Evaluation of Growth-Promoting Effects of Silver Nanoparticles and Magnetic Water in Broilers

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Abstract:
Previously, we evaluated the impact of silver nanoparticles (Nano-Ag) on myogenesis-related gene expression during chicken embryogenesis, where the injection of 20 ppm of Nano-Ag resulted in gene expression alterations accompanied by hyperplasia of skeletal muscle fibers. We conducted the current study to furtherly investigate the effect of Nano-Ag on the post-hatch expression of myogenesis-related genes and its reflection on muscle development and productive performance of birds. Nano-Ag was administered in normal or magnetic water for 35 days. Productive performance parameters such as body weight (BW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), and European production efficiency factor (EPEF) were investigated. The expression of selected myogenesis-related genes was assessed at the mRNA and protein levels, and breast muscle development was also investigated. The results indicated no effect of Nano-Ag and magnetic water on BWG and FI. However, the group that was in ovo injected with 20 ppm Nano-Ag and got 20 ppm Nano-Ag in magnetic water had the lowest FCR, EPEF, and BW. In ovo injection of 40 ppm Nano-Ag followed by post-hatch administration of 40 ppm Nano-Ag in normal water increased the expression of myogenic determination factor 1 (MYOD1) mRNA and protein in breast muscles by 4.0 and 1.8 folds, respectively, and resulted in 19.9% more muscle fibers than the control group. The gene expression was raised at the mRNA level, but not at the protein level, when magnetic water was used alone and decreased the number of muscle fibers by 26.47% than the control group. Nano-Ag in combination with magnetic water is a committed growth-promoting agent in broiler production at a low dose.

Keywords: Silver nanoparticles, Broiler production, Magnetic water, MRFs, Productive performance

Introduction:

Because of their chemical and physical characteristics, nanoparticles play a key role in cattle and poultry production. Nanoparticles may have a positive impact on cattle and poultry digestive efficiency, immunity, and performance, according to a number of studies [1]. Nano-Ag is tiny enough to enter the cell as well as the nucleus, where it may interact with DNA molecules or proteins associated with DNA, causing changes in gene expression patterns[2]. Furthermore, Fondevila[3] examined the antibacterial selective action, low toxicity, and low danger of environmental contamination of Nano-Ag when used as an animal feed additive. Nano-Ag was used as a disinfectant and reduce ammonia and nitrogen oxide emissions in animal production because of its antibacterial capabilities[4]. In addition, the use of Nano-Ag in chickens and rabbits resulted in an improvement in productivity and an increase in the immunological response[5]. In addition, owing to its anti-inflammatory, antibacterial, and immune-stimulating capabilities, Nano-Ag might be deemed a safe dietary supplement for broilers[6]. Moreover, Fouda et al.[7] reported that adding Nano-Ag to the drinking water of broilers accelerates muscle cell growth and maturation via enhancing the expression of myogenesis-related genes. These genes include fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and myogenic determination factor 1 (MYOD1)[8]. Satellite cells that express myogenic factor 5 (MYF5) and myogenic factor 1 (MYOD1) are primarily responsible for activating, proliferating and differentiating into new muscle fibers during the early days of post-hatch life[9]. Nano-Ag stimulated both muscle proliferation and differentiation by increasing FGF2 expression and MYOD1 expression, respectively[10].

On the other hand, Water is typically overlooked as a nutrient, although it is critical to the growth and productivity of broiler flocks. Increasing the fluidity and dissolving power of solutions by subjecting the water to a magnetic field enhances the biological activity of solutions and the performance of animals[11]. Based on the results of[12], The magnetic samples provide benefits such as a greater meat-to-fat ratio, increased livability and EPEF, and a reduction in mortality and illness incidence. Magnetic water might alter the oxidant-antioxidant equilibrium, hence reducing oxidative stress[13]. Also, it improves nutrient digestibility[14]. Chickens may benefit from the use of magnetic drinking water, which might improve their health and production. Some egg production traits may be improved by using magnetic water with a high degree of gauss (greater than 1000 gauss)[15]. For broilers, magnetic water improves growth, feed efficiency,

and water consumption[16]. Also, Hafizi et al[13] demonstrated that by lowering malondialdehyde, boosting the activity of superoxide dismutase in the heart and kidneys, and decreasing nitric oxide, magnetic water might change the oxidant-antioxidant balance and reduce oxidative stress. Another study showed that magnetic water had no influence on performance, carcass composition or immune system[17], the productive characteristics of broiler chickens[18], livability, feed conversion ratio (FCR), body weight gain (BWG), and feed intake (FI)[19].

In the current study, we looked at how Nano-Ag affected muscle development through post-hatch life by modifying the expression patterns of myogenesis-related genes. With and without the use of magnetic water, effects were studied at the transcriptional and translational levels. The impacts of Nano-Ag and magnetic water on productive performance parameters in broilers, such as body weight (BW), body weight gain (BWG), FI, FCR, and European production efficiency factor (EPEF), were also investigated.

Material and methods:

Following our pre-hatch experiment[20], we did this experiment at the Department of Animal Wealth Development, Faculty of Veterinary Medicine, Suez Canal University, Egypt. All procedures involving animals were conducted in accordance with guidelines from the scientific research ethics committee, Faculty of Veterinary Medicine, Suez Canal University and approved with number 2020047.

Nanoparticles

Nano-Ag hydrocolloid solution with 50 ppm of Nano-Ag was obtained from Nanoworld, Egypt. Diluting the original concentration (50 ppm) in distilled water yielded two distinct concentrations of Nano-Ag working solution (20 and 40 ppm). ($MV_{stock} = MV_{diluted}$).

Method of preparation:

The chemical reduction process [21] was used to make nano-Ag from silver nitrate salt (AgNO3) (99.9%), which was dissolved in clean water. The nanoparticles were formed by irradiating AgNO3 solution in ethanolic medium with PVP as a stabilizing agent in a microwave oven. In the presence of a microwave, ethanol functions as a reducing agent.

Characterizations:

1. Optical Properties: A Perkin Elmer Lambda 40 spectrophotometer was used to acquire the UV-Vis absorption spectra. Figure (1A) shows a strong surface plasmon resonance band for the synthesized Nano-Ag at 401 nm, demonstrating the creation of spherical silver with tiny size.

2. Morphology: Size & Shape: A 200 kV accelerating voltage was used for TEM using a JEOL JEM-2100 high resolution transmission electron microscope. An amorphous copper grid was covered with a drop

of extremely dilute sample solution and let to evaporate at room temperature. Nano-Ag was found in the colloidal at a concentration of 50 ppm, with a particle size of 9 nm by TEM., Figure 1B).

Experimental design

Ten groups of one-day-old chicks (Indian river broiler line) were used. Six groups of one-day-old chicks (Indian river broiler line) received in ovo injections with distilled water (placebo), whereas the remaining four groups got in ovo injections with 20 and 40 ppm of Nano-Ag. All injections were performed on day one of incubation (Figure 2). Pens with a lamp heater and deep litter were used to house the chicks. The temperature in the brooding pen ranged from 33 to 35 ˚C. It was 34-32 ˚C for the first week, then 32-28 ˚C for the second week, 28-26 ˚C for the third week, and finally 26-24 ˚C for the fourth week before dropping to 24-18 ˚C for the fifth week [22]. Lighting was set at a ratio of 23:1 (light to dark) during the first 6 days. The birds were given a commercial diet ad libitum for the study duration. The composition of the ration differed according to the developmental stage of birds. For the first 21 days after hatching, a commercial starter mixture with 23% crude protein (CP), 3.67% crude fat (CF), 2.47% crude fiber and 2980 kcal energy was used, while a grower mixture with 21% CP, 5.89% CF, 2.39% crude fiber and 3150 kcal energy was used from the 22nd day until the end of the experiment. The birds had free access to water either normal or magnetic with Nano-Ag (20 or 40 ppm concentration) or without it from day one to day 35. The data of BW and FI were recorded weekly for all birds from day one until day 35 of age.

Dissection procedure

At the end of the experiment after 5 weeks, the birds were weighed, slaughtered and the samples were taken. Samples from breast muscles (n=100, 10 from each group) were dissected and divided into three parts for gene expression analysis, histological examination, and western blot analysis. The samples were preserved as described by Husseiny et al.[20].

Productive performance parameters

BWG, FI, FCR, and EPFE were calculated according to the following formulas [23]:

BWG (g) = Final BW (g) at the end period – Initial BW (g) at start.

FI (g/bird) = (Feed offered – Feed residue)/No. of bird.

FCR (g feed/g gain) = Total feed consumed / Live body weight

$$EPEF = (\text{livability}\ \% \times BW\ (Kg))/(Age\ (d) \times FCR) *100$$

Real-time PCR technique for measuring gene expression.

According to Husseiny et al.[20], homogenization of breast muscle tissue (n=10 samples/group) was performed and total RNA was extracted, quantified, and reverse

transcribed. Next, real-time PCR was carried out using cDNA and gene specific primer pairs (BIONEER, USA; Table 1) mixed with Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific, Lithuania) in a Step One Plus real-time PCR system. A melting curve program (95 ˚C for 15 s, 60 ˚C for 1 min, and 95 ˚C for 15 s) was applied to verify the specificity of the product.

Western Blot Analysis

To evaluate whether Nano-Ag affects gene expression during post-hatch development (at the end of broiler production cycle), protein electrophoretic patterns were detected and monitoring via SDS-PAGE (15%) technique[26]. TriFast (Peqlab, VWR International, LLC, PA, USA) was used to extract soluble proteins from one broiler chicken in each group[20]. 50-100 mg of tissues were homogenized and precipitated. The protein pellet was washed three times, centrifuged, and dissolved in polyacrylamide gel containing 1 percent sodium dodecyl sulfate 30 µg of protein per lane were separated using 15% SDS–PAGE to separate the samples. The samples were then transferred to a HybondTM nylon membrane (GE Healthcare, VWR International, LLC, PA, USA) and incubated in blocking solution. The membrane was incubated in antibody solution containing anti-MYOD1 (abcam ab16148, Abcam plc, Cambridge, UK) and anti-MYF5 (abcam ab125301, Abcam plc, Cambridge, UK). Anti- beta actin primary antibody (abcam ab228001, Abcam plc, Cambridge, UK) was used for data normalization. After washing, the membrane was incubated in an antibody solution containing the appropriate dilution of HRP-conjugated secondary antibody, and then washed again. Totallab analysis software, www.totallab.com (Ver.1.0.1), was utilized for data analysis.

Histological examination of breast muscle tissues

The myofibers in the breast muscles of the 10 groups were studied histologically and statistically (three birds were sampled from each group, three sections from each bird sample, 9 total sections for each group). The breast muscles were taken and kept in 10% formalin at the end of the broiler production cycle, and serial histological sections of 6 µm thickness were produced. Myofibers were counted, and cell counts and average size were calculated [20]. The photographs were taken using a Leica ICC50 HD bright field microscope with built-in camera (Leica Microsystems, Wetzlar, Germany).

Statistical analysis

BW data were analyzed using a mixed design ANOVA repeated measurement method that took into account the effects of treatment, age (period), and treatment-age interactions. To identify the impact of treatment, data on growth

performance, gene expression, and histological inspection were examined using one-way analysis of variance (ANOVA) tests – generalized linear model approach in IMB SPSS statistics version 22.0. Duncan's multiple range test was used to detect the significant difference between groups at $p < 0.05$, and all data were presented as the mean ± standard error (SEM).

Table 1: *Primer sequences used in the current study.*

Gene *	Accession number	Primer sequence *	Amplicon size (bp)	Reference
MYOD1	NM_204214.2	FP: 5′ GAATCACCAAATGACCCAAAG 3′ RP: 5′ CTCCACTGTCACTCAGGTTTC 3′	185	This study
MYF5	NM_001030363	FP: 5′ AGGAGGCTGAAGAAAGTGAACC 3′ RP: 5′ TAGTTCTCCACCTGTTCCCTCA 3′	155	This study
MYF6	NM_001030746	FP: 5′ CCCCTTCAGCTTCAGCCC 3′ RP: 5′ CTCATTTCTCCACCGCCTCTTC 3′	242	This study
MYOG	N M_204184	FP: 5′ AATCCTTTCCCACTCCTCTCCA 3′ RP: 5′ TTGGTCGAAGAGCAACTTGG 3′	176	This study
MEF2A	NM_204864	FP: 5′ TCGGTGCGAAGTTTTCCTCT 3′ RP: 5′ CTGTTCCGTTCGTCCATTATTC 3′	250	This study
ACTB	"NM_205518.2"	FP: 5′ GTCCACCTTCCAGCAGATGT 3′ RP: 5′ ATAAAGCCATGCCAATCTCG 3′	169	[24]

*Abbreviations: myogenic determination factor 1, MYOD1; myogenic factor 5, MYF5; myogenic factor 6, MYF6; myogenin, MYOG; myocyte enhancer factor 2A, MEF2A; beta-actin; ACTB.

The target gene expression in the treated groups was normalized to the control group, and to the beta actin (the housekeeping) gene [25].

ΔCT (a target sample) = CT (target gene) − CT (reference gene)

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$\Delta\Delta CT = \Delta CT$ (a target sample) − ΔCT (a reference sample)

$2^{-\Delta\Delta CT}$ = normalized expression ratio

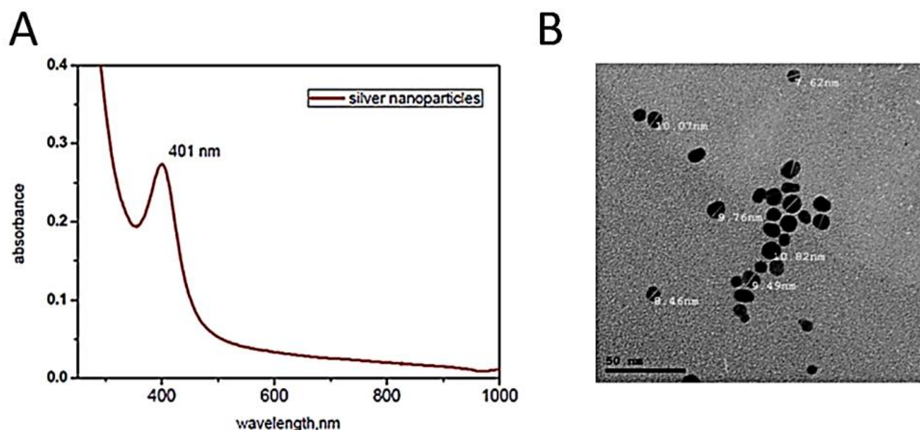

Figure 1 A & B Error! Reference source not found. UV-Vis absorption spectra of a sharp band for the prepared Nano-Ag at 401 nm (**A**). Image of TEM showing the size (9±0.5 nm), shape (spherical), and homogeneity of silver nanoparticles (**B**).

Magnetic water was prepared by using a liquid magnetic reactor from Nefertari Biomagnetic, 10th of Ramadan City, Sharkia, Egypt (www.magnetic.nefertari2.com).

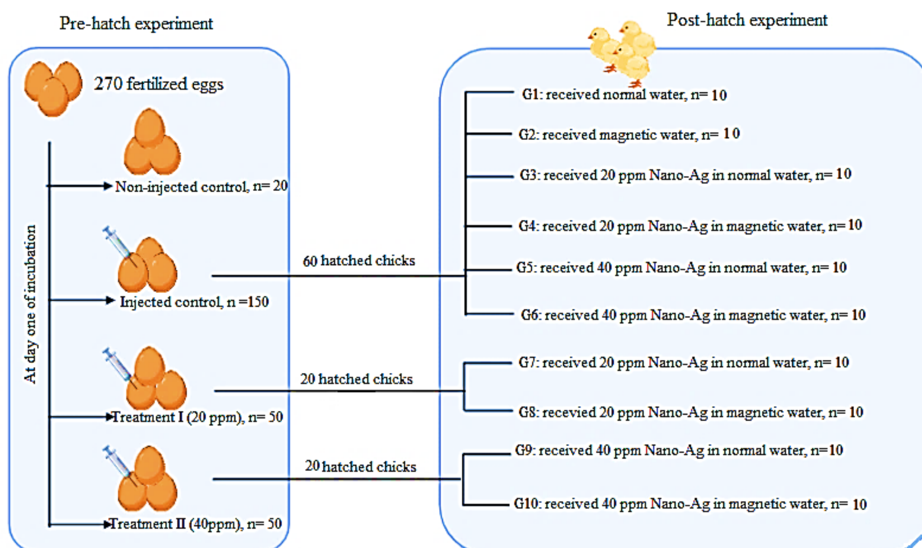

Figure 2: Experimental design for the post-hatch experiment. Ten groups were created from the total hatched chicks (n=100). The chicks hatched from in ovo injected eggs with placebo (n= 60) were divided into six groups(G1-G6). The chicks hatched from in ovo injected eggs with 20 ppm Nano-Ag were

divided into two groups (G7 and G8) while the chicks hatched from in ovo injected eggs with 40 ppm Nano-Ag were divided into two groups (G9 and G10).

Results:

Growth performance, body weight gain, feed intake, feed conversion ratio, and European production efficiency factor

The effect of treatments on body weight (BW) (kg) was not significant for all groups except G8 (2.18 ± 0.04) and G10 (1.95 ± 0.14) which was significant (P< 0.05). Also, there was no significant difference in BWG (kg) and FI (kg) between groups (P< 0.05). A significant difference was detected for FCR where G8 had the lowest value (1.460 ± 0.022) while G4 had the highest value (1.598 ± 0.032) (P<0.05). The highest significant EPEF value was reported in G8 (436.80 ± 7.50) while the lowest one (348.36 ± 12.82) was recorded in G4 (

Table ***2***).

Gene expression analysis

The results indicated that the MYOD1 mRNA fold expression was increased in 40 ppm/ 40 ppm/ normal water group (G9) by 4.00 ± 0.42, magnetic water group (G2) by 3.17 ± 0.60, and 20 ppm/ normal water group (G3) by 2.88 ± 0.73 without significant difference between the groups (*P*<0.05); while there was no significant difference between normal water group (G1) (1.00 ± .00), 20 ppm/ magnetic (G4) (2.05 ± 0.24), 40 ppm/ normal water group (G5) (0.56 ± 0.1), 40 ppm/ magnetic water group (G6) (1.22 ± 0.55), 20 ppm/ 20 ppm/ normal water group (G7) (1.97 ± 0.60), 20 ppm/ 20 ppm/ magnetic water group (G8) (1.56 ± 0.34), and 40 ppm/ 40 ppm/ magnetic water group (G10) (1.47 ± 0.03), *P*<0.05, Table *3*.

For MYF5 mRNA expression, there was great variation in the fold expression which was significantly higher in 20 ppm/ 20 ppm/ normal water group (G7) (20.68 ± 0.41) than in the normal water group (G1) (1.00 ± .00), followed by the magnetic water group (G2) (15.42 ± 6.44) then 40 ppm/ 40 ppm/ normal water group (G9) (13.25 ± 0.19), and 40 ppm/ magnetic water group (G6) (12.81 ± 0.89), *P*<0.05. While for the other groups, although the fold expression was increased, there was no significant difference, *P*<0.05 (Table 3). For MYF6, MYOG, and MEF2A mRNA expression, the fold expression increased but without significant difference between the different treated groups, *P*<0.05 (Table 3).

Western Blot Analysis

The MYOD1 and MYF5 protein expression levels were detected in the experimental groups. For MYOD1, the different treatments

reflected different MYOD1 activity levels. Control group (G1) showed the lowest activity. The highest MYOD1 content was remarked in 40 ppm/ 40 ppm/ magnetic water (G10) with 2.04 folds of the control group. The lowest content was showed by the magnetic water group (G2) which reflected 0.98 fold of MYOD1 content. The 40 ppm/ 40ppm/ normal water group (G9) contained superior MYOD1 content (1.8 folds) compared with other groups. MYOD1 content in 20 ppm/ 20 ppm/ magnetic water (G8) (1.6 folds) was more than the content in other groups (Figure 3, Table 4).

For MYF5 protein expression level, G10 group showed the highest MYF5 content with 2.27 folds compared with the control group (G1). On the contrary, G2 group reflected the lowest MYF5 content with 1.03 folds. G9 remarked with distinguishable increasing MYF5 level (2.0 folds) comparing with other groups. According to the MYF5 content, all the other samples were arranged descendingly with 1.5, 1.39, 1.13, 1.07 and 1.04 folds for G4, G5, G7, G8, and G6 group, respectively (Figure 3, Table 4).

Histological examination

According to statistical analysis of histological samples from day 35 of age shown in Table *5*, the breast muscle of G9 group (in ovo injected with 40 ppm and received 40 ppm Nano-Ag in normal water) exhibited 19.9 % more muscle fibers (222.89 ± 9.08) than G1 group (control group received normal water) (185.89 ± 24.71), followed by G7 group (20 ppm/ 20 ppm/ in normal water) (217.67 ± 11.37) with 17.09 %. While G2 group (magnetic water group) (136.67 ± 7.18) exhibited 26.47 % fewer muscle fibers than the control group (G1). However, the combination of magnetic water with Nano-Ag showed less muscle percentage than magnetic water alone and that difference between the combinates and control group is non-significant, $P<0.05$.

For cross-sectional area of muscle fibers, there was a significant difference between G3 group (20 ppm/ normal water group) (818905.33 ± 37375.37 µm²) and G1 control group (656539.33 ± 66859.20 µm²) and G2 magnetic water group (588905.56 ± 57767.36 µm²), $P<0.05$. Also, there was a significant difference between G2 (magnetic water group) and both G6 (40 ppm/ magnetic water) (801591.89 ± 49266.59 µm²), and G7 (20 ppm/ 20 ppm/ normal water) (763745.67 ± 37991.82 µm²), $P<0.05$ (Figure 4).

For the average size of muscle fiber, it was larger in G2 magnetic water group (5010.00 ± 554.54 µm²) followed by G10 (40 ppm/ 40 ppm/ magnetic water group) (4873.22 ± 622.79 µm²), followed by G4 (20 ppm/ magnetic water group) (4506.56 ± 82.45 µm²), then G3 (20 ppm/ normal water group) (4293.56 ± 566.10 µm²), then G6 (40 ppm/ magnetic water) (4243.67 ± 525.18 µm²), and G5 (40 ppm/ normal water) (4205.67 ± 431.41 µm²), than

that in the G1 control group (4092.56 ± 559.91 µm²), *P*<0.05.

The percentage of muscle fiber area was higher in G3 (20 ppm/ normal water group) (64.77 ± 3.27) and G6 (40 ppm/ magnetic water group) (64.64 ± 3.81), followed by G7 (20 ppm/ 20 ppm/ normal water group) (60.68 ± 2.98) and G10 (40 ppm/ 40 ppm/ magnetic water group) (60.56 ± 4.17) but it was non-significant, *P*<0.05 and lower in G1 (control group) (50.24 ± 4.62) and G2 (magnetic water group) (47.02 ± 2.64).

Table 2: *Least square means and their standard errors for the effect of treatments on body weight (BW) feed intake (FI), feed conversion ratio (FCR) and European production efficiency factor (EPEF) in different treated groups.*

Group	BW* (kg)	BWG* (kg)	FI* (kg)	FCR*	EPEF*
G1	$2.14^{ab} \pm 0.07$	$2.10^{a} \pm 71.97$	$3.09^{a} \pm 57.64$	$1.469^{cd} \pm 0.013$	$416.0^{ab} \pm 13.95$
G2	$2.13^{ab} \pm 0.07$	$2.09^{a} \pm 69.62$	$3.09^{a} \pm 57.53$	$1.486^{bcd} \pm 0.053$	$394.91^{abc} \pm 12.82$
G3	$2.12^{ab} \pm 0.04$	$2.07^{a} \pm 40.24$	$3.09^{a} \pm 57.31$	$1.489^{bcd} \pm 0.022$	$396.46^{abc} \pm 7.51$
G4	$2.02^{bc} \pm 0.07$	$1.98^{a} \pm 74.39$	$3.09^{a} \pm 57.54$	$1.598^{a} \pm 0.032$	$348.36^{d} \pm 12.82$
G5	$2.06^{b} \pm 0.06$	$2.01^{a} \pm 60.76$	$3.09^{a} \pm 57.65$	$1.543^{abcd} \pm 0.044$	$360.40^{cd} \pm 10.66$
G6	$2.09^{ab} \pm 0.05$	$2.04^{a} \pm 49.49$	$3.09^{a} \pm 57.49$	$1.563^{ab} \pm 0.033$	$387.20^{bcd} \pm 9.09$
G7	$2.08^{ab} \pm 0.05$	$2.03^{a} \pm 52.41$	$3.09^{a} \pm 57.50$	$1.569^{ab} \pm 0.030$	$366.0^{cd} \pm 9.27$
G8	$2.18^{a} \pm 0.04$	$2.14^{a} \pm 37.50$	$3.09^{a} \pm 57.49$	$1.460^{d} \pm 0.022$	$436.80^{a} \pm 7.50$
G9	$2.07^{ab} \pm 0.06$	$2.03^{a} \pm 57.91$	$3.09^{a} \pm 57.71$	$1.573^{ab} \pm 0.022$	$370.08^{cd} \pm 10.34$
G10	$1.95^{c} \pm 0.14$	$1.90^{a} \pm 143.66$	$2.92^{a} \pm 8.98$	$1.559^{abc} \pm 0.022$	$361.17^{cd} \pm 26.68$

* Means in the same column followed by the same superscript letters are not significant (One-way ANOVA, p < 0.05)

Table 3: *Least square means and their standard errors for the effect of treatments on myogenic regulatory factors (MRFs) and myocyte enhancing factor 2A (MEF2A) gene expression at the end of broilers' production cycle.*

Groups	MYOD1*	MYF5*	MYF6*	MYOG*	MEF2A*
G1	$1.00^{d} \pm .00$	$1.00^{d} \pm 0.00$	$1.00^{a} \pm 0.00$	$1.00^{a} \pm 0.00$	$1.00^{a} \pm 0.00$
G2	$3.11^{ab} \pm 0.60$	$15.42^{ab} \pm 6.44$	$9.91^{a} \pm 5.48$	$1.68^{a} \pm 0.37$	$57.33^{a} \pm 2.57$
G3	$2.88^{abc} \pm 0.73$	$1.01^{d} \pm 0.57$	$4.56^{a} \pm 3.34$	$1.43^{a} \pm 0.47$	$19.39^{a} \pm 11.83$
G4	$2.05^{bcd} \pm 0.24$	$1.35^{d} \pm 0.84$	$3.990^{a} \pm 3.65$	$2.61^{a} \pm 0.1$	$13.67^{a} \pm 7.52$
G5	$0.56^{d} \pm 0.1$	$10.81^{bcd} \pm 5.39$	$3.61^{a} \pm 0.18$	$2.39^{a} \pm 0.64$	$13.28^{a} \pm 3.43$
G6	$1.22^{d} \pm 0.55$	$12.81^{abc} \pm 0.89$	$5.79^{a} \pm 3.67$	$1.63^{a} \pm 0.25$	$50.73^{a} \pm 41.06$
G7	$1.97^{bcd} \pm 0.60$	$20.68^{a} \pm 0.41$	$10.18^{a} \pm 0.99$	$2.25^{a} \pm 0.08$	$25.62^{a} \pm 3.20$
G8	$1.56^{cd} \pm 0.34$	$3.068^{d} \pm 0.45$	$2.19^{a} \pm 0.42$	$1.80^{a} \pm 0.22$	$9.37^{a} \pm 3.54$

G9	4.00^{a} ± 0.42	13.25^{abc} ± 0.00	4.67^{a} ± 2.075	2.10^{a} ± 1.11	33.21^{a} ± 25.44
G10	1.47^{cd} ± 0.00	3.75^{cd} ± 2.81	2.27^{a} ± 0.58	1.58^{a} ± 0.32	6.73^{a} ± 3.80

* Means in the same column followed by the same superscript letters are not significant (One-way ANOVA, $p < 0.05$), myogenic determination factor (MYOD1), myogenic factor 5 (MYF5), myogenic factor 6 (MYF6), myogenin (MYOG).

Table 4: *(A) Data parameters of MYOD1 protein expression level for the control group and treated groups normalized to beta actin (reference protein) referred to lane %, and molecular weight (MW) of MYOD1. (B) Data parameters of MYF5 protein expression level for the control group and treated groups normalized to beta actin (reference protein) referred to lane %, and molecular weight (MW) of MYF5.*

		Marker	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
(A) MYOD1	Lane %	7.91	26.53	26.25	30.61	39.73	34.64	37.51	40.98	41.24	48.02	52.79
	MW (kDa)	200.00	36.30	35.97	36.30	35.97	35.31	34.98	34.98	34.65	34.33	34.00
(B) MYF5	Lane %	12.07	43.27	42.94	45.11	65.20	59.95	44.89	48.65	45.92	88.15	97.58
	MW (kDa)	200.00	27.41	28.10	26.70	28.10	26.70	27.41	26.70	26.70	25.97	24.47

Lane % is the amount of gene expression in the target samples divided by the amount of beta actin expression in the same sample.

Table 5: *Least square means and their standard errors for the effect of treatment (Nano-Ag with 20 ppm and 40 ppm) on the count, cross-sectional area (µm²), average size (µm²) and % area of muscle fibers from samples at the end of the broiler production cycle.*

Group	Count*	Cross-sectional area* (µm²)	Average size* (µm²)	% Area*
G1	185.89^{a} ± 24.71	656539.33^{bc} ± 66859.20	4092.56^{abc} ± 559.91	50.24^{bc} ± 4.62
G2	136.67^{b} ± 7.18	588905.56^{c} ± 57767.36	5010.00^{a} ± 554.54	47.02^{c} ± 2.64
G3	207.78^{a} ± 11.71	818905.33^{a} ± 37375.37	4293.56^{abc} ± 566.10	64.77^{a} ± 3.27
G4	176.44^{ab} ± 14.15	691646.77^{abc} ± 58053.18	4506.56^{ab} ± 82.45	54.20^{abc} ± 4.81
G5	201.33^{a} ± 23.60	694868.11^{abc} ± 17132.05	4205.67^{abc} ± 431.41	56.83^{abc} ± 1.32
G6	196.00^{a} ± 10.58	801591.89^{ab} ± 49266.59	4243.67^{abc} ± 525.18	64.64^{a} ± 3.81
G7	217.67^{a} ±11.37	763745.67^{ab} ± 37991.82	3308.33^{bc} ± 112.05	60.68^{ab} ± 2.98
G8	197.00^{a} ±8.26	667837.67^{abc} ± 28208.70	3784.78^{abc} ± 195.31	53.15^{bc} ± 1.64
G9	222.89^{a} ±9.08	683141.67^{abc} ± 20235.34	3019.56^{c} ± 39.67	53.57^{bc} ± 1.76
G10	180.33^{a} ±9.35	730314.11^{abc} ± 61363.73	4873.22^{a} ± 622.79	60.56^{ab} ± 4.17

* Means in the same column followed by the same superscript letters are not significant (One-way ANOVA, $p < 0.05$).

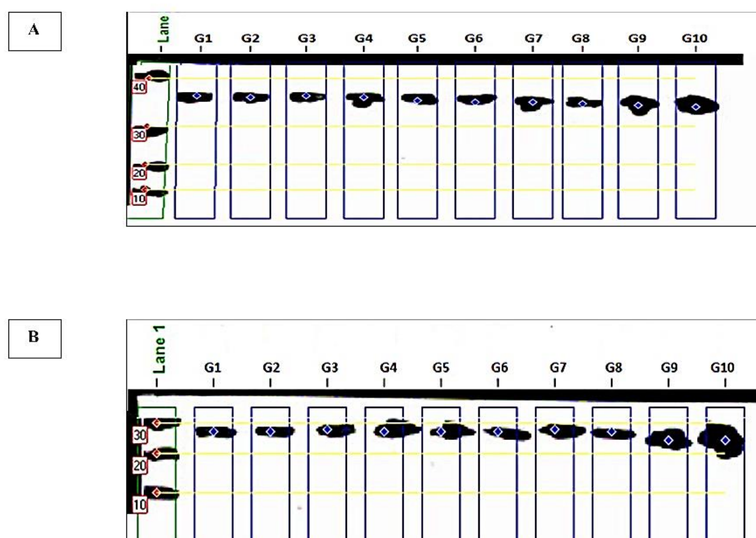

Figure 3: (A) Molecular weight (MW) calculation of MYOD1 protein expression level for the control group and treated groups after broiler breeding cycle, using protein ladder (200 kDa). (B) Molecular weight (MW) calculation of MYF5 protein expression level for the control group and treated groups after broiler breeding cycle, using protein ladder (200 kDa).

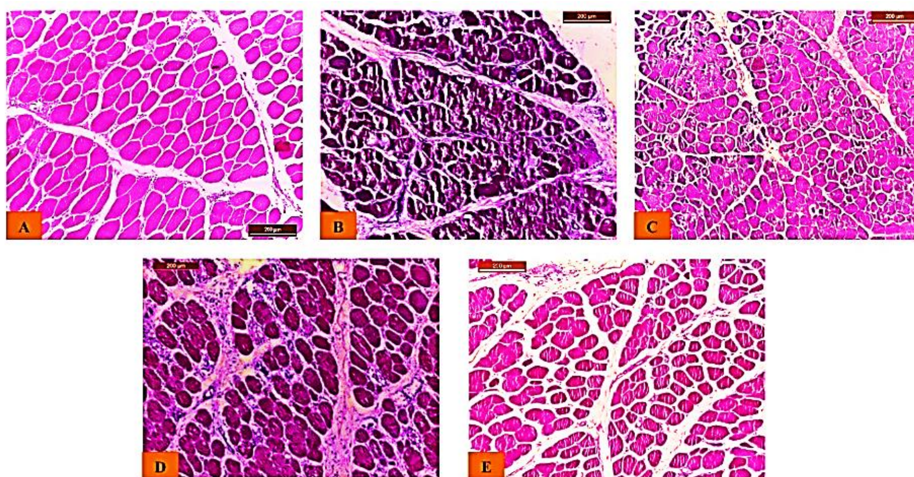

Figure 4: Histological sections from G1 (control group) (A), G2 (magnetic water group) (B), G3 (20 ppm/ normal water) (C), G7 (20 ppm/ 20 ppm/ normal water) (D), and G9 (40 ppm/ 40 ppm/ normal water) (E).

Discussion:

More research is required to follow up on the impact of Nano-Ag on gene expression and muscle growth after hatching[20]. Therefore, the present study was performed. Our findings revealed that Nano-Ag at a dosage of 20 ppm improves growth rate significantly than Nano-Ag at a dose of 40 ppm. As detected in the pre-hatch experimental results of chicken embryos[20], a low dose of 20 ppm Nano-Ag on the first day of incubation may alter the expression of genes associated with myogenesis, resulting in better muscle growth. Magnetic water had no significant negative effect on the growth of groups that received magnetic water alone or in combination with Nano-Ag from that in the control group.

Contrary to popular belief, recent research on broilers fed diets containing Nano-Ag found no improvement in growth performance[27-29]. Our results of BWG, and FI agree with Ahmadi[30] where there was no significant effect on such economic traits. Similar findings were reported before[5] where BWG, FI, and FCR in rabbits were unaffected by nano-Ag administration. Low-dose Nano-Ag treatment resulted in better FCR and EPEF in G8 than in other groups, which may be due to the higher final BW and lower FCR detected. This is in line with what we've seen before[5] which shows that administration of Nano-Ag had a significant impact on the final BW of rabbits exposed to high ambient temperatures.

Consisted with our findings, Andi et al.[31] found that broilers treated with Nano-Ag (20 ppm) had a higher BW and BWG than control group. The biological action of Nano-Ag on pathogenic bacteria and the stimulation of digestive enzymes in the intestines, which enhanced nutritional absorption, are responsible for this rise[32].

In this study, there was a negative impact on the growth performance at the higher dose of Nano-Ag (40 ppm in magnetic water). This is consistent with the results of[33] where the broilers received up to 12 ppm Nano-Ag in their drinking water, a deterioration in growth performance was observed. The growth performance was negatively affected, but the general health status of the birds in the treated groups was not affected which is a significant observation in our study. However, Sawosz et al.[34] and Vadalasetty et al.[33] showed that using Nano-Ag in drinking water at 50 ppm, reduced broiler development, impaired the immune system, and showed no antimicrobial effect. [1]suggested an explanation for this deterioration, stating that Nano-Ag treatment likely caused a disruption in protein catabolism, as evidenced by a reduction in the activity of the liver enzymes AST and ALT. Broiler chickens' postnatal growth performance and energy metabolism were unaffected by different concentrations of Nano-Ag (10 and 20 ppm) in their drinking water[35].

For histological examination, the

quantitative analysis of myofiber count, cross sectional area, average size and percentage area revealed that Nano-Ag with two different doses (20 ppm & 40 ppm) had no negative effect on breast muscle structure. This result is consistent with[7] where the treatment had no significant impact on the ultrastructural analysis of breast muscle. On the contrary, [36]revealed that Nano-Ag increased the MYOD1 expression, which controls embryonic myogenesis in adult tissue. In turn regulate muscle cell differentiation.

It is important to note that the treatment of Nano-Ag had no influence on broiler growth; however, there have been reports indicating that Nano-Ag may accelerate the growth and development. An explanation of the contradictory findings was provided by[5] who stated that this might be attributed to changes in Nano-Ag size, dosage, exposure period, and preparation process, as well as species differences (quail, chicken, and pig), form differences (colloidal vs. powder), and delivery mechanism (water vs. food)[35]. Nano-Ag in the colloidal form is less stable and loses its efficiency via the drinking water[37].

In addition to the impact of Nano-Ag on the gene expression and the growth performance in broilers, the effect of magnetic water either alone or in combination with Nano-Ag was studied. The current study revealed that the magnetic water had no effect on BW, FI, FCR, or EPEF. However, the magnetic water upregulated the expression of MYOD1 and MYF5 genes essential for post-hatch muscle development at the level of transcription but not translation, which in turn was reflected on body weight. This result agreed with[18] study which demonstrated that there were no significant differences between magnetic water treatments and tap water concerning studied traits (BW, BWG, FI, FCR, mortality, viability, and production index).

Although, magnetic water groups exhibited less muscle fibers than the control group, they had nearly the same BW. This can be illustrated as magnetic water follows another compensatory strategy through the induction of hypertrophy of muscle fibers (increase the diameter of muscle fiber). This contradicts[12] where the magnetic treatments exhibited more meat (200 g) compared to non-magnetic samples, increased EPEF, and reduced FI. There may be an explanation for our result which may be referred to the low magnetic capacity of the magnetic water reactor used in the experiment or as mentioned by Baker and Judd[38] that continuously recirculating systems, which allow the process water to be treated again, were more effective (especially in the industrial boilers).

To be more specific about the results in the current study, the broilers that were injected with 20 ppm Nano-Ag in ovo and received 20 ppm Nano-

Ag in magnetic water (G8) exhibited the highest body weight among all the experimental groups. This can be illustrated through the effect of Nano-Ag on the gene expression of the key factors (MYOD1 and MYF5) involved in muscle development at the mRNA level and at the protein level where their expression was upregulated. In addition, G8 generated from treatment I (in ovo injected with 20 ppm on day 1 of incubation); the group which revealed significant upregulation of MYOD1 on the mRNA and protein levels also 31.4% more muscle fibers (hyperplasia) [20]. Also, the magnetic water in turn adverse the negative effect of Nano-Ag (induce oxidative stress) to a certain limit. According to[39], the oxidant-antioxidant balance might be efficiently influenced by magnetic water. On the other hand, although G9 (40 ppm/ 40 ppm/ normal water) upregulated the expression of MYOD1 and MYF5 with higher fold expression at the mRNA and protein levels, the weight was nearly the same as G8 (20 ppm/ 20 ppm/ magnetic water). This can be attributed to the disturbances in protein catabolism as mentioned above. Also, G9 generated from treatment II (in ovo injected with 40 ppm at day 1 of incubation); the group that exhibited hypertrophy of muscle fibers due to increase the average size of myofibers during embryogenesis[20].

Away from the effect of treatment with Nano-Ag and magnetic water, the different groups had a very narrow range for body weights. This may be due to the immediate feeding of the birds upon arrival to the farmhouse without waiting 24h until complete absorption of the remnant of the yolk sac. Early post-hatch nutrition is so important for optimal satellite cells (SC) mitotic activity and muscle development in broiler chicks[40]. Restriction of feed during the initial post-hatch period, which is mostly mediated by the SC, may have an impact on post-hatch muscle development.[41]. The mild effects of Nano-Ag in this study could be partially attributed to the number of birds used since large number could reveal more variations in body weights and gene expression. In addition, the good health status due to the restricted management (good hygienic control) followed during the broiler breeding cycle which indicated by the lack of morbidity and mortality as in[35].

Conclusion:

In broiler production, the use of Nano-Ag at a low dosage (20 ppm) is more effective than at a high dose (40 ppm). Since it promotes the expression of important genes involved in post-hatch muscle growth without the side effects of a large dosage and at a cheap cost. Additionally, the use of magnetic water may enhance the birds' overall health. Based on the pre-hatch and post-hatch data, Nano-Ag might be

utilized commercially to increase chicken output.

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Ahmed Elaswad: contributed to the research design, analyzed the data, interpreted the results of the experiments, edited and revised manuscript.

Karim Khalil :performed the histological and quantitative analyses of the muscles.
Walaa A. Husseiny : performed the experiments, analyzed the data, prepared the figures and tables, drafted the manuscript.s
Adel A.S. El Nabtiti : contributed to the research design, analyzed the data, interpreted the results of the experiments, edited and revised manuscript.

Data Availability: The datasets generated during the current study are available from the corresponding author on reasonable request.

Ethics approval: This study was performed in line with the principles of the scientific research ethics committee, Faculty of Veterinary Medicine, Suez Canal University with number 2020047.

تقييم التأثيرات المعززة للنمو لجسيمات الفضة النانوية والمياه الممغنطة في دجاج التسمين

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الملخص

في السابق ، قمنا بتقييم تأثيرات جسيمات الفضة النانونية (Nano-Ag)على التعبير الجيني المرتبط بتكوين العضلات أثناء التطور الجنيني للدجاج ، حيث أدى حقن 20 جزء في المليون من جسيمات الفضة النانونية إلى تغييرات في التعبير الجيني مصحوبة بتضخم ألياف العضلات الهيكلية. لقد أجرينا الدراسة الحالية لمزيد من التحقق في تأثير جسيمات الفضة النانونية على تعبير ما بعد االفقس للجينات المرتبطة بتكوين العضلات وانعكاسه على نمو العضلات والأداء الإنتاجي للطيور. تم إعطاء Nano-Agفي ماء عادي و ماء ممغنط لمدة 35 يومًا. تم فحص معايير الأداء الإنتاجية مثل وزن الجسم (BW)، وزيادة وزن الجسم (BWG) ، وكمية العلف (FI) ، ومعدل تحويل العلف (FCR) ، ومعامل الكفاءة الإنتاجية الأوروبي (EPEF) .تم تقييم التعبير عن الجينات المختارة المرتبطة بتكوين العضلات على مستويات RNA (الحمض النووى الريبوزى) والبروتين ، كما تم فحص نمو عضلات الصدر. أشارت النتائج إلى عدم وجود تأثير لـ Nano-Ag والمياه الممغنطة على زيادة وزن الجسم (BWG) وكمية العلف المستهلكة ومع ذلك ، تم تسجيل أدنى معدل لمعامل التحويل الغذائى(FCR) وأعلى معدل لمعامل الكفاءة الإنتاجية الأوروبي (EPEF) و وزن الجسم(BG) في المجموعة التي تم حقنها اثناء تفريخ البيض بـ 20 جزء في المليون من Nano-Ag وحصلت على 20 جزء في المليون من Nano-Ag في الماء الممغنط اثناء التسمين. بينما في المجموعة التى تم حقنها اثناء تفريخ البيض بـ 40 جزء في المليون من Nano-Ag متبوعًا بإعطاء 40 جزء في المليون من Nano-Ag في الماء العادي ، زاد التعبير عن عامل التحديد العضلى رقم 1(MYOD1) فى mRNA والبروتين في عضلات الصدر بمقدار 4.0 و 1.8 مرة ، على التوالي ، و أدى إلى زيادة في الألياف العضلية بنسبة 19.9٪ مقارنة بالمجموعة الضابطة. زاد الماء الممغنط وحده من التعبير الجيني على مستوى mRNA ولكن ليس على مستوى البروتين وقلل عدد ألياف العضلات بنسبة 26.47٪ مقارنة بالمجموعة الضابطة.

جسيمات الفضة النانوية مع الماء الممغنط هو عامل مشارك لتعزيز النمو في إنتاج دجاج التسمين بجرعة منخفضة.

الكلمات الدالة

جسيمات الفضة النانوية، إنتاج دجاج التسمين؛ مياه ممغنطة ، الجينات أعضاء عائلة العوامل المنظمة للعضل(MRFs). الأداء الانتاجى