	1.64	3.52	1.29	0.21	0.19
Sig.	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**

[a and b]: *Means in the same row having different superscripts differ significantly at P≤0.05. SEM: Standard error of the means. NS: Not significant; *, ** and ***: Significant at P≤0.05, 0.01 and 0.001, respectively.*

Table (5) showed significant increases in serum concentrations of IgG, IgM, IgA, CAT, TAC, SOD, T3, T4 and FSH in the treatment groups fed the RSM-supplemented diets compared to the control group

(P<0.01). However, results showed significant reduction in the serum levels of ALP and MDA concentration in the supplemented groups as compared to control diet.

This effect may be due, in part to that RSM had an antioxidant activity as evidenced by decreasing serum level of the lipid peroxidation by-product (MDA), increasing content of reduced glutathione and restoring activities of antioxidant (SOD and CAT) enzymes.

In agreement with the present finding, Shalaby and Hammouda (2014) reported that feeding RSM-diet significantly increased the activity of SOD and CAT enzymes in when compared with control group.

Thyroid hormones are important for the growth and development of the body and regulation of metabolism (Huang *et al.*, 2008). An increase in the production of thyroid hormones reflects an elevated activity of thyroid gland. Various pathological and physiological factors have been known to affect the concentration of these hormones and their metabolic activities. Serum levels of T4 and T3 of chicks fed RSM-containing diets were significantly (P<0.05) lower than those of the control group (Al-Mayali and Hasan, 2016). The reduction in of T4 could be due to the inhibition of 5-deiodinase (type 1) activity, which is responsible for the conversion of T3 to T4 (Maiti and Kar, 1997). Kale *et al.* (2007) mentioned that T3 and T4 secretion is in a negative correlation with the level of oxidative stress. On the other side, the antioxidant activity of *Eruca sativa* extract due to its constituents of flavonoids, saponin and vitamin C (Gauthaman *et al.*, 2003).

The observed increase in the levels of T3 and T4 in our study may be due to an increase in the activity of thyroid gland by some compounds present in RSM. This explanation agrees with that reported by Al-Shaikh *et al.* (2014) who found that feeding albino-mice on RSM-containing diets had significant effects on physiology and function of follicular cells of the thyroid gland. RSM may also cause enhancement of the transport of sodium-iodide and increased absorption of iodide resulting in increased production of T3 and T4 and may influenced iodo-tyrosine deiodinase in rabbits fed RSM-containing diets (Yadav *et al.*, 2016). In the fact, glucosinolates which are present in RSM have several biological activities including anti-carcinogenic, anti-fungal and anti-bacterial as well as their antioxidant properties (Kim *et al.*, 2004).

Khoobchandani *et al.* (2012) reported that parsley and *Eruca sativa* oil given orally has been reported to significantly increase the concentration of T4 and T3 in hypothyroidism in rats as compared to untreated rats. It is apparent that recovery of thyroid parenchyma is related to protection offered by *Eruca sativa* (Khoobchandani *et al.*, 2012) against hyperplastic changes that well known to be associated with hypothyroid status.

These results are in agreement with other researches using different medicinal plants like rocket which might reflect their antioxidant effects (El-Missry and El- Gindy, 2000), or due to the presence of phenolic compounds which exerted their modulator actions (Saleh *et al.*, 1995) and improvement of the status of antioxidant enzymes (Martinez-Sanchez *et al.*, 2007 and Ettebong and Nwafor, 2009).

Few observations reported that the presence of sterols, flavonoids, quercetin and saponins in *Eruca sativa* which can scavenge or remove free radicals which may cause an improvement the fertility and testicular functions (Agarwal and Allamaneni, 2011and Ansari *et al.*, 2014). *Eruca sativa* increased levels of serum FSH concentration (Nowfel and Al-Okaily, 2017).

Recently, Al-Tohamy *et al.* (2010) showed that the presence of glucosinolates (the major glucosinolate is Erucin) and other stimulant materials in *Eruca sativa* seeds have several biological activities and potentially capable for protecting the cells against oxidative stress resulting in an amelioration in semen characters and fertility in male rabbits (Talalay and Fahey, 2001).

Accordingly, the positive actions of *Eruca sativa* on the hormonal profile support the folkloric beneficial effect of the plant in the management of reproductive dysfunction.

It is reported that rocket seed oil is widely used by males to improve their sexual performance; the valuable effects of the oil are usually related to its fatty acid contents (Salem and Moustafa, 2001).

Hussein (2013) and Salem and Moustafa (2001) demonstrated that *Eruca sativa* leaves (which contain steroids, alkaloids, flavonoids, terpenes, glycosides and saponins) and seed oil has beneficial effects on fertility and male reproductive system. Moreover, several studies have shown that the aqueous extract as well as ethanolic extract of *Eruca sativa* plant increase spermatogenesis (Hussein, 2013 and El-Gayar *et al.*, 2016). Intake of *Eruca sativa* leaves as a salad may be helpful for diabetic patients to minimize the reproductive performance deterioration (Ansari *et al.*, 2014).

Inversely, results as shown in Table (5) revealed positive effects of enriching diets with nano-chitosan on the antioxidant defense system compared to control one. The present results showed a positive effect in serum IgG, IgM, IgA, TAC, SOD and T4.

Conversely, our results showed reduction in serum ALP and MDA. On the other hand, dietary inclusion of nano-chitosan led to a significant increase in serum levels of CAT, T3 and FSH as compared to the control group.

On the other side, the interactions between added dietary RSM and fortification with nano-chitosan in Table (5) revealed insignificant (P<0.01) effects on serum levels of antioxidant defense and immunity system parameters in all groups.

Evaluation of gene expression activity:

As confirmed by previous results, the expression levels for *IGF*-1, *PPARα* and *PPARγ* genes in the liver tissue of different experimental groups were analyzed and normalized to the expression of β-*act* and *GAPDH* genes. The relationship between normalized data to β-*act* and *GAPDH* genes (Fig. 3) was linear with a highly significant positive correlation (r=0.999**), demonstrating high accuracy of the measurement and gives higher reliability to our results.

Relative expression patterns of *IGF*-1, *PPARα* and *PPARγ* genes in liver tissue taken immediately after slaughter from broiler chickens fed on SBM and RSM without or with nano-chitosan were illustrated in (Fig. 4).

Insignificantly differences for gene expression levels of *IGF*-1 and *PPARα* genes were recorded among all experimental birds. However, the chicks fed RSM diet displayed the best in terms of the transcription level for the *IGF*-1 gene. This refers that the activity of growth factor IGF-1 and fatty acid oxidation was not affected with feeding on the RSM-diets without or with nano-chitosan, though the activity of growth factor *IGF*-1 was enhanced by feeding on RSM-diet. While, the *PPARγ* gene showed a decreases in transcriptional activity in chickens fed on RSM without or with nano-chitosan. Such a decrease was greater and significant in chickens fed on RSM-diet with nano-chitosan (mean of FC was 0.145).

Correlation coefficients shown in Table 6 may dictate that there was no high correlation between *IGF*-1 activity and the performance traits of the experimental chicks, except in feed intake trait which its correlation was significantly positive. Insulin-like growth factor-1 is one of the principal binding proteins that have biological functions involved in growth, development, and differentiation. Rechler (1993) reported that the Insulin-like growth factors (IGF) are polypeptides that play an imperative role in cell growth and differentiation. Chesik *et al*. (2007) showed that IGF binding proteins extend the half-life of the IGFs and have shown to either inhibit or stimulate the growth promoting effects of the IGFs on cell culture. Beccavin *et al.* (2001) noticed that growth rates were associated with IGF-1 activity, supporting the hypothesis of a stimulatory role for both IGFs-1&II during post-hatching growth of chickens. Guernec *et al.* (2004) demonstrated the IGF-1 was sensitive to nutrient supply in hatching chicks, and also that fasting reduced IGF-1 mRNA levels in muscles of older chickens. All of this information supports our results which have shown that growth rates were not affected by feeding the RSM-diets without or with nano-chitosan. The lack of a harmful effect on growth rate, reported here, would reflected on IGF-1 expression activity that was reported to be sensitive to the feeding system.

Table (6): Correlation coefficients between performance traits and means of relative expression for *IGF*-1, *PPARα* and *PPARγ* genes recorded for all experimental groups of broilers.

	FBW	BWG	FI	FCR	T3	T4	T. lipids	TG	Chol	LDL	HDL
IGF-1	-0.244	-0.242	0.948*	0.528	0.135	0.291	0.083	-0.738	-0.088	0.242	-0.299
PPARα	-0.135	-0.131	0.810	0.397	-0.218	-0.398	0.400	-0.286	0.200	0.147	0.004
PPARγ	-0.936*	-0.935*	0.258	0.896	-0.962*	-0.803	0.993**	0.565	0.974*	0.942*	-0.837

* and ** significant values at 0.05 and 0.01 probability levels, respectively.

Also, from Figure 3 and Table 6 can notice that gene expression activity of *PPARα* was not affected by feeding the RSM-diet without or with nano-chitosan and that the change in the expression activity of the gene was not correlated with any change in estimates of performance traits. These results may reflect the rate of fatty acid (FA) oxidation which increased by increasing gene expression of *PPARα* (Bell *et al*., 1998 and Pineda *et al*., 1999), since the latter was not affected by feeding on RSM-diet without or with nano-chitosan.

The significant positive correlation coefficients (0.993**, 0.974* and 0.942*) between *PPARγ* relative gene expression in liver tissue and serum contents of total lipids, total cholesterol and low-density

lipoprotein (Table 6), confirms the lipogenic effect of *PPARγ*. By contrast, the relationship between *PPARγ* relative gene expression and final body weight, body weight gain and T3 hormone concentration were significantly negatively correlated (-0.936*, -0.935* and -0.962*, respectively).

The peruse of results (Table 6) and the results presented in Figure (4) for *PPARγ* relative gene expression under the influence of feeding the experimental diets, can help us to find a beneficial effect of added dietary RSM with nano-chitosan which regulated the metabolic processes of lipids in favor of broiler chicks. These results correspond to the findings of Abozid *et al.* (2014), who illustrated that rocket seed contained high amount of ω-3 and ω-6 fatty acids. These fatty acids have been reported to act as a hypolipidemic agent. Also, other previous reports have illustrated that dietary ω-3 and ω-6 fatty acids inhibit lipogenesis.

The most role of *PPARγ* is initiating adipocyte differentiation and increasing fat deposits (Tontonoz *et al.*, 1993 and Hua *et al.*, 1995). Conclusively, feeding the RSM diets had a significant effect in reducing the lipogenesis in broiler chicks. This is extremely important for the health and nutritional value of their products.
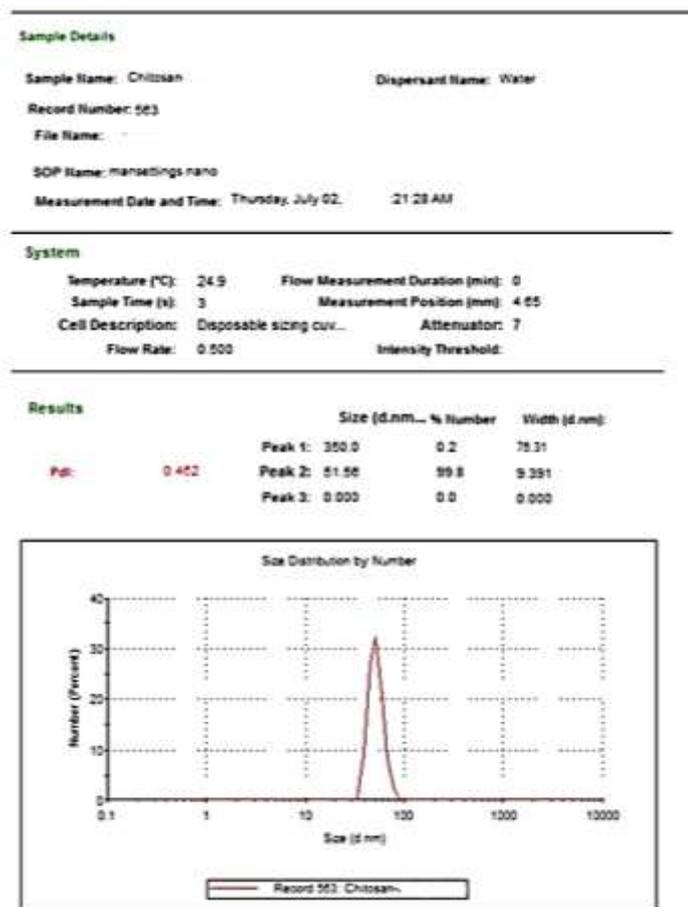

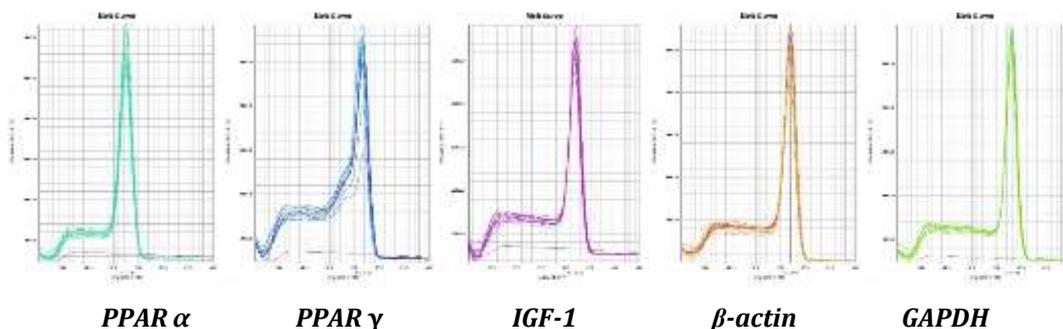**Fig. 1: Zeta sizer showed the size of nano-chitosan was between 30 ~ 40 nm.**

| *PPAR α* | *PPAR γ* | *IGF-1* | *β-actin* | *GAPDH* |

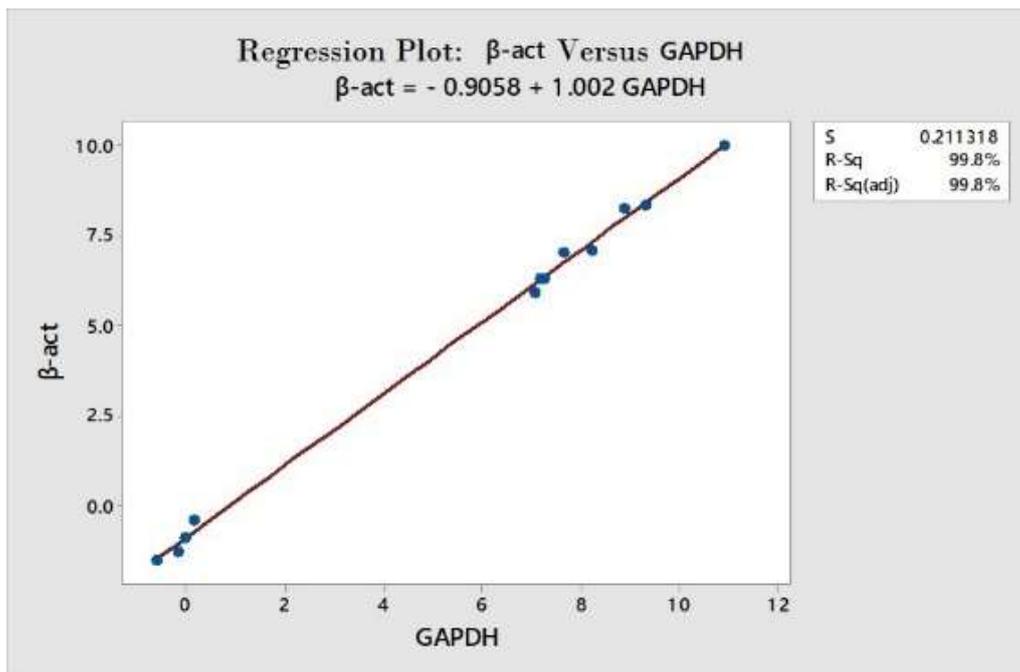**Fig. (2): Melting curves of single amplicon for target and reference genes of interest in this study.**

Fig. (3): Relationship between ΔC$_T$ values for all interesting genes normalized to β-*act* and *GAPDH* gene expressions.

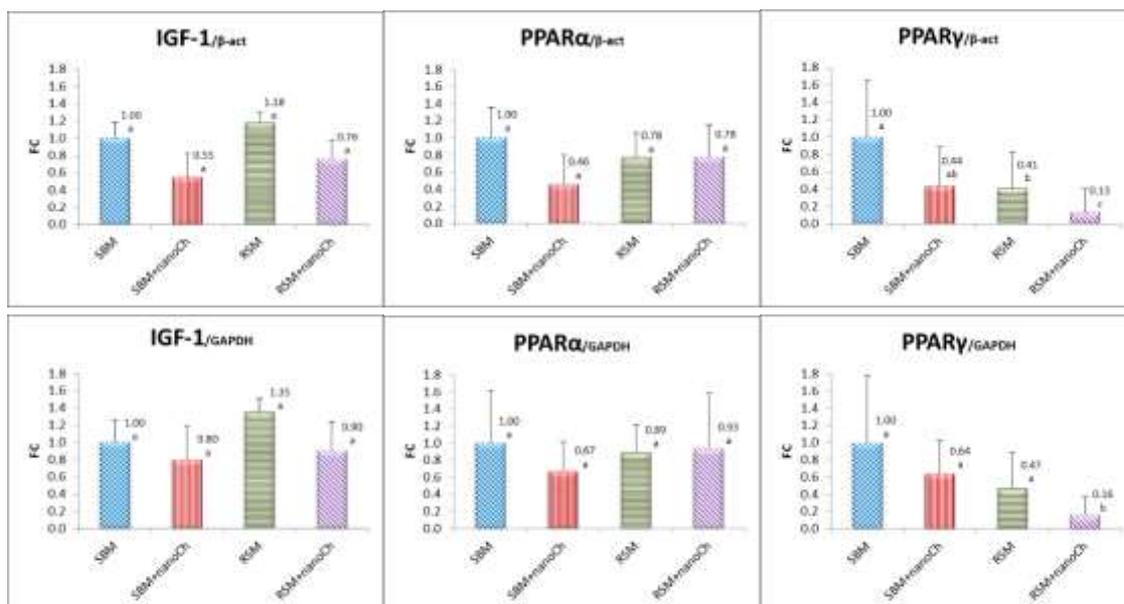**Fig. (4): Effect of different experimental diets on expression of *IGF*-1, *PPARα* and *PPARγ* genes normalized to the expression of β-*act* and *GAPDH* reference genes in liver tissue.**

CONCLUSION

It could be suggested using rocket seed meal (RSM) in broiler chicks diet up till 7.5 % singly or in combination with nano-chitosan without any adverse effect on performance, blood biochemical and immunological responses and related gene expression criteria. Besides, a significant effect in reducing the lipogenesis in broiler chicks, this is extremely important for the health and nutritional value of their products.

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تأثير التغذية على العلائق المحتوية على كسب بذور الجرجير والمدعمة بالنانوشيتوزان على الأداء الإنتاجى والتعبير الجينى المرتبط بالنمو وتكوين الليبيدات فى كتاكيت التسمين

هيام محمد أبو المعاطي*، محمد حسن عبد العزيز**، محمد زكريا الدياسطى**، إيمان أحمد السعيد***
*قسم إنتاج الدواجن ــ كلية الزراعة ــ جامعة المنصورة ــ مصر.
**قسم الوراثة ــ كلية الزراعة ــ جامعة المنصورة ــ مصر.
*** قسم إنتاج الدواجن ــ كلية الزراعة ــ جامعة دمياط ــ مصر.

أجريت الدراسة لتقييم تأثير تغذية كتاكيت التسمين من سلالة كوب ٥٠٠ عمر يوم غير مجنسة على علائق تحتوى على مصدرين من البروتين النباتى (كسب فول الصويا وكسب بذور الجرجير) المدعمة بالنانوشيتوزان أو بدونه خلال مرحلتى البادئ والنامى حتى الإنهاء وذلك على النمو ومقاييس سيرم الدم وكذلك أنماط التعبير عن الجينات المرتبطة بالنمو وتكوين الليبيدات (PPARα, PPARγ IGF-1,) فى أنسجة الكبد. حيث تم توزيع ٢٢٠ كتكوت عشوائياً على أربع علائق(معاملات تجريبية) (تضمنت كل معاملة خمس مكررات بكل مكررة ١١ كتكوت) فى مزرعة أرضية تحت ظروف صحية.

وأوضحت النتائج أن كتاكيت اللحم المغذاه على ٧٫٥٪ كسب بذور الجرجير حققت أفضل وزن نهائي للجسم، أفضل زيادة وزنيه، وأفضل معامل تحويل غذائي مقارنة بمجموعة الضبط. أما الكتاكيت المغذاه على العلائق المحتوية على النانوشيتوزان فاستهلكت أقل كمية من الغذاء ومع ذلك حققت وزن جسم نهائي أعلى وزيادة وزنيه مقارنة بالمجموعة الضابطة. الطيور المغذاه على العلائق المحتوية على كسب بذور الجرجير سجلت نقصا معنويا فى مستويات سيرم الدم من الجليسريدات الثلاثية والدهون الكلية والكوليستيرول الليبوبروتينى منخفض الكثافة ونشاط إنزيمات الكبد مقارنة بالمجموعة الضابطة. بينما لم يكن هناك فروق معنوية فى مستويات السيرم من الألبيومين، الكوليستيرول الكلى، والكوليستيرول الليبوبروتينى مرتفع الكثافة.

كما أحدثت التغذية على العلائق المحتوية على كسب بذور الجرجير زيادة معنوية فى تركيزات الدم من الجلوبيولينات المناعية، وهرمونى الغدة الدرقية ونشاط انزيمات مضادات الأكسدة ونشاط إنزيمات السوبر أوكسيد ديسميوتيز، والكتاليز مقارنة بالمجموعة الضابطة.

لم يكن هناك فروق معنوية فى التعبير الجينى بين المجموعات التجريبية المختلفة فيما يخص نشاط جينى IGF-1 وPPARα . بينما أظهر جين PPARγ انخفاضًا معنويًا في نشاط النسخ في الدجاج الذي يتغذى على كسب بذور الجرجير المدعم بالنانوشيتوزان. ويمكن أن نستنتج من هذه الدراسة أنه يمكن تغذية كتاكيت اللحم على علائق تحتوى على ٧٫٥٪ كسب بذور الجرجير بمفرده أو مدعم بالنانوشيتوزان دون حدوث تأثيرات سلبية على الأداء الإنتاجى و معايير الدم أو التعبير الجينى ذو الصلة مع تأثير مفيد لعليقة كسب الجرجير المدعم بالنانوشيتوزان يتمثل في خفض مستويات الليبيدات في كتاكيت التسمين.