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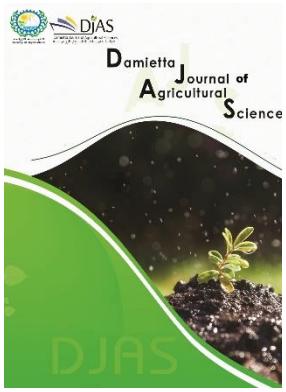
Genetic Effects of Dietary Protein Sources on Gene Expression and Inherited Traits in Broiler Chicks.

Norhan M. Sharshera [1] and Eman A. El-Said [2]
[1]Agric. Biotechnology Department, Faculty of Agriculture, Damietta University, Egypt
[2]Animal,Poultry and Fish Production Department, Faculty of Agriculture, Mansoura University, Egypt



ABSTRACT

A feeding experiment was conducted to elucidate the impact of partially substituting soybean meal with three alternative plant protein sources (rocket seed meal, Nigella sativa meal, and coconut meal) or their combination on gene expression and inherited traits and blood lipid profile. A total of 225 one-day-old Ross 308 broiler chicks were randomly allocated into five dietary treatments: a control diet containing soybean meal and four experimental diets incorporating the respective plant protein sources. The results demonstrated that the growth responses of the birds fed the alternate protein diets significantly improved in live body weight (LBW), body weight gain (BWG), feed consumption (FC), and feed conversion ratio (FCR) compared to the control. Furthermore, serum concentrations of immunoglobulins (IgG, IgM, and IgA) were significantly increased in all treatment groups relative to the control . Additionally, the inclusion of alternative protein sources significantly increased total antioxidant capacity (TAC) and reduced malondialdehyde (MDA) levels. The strongest antioxidant response was observed in diets containing rocket seed meal (RSM) and Nigella sativa meal (NGM).

Significant variations were observed in the expression of IGF-1, PPARα, and PPARγ genes among the dietary groups. The findings indicated that untraditional protein sources modulated lipid metabolism and growth-related pathways. Treatments upregulated IGF-1 and PPARα expression while downregulating PPARγ transcription. Diets containing rocket seed, Nigella sativa, and coconut meals proved particularly effective in optimizing the expression of these target genes.

Key words: *Gene expression, IGF-1; PPARs; lipids; antioxidant Genetic Immunity*

INTRODUCTION

One of the major challenges in poultry production is providing nutritionally balanced diets that meet the growth and physiological requirements of birds (Wilhelmsson et al., 2019). Feed accounts for approximately 70–75% of total production costs (Pires Filho et al., 2021), with dietary protein mainly supplied by soybean meal—representing the most expensive component. Feed cost is strongly influenced by both the level and the source of dietary protein, which is the most expensive component of animal feed (Singh et al., 2006). Protein plays a fundamental role in the synthesis of structural body components (Sabino et al., 2004), owing to the amino acid bioavailability it provides (Vasconcellos et al., 2015). Inadequate protein levels or reliance on sources with low biological value can lead to impaired growth, poor feed efficiency (Wen et al., 2018), reduced carcass quality, and detrimental effects on bird health (De Figueiredo Vasconcellos et al., 2010).Utilization of untraditional protein sources is the conclusive resolution to raise cost of production and feed cost. Among alternative protein sources, coconut meal, a by-product of coconut oil extraction is notable for its high dietary fiber content .Panigrahi et al. (1987) reported satisfactory broiler growth performance when 25% of the diet was replaced with copra meal supplemented with 0.5% lysine. Nutritionally, coconut meal contains approximately 21% protein, 5% lignin, 48% carbohydrates, and 5.7% lipids (Sundu & Dingle, 2003), in addition to 25–30% mannan, present as both mannan and galactomannan forms. Copra water is a traditional but underutilized product

(Jordana, 2000), with recognized nutritional and medicinal applications (Ediriweera, 2003). Coconut meal is protein sources and enriched with essential amino acids, especially lysine, methionine, isoleucine and leucine (CNPSA 1991).

Eruca sativa (rocket) has long been used in traditional medicine for its antiscorbutic, diuretic, antimicrobial, and aphrodisiac properties (Grieve, 1959; Boulos, 1983). It is also commonly used as a culinary spice (Simoes et al., 2009) and is recognized as a natural source of antioxidants (Hassein, 1985; Yani et al., 1998) and oil production (Lamy & Schroder, 2008). Rocket leaves and seeds conserved against oxidative damage by reducing free radicals and enhancing antioxidant enzymes activity (Alam and Jabbar, 2007). Essential oil from Rocket seeds is characterized by a rich in compounds containing sulfur and nitrogen (Miyazawa et al., 2002). Which, is used as an appetizer, urine and phlegm discharger, sexual power enhancer and blood cleaner (Kim et al, 2004).

Nigella sativa (black seed), a member of the Ranunculaceae family, is an annual herbaceous plant widely valued for its medicinal, aromatic, and nutritional properties (Randhawa, 2008). Numerous in vivo and in vitro studies have shown that its bioactive constituents possess anti-inflammatory, analgesic, hepatoprotective, nephroprotective, and antimicrobial properties (Yildiz et al., 2008; Pichette et al., 2012; Morsi, 2000).Nigella sativa (NS) has been reported to possess activity against coccidial parasites and helminths (Maqbool et al., 2004; Rahman and Nada, 2006; Baghdadi and Al-Mathal, 2011). Nigella sativa is among the most important medicinal plants, because of its beneficial actions, as a natural alternative to antibiotics for promoting animal health and enhancing both the yield and quality of animal-derived products. Furthermore, Nigella sativa seeds are rich in protein, containing over 30% (El-Ayek, 1999), making them a versatile feed ingredient., and inclusion in balanced rations has been shown to enhance feed intake (Abdullah and Al-Kuhla, 2010), and overall nutritive value in livestock production systems (Shewita and Taha, 2011).The expression levels of nuclear hormone receptors such as peroxisome proliferator-activated receptors (PPARs) and growth factors like insulin-like growth factor 1 (IGF-1) in liver and muscle tissues serve as molecular indicators of dietary effects on metabolism and growth (Wahle et al., 1995; 2003). PPARα and PPARγ regulate lipid metabolism, fatty acid oxidation, and adipogenesis, while IGF-1 is directly involved in myoblast proliferation and differentiation (Coleman et al., 1995; Noguchi, 2005; Xu et al., 2003).By upregulating the expression of the enzymes carnitine palmitoyl transferase and acyl-coenzyme A oxidase, Peroxisome Proliferator-Activated Receptorα (PPARα) increases the oxidation of fatty acids (Bell et al., 1998 and Pineda et al., 1999).PPARγ role in the production of tissues and fat cells (adipogenesis) in chickens is evidence of its significant metabolic impact (Navidshad and Royan, 2015). Understanding how coconut oil, Nigella sativa, and Eruca sativa affect these genes may help optimize broiler.Therefore, the present study aimed to evaluate the effects of dietary inclusion of Eruca sativa, coconut meal (copra), and Nigella sativa as alternative protein sources on growth performance (body weight gain, feed intake, and feed conversion ratio), immune responses, oxidative status, and the expression of lipid metabolism-related genes in broiler chicks.

MATERIALS AND METHODS

The experiment was conducted on a private poultry farm located in Damietta Governorate, Egypt, where the ambient temperature during the study period ranged between 33°C and 22°C. All experimental procedures related to animal care and management complied with the ethical standards of the Local Experimental Animal Care Committee and were approved by the Institutional Ethics Committee, Faculty of Agriculture, Damietta University, Egypt

Experimental design

A total of 225 one-day-old Ross 308 broiler chicks with uniform initial body weight were randomly assigned to five dietary treatments. The experimental design involved partial substitution of soybean meal with three alternative plant protein sources—rocket seed meal (RSM), Nigella sativa meal (NGM), and coconut meal (CM) or their combination (RSM + NGM + CM). Each treatment group consisted of 45 chicks, equally distributed into three replicates (15 birds per replicate). Diets were formulated to meet the nutrient requirements for broiler chickens according to NRC (1994).

Feed and water were provided ad libitum throughout the experimental period. The ambient temperature was maintained at 34 °C during the first week and gradually reduced to 21 °C by the end of the trial.

Criteria of response

Broiler chicks were weighed at day old and 35 days of age. At the end of the experimental period, live body weight (LBW) and feed consumption (FC) of broiler chicks were recorded, body weight gain

(BWG) and feed conversion ratio (FCR) were also calculated.

At slaughter, blood samples were collected from five birds per treatment group. Serum was separated by centrifugation at 3500 rpm for 20 min then stored at −20 °C until biochemical analyses were performed.

Table (1). Components of the broiler chicken diets for alternative plant protein sources

Ingredients (%)	Starter diet(0–3weeks)					Grower diet(3-6weeks)				
	Control	CM	RSM	NGM	CM+RSM+ NGM	Control	CM	RSM	NGM	CM+RSM +NGM
Yellow corn, ground	61.5	58.4	59.4	59.5	58.99	67.2	64.2	64.7	65.2	64.89
Soybean meal (44% CP)	16.0	19.0	16.67	16.67	16.5	15.0	15.0	15.0	15.0	15.0
Vegetable oil	1.0	1.0	1.0	1.0	1.3	1.0	1.0	1.0	1.0	1.0
Di calcium phosphate	1.8	1.8	1.8	1.8	1.8	1.5	1.5	1.5	1.5	1.5
Ground limestone	1.8	1.8	1.8	1.8	1.8	1.5	1.5	1.5	1.5	1.5
Corn gluten meal	16.5	19.3	18.3	18.5	18.7	2.0	5.2	5.2	5.2	5.07
Common salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Vit. and min. premix	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Lysine HCl	0.0	0.0	0.0	0.0	0.0	0.4	0.4	0.4	0.4	0.4
Dl-methionine	0.0	0.6	0.6	0.6	0.6	0.0	0.0	0.0	0.0	0.0
CM	0.0	3.0	0.0	0.0	1.78	0.0	3.0	0.0	0.0	1.67
RSM	0.0	0.0	3.33	0.0	1.78	0.0	0.0	3.0	0.0	1.67
M	0.0	0.0	0.0	3.33	1.78	0.0	0.0	0.0	3.0	1.67
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Each 3 kg of premix contained vit. A, 12,000 IU; vit. D3, 2200 IU; vit. E, 10 mg; vit. K3, 2.0 g; vit. B1, 1.0 g; vit. B2, 5.0 g; vit. B6, 1.5 g; vit. B12, 10 mg; pantothenic acid, 10 mg; niacin, 30 mg; folic acid, 1.0 g; biotin, 50 mg; choline chloride, 300 mg; Mn, 60 mg; Zn, 50 mg; Cu, 10 mg; Fe, 30 mg; I, 1.0 g; Se, 100 mg; Co, 100 mg; and CaCO3, 3g. CM coconut meal, RSM rocket seed meal, NGM Nigella sativa meal , ME metabolizable energy

Blood biochemical parameters

Serum concentrations of total lipids, triglycerides (TG), high-density lipoprotein (HDL), total cholesterol (TC), and low-density lipoprotein (LDL) were determined spectrophotometrically using commercial kits (Biodiagnostic Co., Giza, Egypt) according to the procedures described by Taha et al. (2019). Serum immunoglobulins (IgG, IgM, and IgA), total antioxidant capacity (TAC), and malondialdehyde (MDA) were also measured using standard commercial assay kits following the manufacturer's instructions.

Gene Expression Analysis:

Immediately after slaughter, liver tissue samples were collected from three birds per treatment, rapidly frozen in liquid nitrogen, and stored at −80 °C until subsequent RNA extraction.

To measure the expression levels of the selected genes, total RNA was taked out from each sample using the Gene JET RNA Purification Kit (Thermo Scientific, cat. no. K0731), following the manufacturer's instructions. A Thermo Fisher Scientific Inc. NanoDrop spectrophotometer was used to assess the isolated RNA's concentration and purity. Ethidium bromide staining electrophoresis was used to identify 18S and 28S rRNA, which should appear as clear, separate bands. DNase I (RNase-Free) was then used to extract the genomic DNA from the RNA preparations.The RevertAidTM First Strand cDNA Synthesis Kit from StepOne Applied Biosystems (Thermo Scientific) contained a cDNA synthesis process that was used. After adding the Ribolock RNase inhibitor, the reaction halted by heating it to 70°C for five minutes. The cDNA was then stored at -80°C until it was required.

Primer Design: Using Primer-BLAST, particular primer pairs (forward and reverse) for target genes (PPARα, PPARγ, and IGF-1) and reference gene (β-actin) were produced in accordance with Abou El-Maaty et al. (2021) and previous findings. These primers were validated on cDNA samples with PCR and gel electrophoresis after being acquired from Invitrogen (Thermo Fisher Scientific).

RT-PCR quantification:

According to the instructions, a quantitative 2-step RT-PCR was executed using an Applied Biosystems® Veriti® 96-Well Thermal Cycler and a Master Mix Maxima SYBR Green qPCR 2x ROX solution. RT-PCR program was as follows:

Initial denaturation: 95 °C for 10 minutes

40 variations of:

Denaturation: 95 °C for 15 seconds

Annealing/extension: 60 °C for 60 seconds (data collection is part of this phase).

By examining the melting curves of the RT-PCR results, the specificity of the target and reference gene primers was evaluated. To ensure that only one amplicon was amplified, these curves were performed

between 55 and 95 degrees Celsius. Each sample displayed a single peak in the melting curve and about the same Tm for the target sequence for each gene being tested. Relative expression levels of the target genes were quantified using the 2^−ΔΔCt method (Livak and Schmittgen, 2001). Expression values were normalized to the β-actin housekeeping gene as an internal reference. Change of fold (FC) Expression was normalized using reference or housekeeping gene as endogenous controls. The output data's fold change (FC) values were calculated in relation to the control (fold relative expression). Target and housekeeping gene CT data were entered into an Excel spreadsheet in Microsoft Office, which included the 2-ΔΔCT method equations, to get the prorated expression values. Gene expression data was compared using SAS 9 software. The significance of differences was determined at $P < 0.05$ using Duncan's Multiple Range Test. The correlation coefficient and linear regression were calculated using the statistical software Minitab17. The ranges listed below were examined:
*Normalized expression levels with respect to β-actin
* The relative expression of genes associated with performance and study

Statistical analysis

Data were analyzed using the General Linear Model (GLM) procedure of SAS software (Version 9.0; SAS Institute, 2009) to evaluate treatment effects. When significant differences were detected ($P \leq 0.05$), mean comparisons were performed using Duncan's Multiple Range Test (Duncan, 1955)., following the statistical model below:

$x_{dk} = \mu + L_d + e_d$

Where:

x_{dk} = An observation, μ = Overall mean,

L_d = tested diet (i = 1, 2, 3, 4 and 5), e_d = Random error.

RESULTS AND DISCUSSION

The effects of feeding the diets supplemented different meal protein sources (RSM, NSM, CM) and their combination as partial replacements for soybean meal on broiler growth performance are summarized (Table 2). Body weight of chicks at day old showed no significant differences ($P \leq 0.01$) among the dietary treatments. Similarly, the end of feeding trial at 35-day, chicks were fed the alternative meal protein sources (RSM, NSM, CM and their combination) exhibited no significant ($P \leq 0.01$) differences in live body weights (LBW) and BWG compared to the control group at slaughtering age

Table (2). Effects of different protein source on growth performance of broiler

Dietary Treatments	IBW	LBW 35-d-old	BWG	FC	FCR
Protein source	(g)	(g)	(g)	(g)	(g:g)
SBM	45.4	2150	2105	3200^{a}	1.52^{a}
RSM	45.2	2245	2200	3020^{ab}	1.37^{bc}
NSM	45.1	2260	2215	3050^{ab}	1.37^{b}
CM	44.8	2230	2185	3000^{b}	1.37^{bc}
Mix (RSM+ NSM+ CM)	44.9	2240	2195	2950^{b}	1.34^{c}
SEM	0.92	72.3	73.95	35.87	0.015
Sig.	NS	NS	NS	**	**

$^{a-c}$: means in the same column bearing different superscript are significantly different ($P \leq 0.05$) NS: not significant, **: significant at $P \leq 0.01$, SEM= Standard errors of the means

Chicks were fed the alternative plant protein sources (RSM, NSM, CM and their combination) exhibited significantly ($P \leq 0.01$) heavier feed consumption (FC) than control group.

Chicks fed the diets containing (RSM, NSM, CM, and their combination) obtained significantly better ($P \leq 0.05$) FCR than the control group (Table 2). However, the combination (RSM, NSM, CM) group exhibited a significant decrease ($P \leq 0.05$) in FCR as against the control and other groups (Table 2). It is widely recognized that high-fat diets can slow the passage rate of feed through the avian gastrointestinal tract, thereby negatively affecting feed consumption (FC). The observed enhancements in the feed conversion ratio (FCR) of broilers fed the tested plant protein sources (RSM, NSM, and CM) in this study align with the findings of Abou El-Maaty et al. (2021). This improvement is likely linked to the higher levels of essential amino acids present in the experimental meals compared to the control, as shown in Table 2. Abou El-Maaty et al. (2021) reported that chicks receiving 7.5% RSM in both starter and finisher diets achieved significantly better FCR values than control one. Similarly, Abdo (2003) demonstrated that substituting soybean meal (SBM) with 10–25% RSM resulted in a superior body weight gain (BWG) relative to that of the control. In another study, Razooqi et al. (2014) found that supplementing broiler

diets with rocket seed (1.0 g/kg) enhanced growth performance compared with un-supplemented diets

The effect of different source of protein (SBM, RSM, NSM, CM and their combination) on blood total lipids and lipids profile concentrations are summarized in Table 3.

Data in Table 3 revealed a significant (P≤0.05) decrease in serum total lipids, TG, Chol and LDL concentration in tested diatry groups compared with control group. However, SBM, RSM, NSM, CM and their combination diet groups obtained a significant (p≤0.01) raised in serum HDL concentration compared with control.

Table (3). Effects of different protein source on serum blood parameters of broiler

Dietary Treatments	T.Lipids	Chol	TG	HDL	LDL	IgG	IgM	IgA
Protein source	**mg/dl**	**mg/dl**	**mg/dl**	**mg/dl**	**mg/dl**	**g/l**	**g/l**	**g/l**
SBM	701^{a}	220.0^{a}	149.9^{a}	50.2^{d}	139.9^{a}	420.54^{d}	174.22^{b}	120.28^{b}
RSM	634^{b}	179.0^{c}	125.8^{c}	72.0^{a}	81.8^{c}	565.71^{a}	232.37^{a}	134.44^{a}
NSM	645^{b}	191.6^{b}	132.7^{b}	67.9^{b}	97.1^{bc}	535.83^{b}	229.06^{a}	133.83^{a}
CM	684^{a}	197.3^{b}	139.7^{b}	59.4^{c}	110.0^{b}	465.79^{c}	185.11^{b}	129.07^{ab}
Mix (RSM+ NSM+ CM)	662^{ab}	194.1^{b}	139.6^{b}	63.8^{b}	102.4^{b}	516.56^{b}	222.86^{a}	138.97^{a}
SEM	21.6	11.2	4.2	2.7	5.4	9.05	4.75	3.35
Sig.	**	**	**	**	**	*	*	**

$^{a-c}$: means in the same column different superscript are significantly different (P≤0.05)
*significant at P≤0.05, SEM= Standard errors of the means

The results indicated that broilers fed the diets supplementing meal protein sources (RSM, NSM and CM and their combination) had significantly higher (P ≤ 0.05) serum IgG levels compared with the control group. Significantly superior serum levels of IgM and and IgA (P≤0.01) were achieved for broiler fed the diets replacement RSM, NSM and the mixture plant protein sources (RSM, NSM and CM) in comparison with control and CM diet. The reducing in cholesterol blood levels may be attributed to the high content of NS from unsaturated fatty acids which may induce the cholesterol excretion into the digestive tract and stimulate the oxidation activity (Khodary et al., 1996). Increasing immune globuline may be related to Nigella sativa components such as carvacrol, nigellimine, nigellicine, thymol, and thymoquinone which have possitive effects in maintaining a physiological balance and therefore enhancing a healthy environment for immunity responses (Alqasoumi et al., 2010).

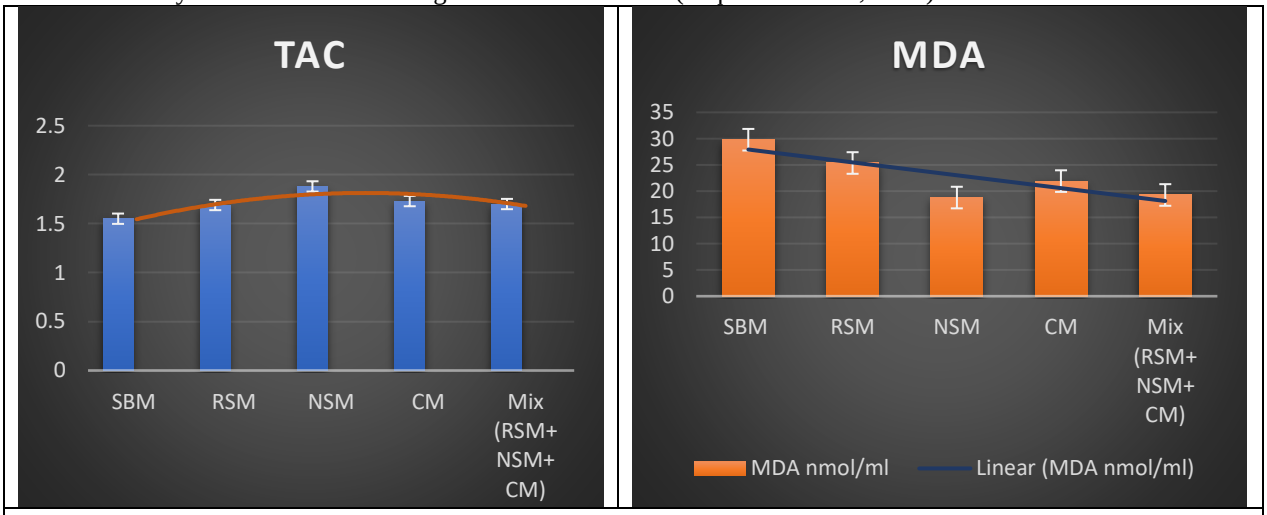


Fig. 1. Effects of different protein sources on serum blood antioxidant levels of broiler

Serum blood criteria of immune response, antioxidant status, and thyroid hormones in birds fed alternative diets with meal plant protein sources (RSM, NSM and CM) and their combination are presented in Table 3.

Notably, chicks fed NSM diet group exhibited significantly higher (P≤0.05) serum total antioxidant activity compared with both the control and other treatment groups. Furthermore, TAC values No significant differences were observed among the other groups irrespective of the plant protein source. Conversely, broilers fed diets containing RSM, NSM, CM, or their combination exhibited significantly lower (P ≤ 0.05) serum malondialdehyde (MDA) levels compared to the control group. These findings are consistent with Shalaby and Hammouda (2014), who

observed that rocket seeds markedly improved the activities of antioxidant enzymes compared with control one.

The beneficial effects of Nigella sativa seeds could be attributed to their bioactive compound, carvacrol, thymoquinone, along with other essential oil constituents such as , 4-terpineol, and anethole. Nigella sativa seeds contain approximately 0.5–1.6% volatile oil, of which thymoquinone represents about 60–80%. Their bioactive compound, exhibits strong antioxidant activity by scavenging various free radicals, including those involved in iron-dependent microsomal lipid peroxidation, and hydroxyl radicals (Attia et al., 2003). Its inhibitory action on lipid peroxidation, a process mediated by free radicals, further highlights its antioxidant potential. Mahmoud and Mansour (2000) demonstrated that the inhibitory action of thymoquinone is concentration dependent, in addition, replacement with Nigella sativa has been shown lower in malondialdehyde (MDA) levels while enhancing the activities of antioxidant enzymes, effects attributed to its antioxidant capacity.

Furthermore, Nigella sativa oil may help preserve cellular ATP by reducing the activities of xanthine oxidase and adenosine deaminase, enzymes considered major sources of oxygen free radicals (Guler et al., 2007).

Gene expression activity

Relative expression patterns of, PPARα, PPARγ and IGF-1 genes in liver tissue, immediately taked after slaughter from Ross 308 broiler chicken strain feeding with treatments of different untraditional protein sources and control, were illustrated in Figures 2,3,4

Significant differences were recorded in the gene expression levels of IGF-1, PPARα, and PPARγ among the experimental birds. This suggests that the activity of growth factor IGF-1 and fatty acid oxidation were influenced by different untraditional Protein Sources. Interestingly, the efficiency of IGF-1 and PPARα were increased by the treatments, while PPARγ gene appeared a decrease in transcriptional action in chickens. High gene expression of PPARα was recorded in rocket seed meal and mix between (RSM,NSM.CM) was the highest in PPARα and *IGF-1* gene expression, while the PPARγ gene expression was the better in Mix then NSM protein source, Blood measurements also agree with gene expression.

Polypeptides known as insulin-like growth factors (IGF) are essential for cell division and proliferation. Chesik et al. (2007) showed that IGF binding proteins either increase or decrease the growth-promoting effects of IGFs on cell culture and extend the half-life of IGFs. By promoting hepatic fatty acid β-oxidation, PPARα activation can indirectly reduce fat accumulation. This is corroborated by research showing that decreased fat storage in liver cells is linked to increased PPARα expression (Yoon, 2009). On the other hand, PPARγ is essential for adipogenesis, or the accumulation of fat. It stimulates the expression of genes involved in the development of fat cells by acting as a major gene regulator in adipose tissue (Royan & Bahman, 2016). Coconut oil or meal, which mainly affects PPARα and PPARγ expression through its fatty acid composition (Xu et al., 2003), the best results demonstrated that coconut oil and mix treatment improved lipid gene expression. Additionally, coconut oil boosted the expression of IGF-1 and IGFBP-1 genes. According to Ibrahim et al. (2022), rocket seed meal (RSM) similarly shown a significant drop in PPARγ expression in the fed group, which is consistent with our findings. Additionally, the RSM-fed chicks showed an impact on the IGF-1 gene's transcription level. According to Abou El-Maaty et al. (2021), this indicates that feeding on the RSM diet increased the activity of growth factor IGF-1 and fatty acid oxidation.

Nigella sativa oil or seed meal enhanced growth performance and immune status in poultry trials, which increased IGF-1 gene expression treatments compared to control (Balbaa et al., 2016, El-Bahr and El-Sabagh ,2014). Nigella sativa seed extract also decreased PPARγ-dependent transcriptional activity in adipocytes, hepatocytes, and myocytes, indicating a direct stimulatory effect (Haas et al., 2022).

Therefore, it was necessary to take advantage of these protein sources to increase the efficiency of production and gene expression of these genes, which have important economic and health implications.

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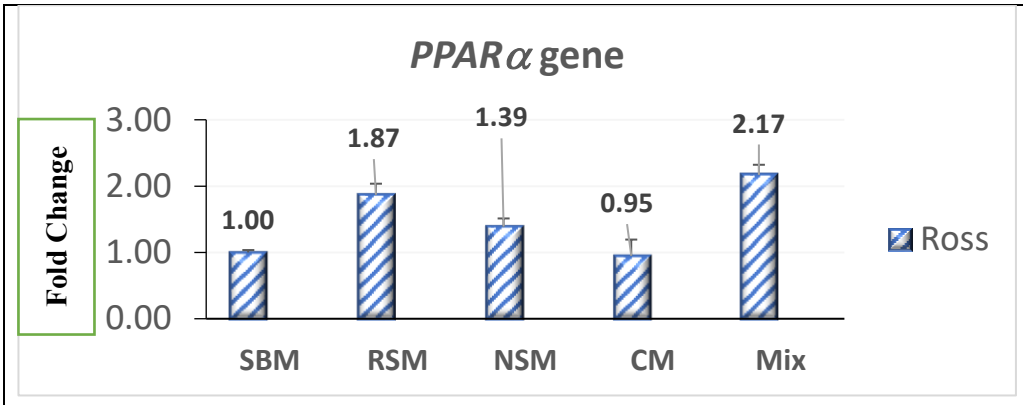

Fig 2: **Effects Of Different Untraditional Protein Sources On *PPARα* Gene Normalized To The Expression B-Act Reference Genes In Liver Tissue.**

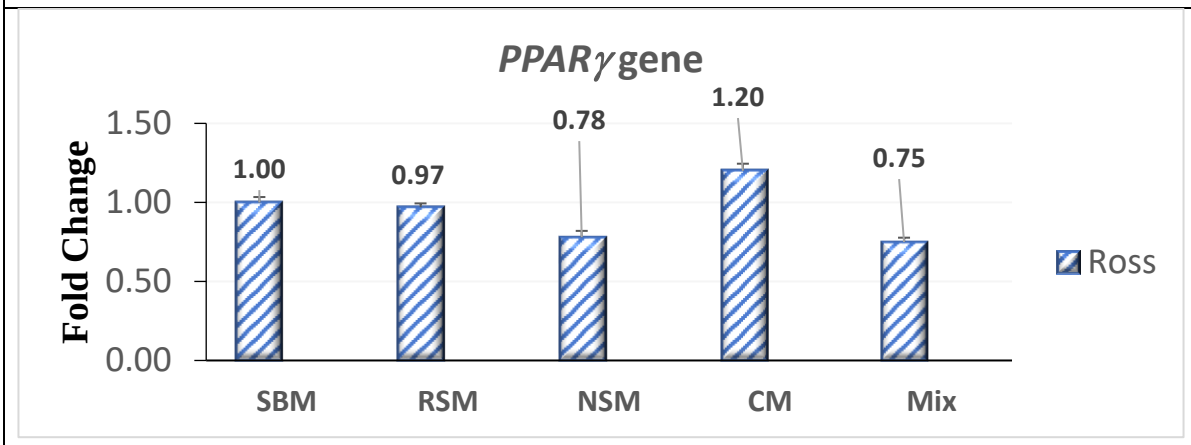

Fig 3: **Effects Of Different Untraditional Protein Sources On *PPARγ* Gene Normalized To The Expression B-Act Reference Genes In Liver Tissue.**

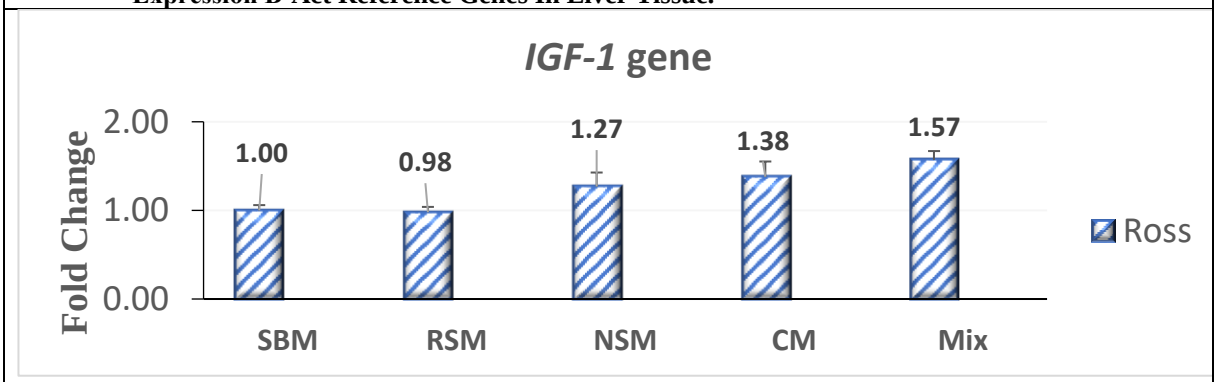

Fig 4: **Effects Of Different Untraditional Protein Sources On *IGF-1* Gene Normalized To The Expression B-Act Reference Genes In Liver Tissue.**

CONCLUSION

This study highlights the potential of alternative plant protein sources to enhance broiler performance, improve antioxidant capacity, and modulate gene expression. These findings support the sustainable use of unconventional protein sources as cost-effective alternatives to soybean meal.

Status, and modulate lipid-related gene expression in broiler chicks. These findings provide strong evidence for the use of unconventional plant protein sources as cost-effective and sustainable alternatives to soybean meal

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

AUTHORS CONTRIBUTION

The authors developed the concept of the manuscript. All authors checked and confirmed the final revised manuscript**.**

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الملخص العربى

التأثيرات الوراثية لمصادر البروتين الغذائي على التعبير الجيني والصفات الموروثة في دجاج اللحم

نورهان محمد شرشيرة[1] ,ايمان احمد السعيد[2]
[1]قسم البيوتكنولوجيا الزراعية ,كلية الزراعة,جامعة دمياط
[2]قسم الانتاج الحيواني والداجنى والسمكي, كلية الزراعة,جامعة دمياط

تؤدى التغذية ببعض المصادر البروتينية النباتية البديلة مثل (كُسب الجرجير، كُسب حبة البركة، وكُسب جوز الهند) أو خليط منها على معدلات النمو، كفاءة التحويلية للغذاء، ودهون الدم. تم توزيع 225 كتكوت تسمين من سلالة Ross 308 عمر يوم إلى خمس مجموعات: مجموعة كنترول تم التغذية على عليقة كُسب فول الصويا، وأربع علائق اختبارية تحتوي على البروتينات النباتية المختارة. وتم قياس بعض القياسات الفسيولوجية وكذلك التعبير الجينى لجينات المتأثرة بالدهون. أظهرت النتائج أن الطيور التي تناولت علائق البروتينات البديلة سجلت تحسنًا ملحوظًا في معدلات النمو (الوزن الحي، الزيادة الوزنية، استهلاك العلف، ومعامل التحويل الغذائي) مقارنة بالمجموعة االكنترول. علاوة على ذلك، أظهرت المعاملات ارتفاعًا في مستويات الأجسام المناعية (IgG, IgM, IgA) في الدم مقارنةً بالكنترول. كما أن المصادر البروتينية النباتية الأربعة أدت إلى زيادة مضادات الأكسدة (TAC) وانخفاض (MDA)، خاصة في العلائق التي تضمنت كُسب الجرجير وحبة البركة حيث سجلت التأثير الأكبر على مستويات النشاط المضاد للأكسدة. كما سُجلت فروق معنوية في مستويات التعبير الجيني لكل من **IGF-1**و **PPARα**و **PPARγ**بين الطيور في المعاملات المختلفة. وأوضح ذلك أن أكسدة الأحماض الدهنية ونشاط عامل النمو IGF-1 تأثرت بمصادر البروتين غير التقليدية المختلفة. حيث ارتفع التعبير الجيني لكل من **IGF-1**و **PPARα** نتيجة المعاملات، بينما انخفض نشاط النسخ لجين **PPARγ**في الدجاج. وقد ثبت أن كُسب الجرجير، وحبة البركة، وكُسب جوز الهند له فعالية في تحسين التعبير الجيني للجينات. لذا نستنتج من الدراسة انه يمكن التغذية ببعض المصادر البروتينية النباتية البديلة مثل (كُسب الجرجير، كُسب حبة البركة، وكُسب جوز الهند) دون حدوث تأثيرات سلبية على الأداء الإنتاجى و معايير الدم أو التعبير الجينى للجينات ذات الصلةحيث يمكن من خلال المعاملات رفع معدلات النمو واكسدة الدهون في جسم الدجاج وكذلك خفض نسبة اللبيدات الضارة .