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Effect of dietary supplementation of phytogenic plant leaves on expression of insulin-like growth factor-1 (IGF-1) in the Japanese quail (*Coturnix japonica*)

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ABSTRACT

Nutrigenomics is an important and emerging field that examines the interactions between feed and genes that affect health and production. The present study aligned with this direction; the expression of insulin-like growth factor-1 (IGF-1) in the muscle of Japanese quail (*Coturnix japonica)* was evaluated following dietary supplementation with phytogenic feed additives. The experimental trial involved 120-day-old Japanese quail chicks reared for 42 days. Birds were randomly allocated into four treatment groups of 30 birds each: T1 (control), fed a basal diet formulated according to ICAR (2013) recommendations; T2, fed basal diet supplemented with 1% Shisham (*Dalbergia sissoo*) leaf powder; T3, fed basal diet supplemented with 1% poplar (*Populus deltoides*) leaf powder; and T4, fed basal diet supplemented with 2% leaf powder comprising 1% Shisham and 1% poplar. At 42 days of age, birds were slaughtered, and muscle tissue samples were collected for IGF-1 gene expression analysis. Total RNA was isolated, and gene expression was quantified using one-step quantitative real-time Polymerase chain reaction (qRT-PCR). IGF-1 expression was found higher in all phytogenically supplemented groups compared to the control, with the highest fold increase observed in the T4 group. These findings suggested that phytogenic leaf supplementation positively influenced muscle protein development, with a synergistic effect observed when multiple phytogenic sources are combined in the diet showing the higher fold increase in the expression level of IGF-1.

Keywords: Gene expression, Insulin like growth factor-1, Japanese quail, Phytogenic supplements

Poultry production plays an essential role in global food security by providing affordable sources of animal protein (Birhanu *et al*. 2023). In India, the poultry sector has expanded rapidly over the past two decades (Gulati and Juneja 2023, Chatterjee and Rajkumar 2015). Globally, poultry output is expected to exceed 37 billion birds by 2050, driven by rising protein demand and the adoption of intensive, automated production systems (Yitbarek 2019). The broiler industry represents a major component of this growth and contributes significantly to meeting rising nutritional requirements (Hatab *et al*. 2019). Japanese quail have gained considerable importance due to their rapid growth, high reproductive efficiency, good meat quality, and minimal space requirements (Priti and Satish 2014). Quail meat contributes approximately 0.20% to global poultry production, while meat-type quails constitute nearly 11.8% of productive poultry populations (Lukanov, 2019). In India, broiler quails introduced in 1974 are typically marketed at five weeks of age and attain slaughter weights of 165–300 g by 35–42 days (Portillo *et al*. 2014). Now a days, nutrigenomics is an emerging field in animal nutrition that focuses on the molecular study of the effects of various feed supplements on the expression profiles of genes responsible for growth and other vital parameters. Poultry growth is regulated by multiple genes, with insulin-like growth factor-1 (IGF-1) and growth hormone (GH) acting as primary endocrine regulators of muscle growth and protein deposition (Kita *et al*. 2005). The expression of IGF-1 is influenced by developmental stage, endocrine regulation, and nutritional status (Hawkes and Grimberg 2015, Thissen *et al*. 1994). Nutritional deprivation has been shown to alter IGF-IR expression, highlighting the IGF system's sensitivity to dietary modulation (Fu *et al*. 2001). Phytogenic feed additives especially leaves of agroforestry trees are increasingly used as alternatives to antibiotic growth promoters to reduce antimicrobial resistance and feed costs, although their effects vary with plant source and inclusion level (Pliego *et al*. 2022, Yang *et al*. 2015). Plant-derived phytochemicals possess nutritive and antioxidant properties and can regulate IGF-1-mediated growth pathways (Naeem and Fatima 2025, Caban *et al*. 2019). Shisham (*Dalbergia sissoo*) and poplar (*Populus deltoides*) leaves are well known to contain appreciable levels of

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Table 1. Information of PCR primers used in the study

S.No.	Name of gene	Primer	Sequence (5'-3')	Product length (bp)	Annealing (°C)	Reference
1.	GAPDH	Forward Primer	AGCTCCAGTAGAAGCAGG GA	148	59	Hatab *et al.* 2024
		Reverse Primer	CCCATTTAGCTGGTGGAGCA			
2.	IGF-1	Forward Primer	CTTGTGGATGGCATGATCT	86	59	
		Reverse Primer	CACCTAAATCTGCACGCT			

nutrients, phytochemicals, antioxidants and digestible components and bioactive compounds, making both tree leaves an alternate resource of feed for animals and poultry (Chaddha and Gupta 2025, Okińczyc *et al*. 2024). These two tree leaves are widely used as animal feed however, their role in regulating IGF-1 expression in Japanese quail remains largely unexplored. The current study, therefore, was designed to explore the effect of dietary supplementation of phytogenic plant leaves at different inclusion levels on the expression of IGF-1 in Japanese quail.

MATERIALS AND METHODS

One hundred and twenty day old healthy Japanese quails were procured from the Instructional Poultry Farm of G. B. Pant University of Agriculture and Technology, Pantnagar, and reared using standard guidelines. The birds were maintained under a cage system with proper housing and managemental practices throughout the 42-day experimental period and subjected to a feeding trial involving dietary supplementation with shisham and poplar leaf powder. The experiment was performed in accordance with the guidelines of the Institutional Animal Ethics Committee. The leaves of shisham and poplar were freshly collected, shade-dried, oven-dried, and ground in a hammer mill to form a fine powder. The obtained powder was stored in a sealed polyethylene bag to prevent moisture and supplemented in the basal diet at the desired level. All 120 individually weighed birds were randomly divided into 4 treatment groups, each with three replicates of ten birds. The four experimental groups were control (T1), which contained only the basal ration; T2, which contained the basal diet with 1% shisham leaves powder; T3, which contained the basal diet with 1% Poplar leaves powder; and T4, which contained the basal diet with 2% (1% shisham and 1% poplar leaves powder). At 42 days of age, the quails were humanely sacrificed for sample collection, the muscle tissue from different birds was collected, and the expression of the candidate gene insulin-like growth factor-1 (IGF-1) was analyzed using a relative quantification approach in real-time PCR using the Step One Real-Time PCR System (Applied Biosystems, USA). Total RNA was extracted from muscle tissues collected from birds in different treatment groups and subjected to SYBR Green-based one-step quantitative real-time PCR (qRT-PCR). RNA isolation was carried out using the TRIzol method with TRIzol® reagent (Invitrogen, USA) in accordance with the manufacturer's instructions. Briefly, 50–100 mg of muscle tissue was homogenized in 1 mL of TRIzol® reagent and incubated for 5 minutes at room temperature (15–30 °C), followed by phenol–chloroform extraction. The upper aqueous phase containing RNA was carefully transferred to a fresh tube, and RNA was precipitated by the addition of isopropanol at a ratio of 0.5 mL per mL of TRIzol® reagent used, followed by centrifugation to obtain an RNA pellet. The pellet was subsequently washed with 75% ethanol, air-dried, and finally dissolved in nuclease-free water. The RNA isolated was quantified for concentration and quality using the Biospectrometer (Eppendorf, Germany). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the endogenous reference gene. The primer pairs specific for the IGF-1 and GAPDH genes used in the present study are listed in Table 1. One-step SYBR Green-based qRT-PCR was performed using the Hi-Quanti One-Step SYBR Green RT-PCR Kit (Himedia, Mumbai, India). Each reaction mixture (20 µL final volume) comprised 10 µL of Hi-SYBR 2× Master Mix, 1 µL of One-Step RT enzyme mix, 1 µL each of forward and reverse primers (10 µM), 3 µL of RNA template, and nuclease-free water to volume. The thermal cycling protocol included reverse transcription step at 50 °C for 15 minutes, initial denaturation at 95 °C for 2 minutes and 30 seconds, followed by 40 amplification cycles consisting of denaturation at 94 °C for 30 seconds and combined annealing and extension at 59 °C for 45 seconds. Melt curve analysis was performed at the end of amplification to verify product specificity. Fluorescence data were collected during each annealing phase. Threshold cycle (Ct) values were exported to Microsoft Excel, and relative gene expression levels were calculated using the $2^{-}\Delta\Delta Ct$ method.

RESULTS AND DISCUSSION

The relative expression of the IGF-1 gene in muscle tissue varied markedly among the dietary treatments, as presented in Figure 1. Figures 2 and 3 represent the amplification plots and the melt curve, respectively, which showed the specific amplification in qRT-PCR. Birds in the control group (T1) exhibited the lowest IGF-1 expression level (0.07), representing the basal expression in non-supplemented group. Supplementation with 1% Shisham leaf powder (T2) resulted in a 1.84-fold increase in IGF-1 expression (0.13), indicating a

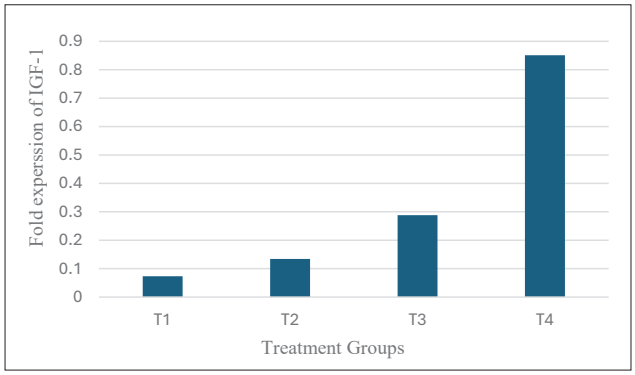

Fig. 1. Expression level of IGF-1 in the muscle tissue of Japanese quail

modest stimulatory effect on muscle growth–related gene activity. A further enhancement was observed in birds fed 1% Poplar leaf powder (T3), which exhibited a four-fold increase in IGF-1 expression (0.29), suggesting a stronger influence on anabolic signalling pathways. The most pronounced upregulation was recorded in the combined supplementation group (T4), which showed a twelve-fold increase in IGF-1 expression (0.85). This marked elevation indicated a synergistic effect of shisham and Poplar leaf powders when administered together. Overall, these results demonstrate that combined shisham and poplar supplementation substantially enhances IGF-1 expression in muscle tissue, thereby supporting improved muscle growth and performance in Japanese quail. The findings of the present study were consistent with earlier reports demonstrating enhanced IGF-1 expression in poultry

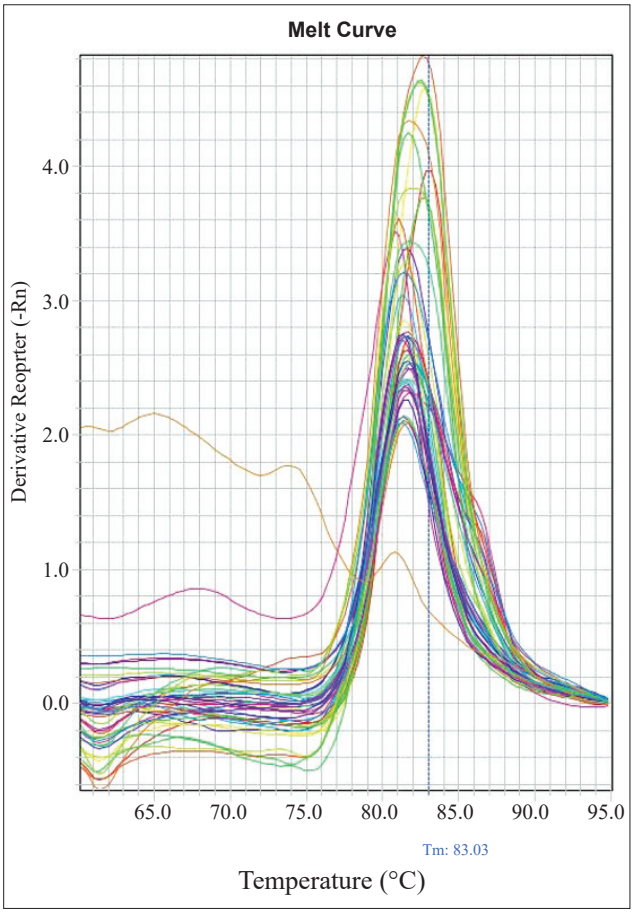

Fig. 3. The melt curve of the relative expression of IGF-1 by real-time PCR showing specific amplification

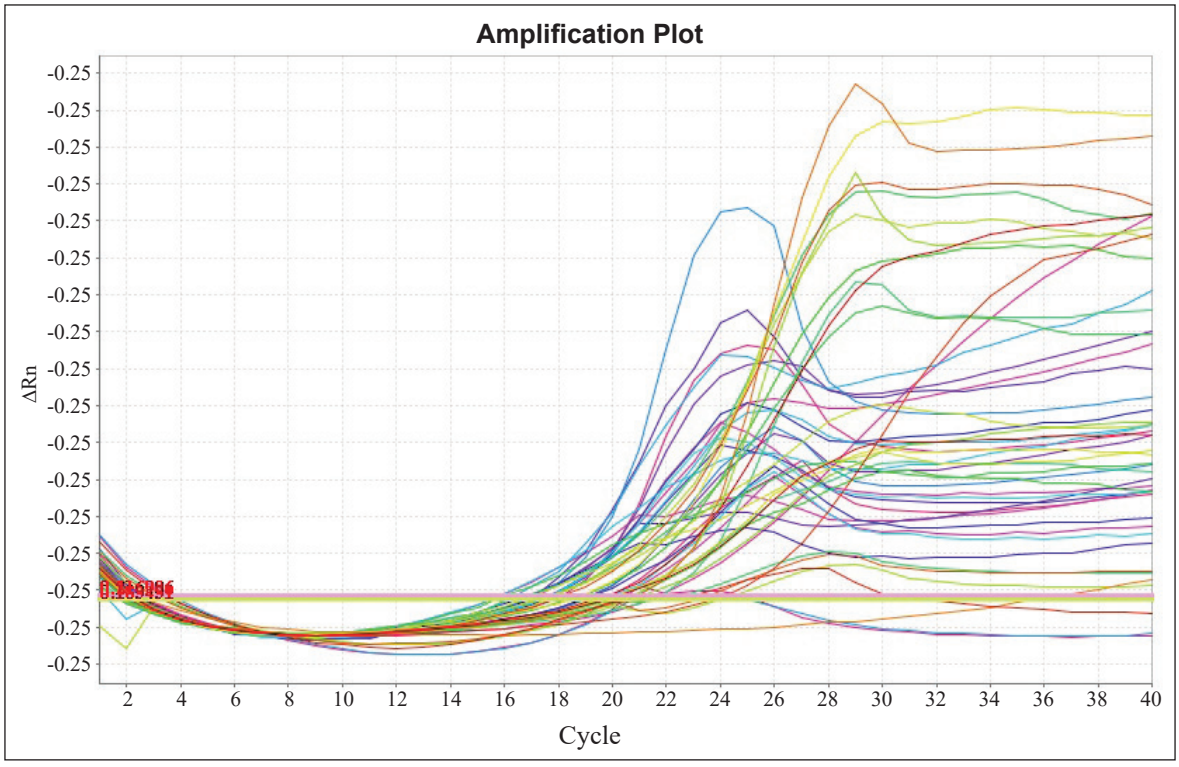

Fig.2. The amplification plots of the relative expression of IGF-1 by qPCR in various experimental groups

following supplementation with plant-derived bioactive compounds. Lv *et al.* (2019), reported that maternal genistein supplementation of shisham leaves significantly increased hepatic IGF-1 expression in offspring chicks through activation of the growth hormone–IGF-1 signalling pathway, thereby promoting growth and muscle development. Similarly, Hour et al. (2024) demonstrated that kaempferol, a phytochemical present in poplar leaves, activates IGF-1 signalling, leading to enhanced myogenic differentiation and muscle protein synthesis, confirming the central role of IGF-1-mediated pathways in skeletal muscle development. Caban *et al.* (2019) reported that plant-derived phytochemicals, particularly polyphenols, modulate the IGF-1 system by influencing IGF-1 synthesis, receptor activity, and downstream signalling pathways. Comparable effects were observed by Kurşun (2025), who reported dose-dependent upregulation of IGF-1 expression in Japanese quails fed *Moringa oleifera* leaf powder, attributing the response to improved nutrient availability, antioxidant protection, and modulation of growth-related molecular pathways. Although the plant sources differed, the underlying mechanism of phytogenic stimulation of IGF-1 signalling remained consistent with the present findings. In a comparable manner, Kamel *et al.* (2021) reported that dietary supplementation with pomegranate peel powder significantly increased hepatic IGF-1 gene expression in Japanese quail, indicating a consistent stimulatory effect of polyphenol-rich phytogenic additives on growth-related gene regulation in quail. In agreement with the present findings, Al-Assaf *et al*. (2025**),** reported a significant upregulation of hepatic IGF-1 gene expression in broiler chickens supplemented with *Morus nigra* leaf powder. Likewise, Mohammed *et al*. (2025) reported increased hepatic IGF-1 expression in broiler chickens supplemented with *Tribulus terrestris* powder, further supporting the role of plant bioactives in regulating the growth hormone–IGF axis at the gene expression level. Further, Mhyson *et al.* (2022) reported a significant upregulation of IGF-1 gene expression in broiler fed spinach powder supplemented diets, with higher inclusion levels producing greater expression compared to the control group. Collectively, these studies reinforce the present observation that phytogenic leaf powders stimulate IGF-1 transcription, thereby contributing to enhanced muscle growth in poultry.

The study concluded that the phytogenic plants shisham (*Dalberiga sisso*) and Poplar (*Populus deltoides*) at 1% dietary supplementation individually or 2% dietary supplementation, supplemented together positively regulated the Insulin like growth factor-1 gene expression in the muscle of Japanese quail and can be used as a natural phytogenic supplement in quails up to this level.

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