International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.9, No.08 pp 21-34, 2016

Effect of Age at mating and silver nanoparticles administration on progeny productive performance and some blood constituents in Japanese quail

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Abstract: A total of 64 males and 192 females from Japanese quail breeders flock (*Coturnix coturnix japonica*) were divided into two equal groups of different ages (old birds; 52 weeks of age (WOA) and young birds; (10 WOA). These subgroups were used in a series of four natural mating groups in a regular and reciprocal manner. In the 1st and 2nd mating groups; old males were paired with old and young females, respectively. While for the 3rd and the 4th groups; young males were paired with old and young females, respectively. The first and the second subgroups of both ages (old and young) were subjected to a treatment of 20 ppm silver nanoparticles (AgNps) in drinking water. The third and fourth ones were used as a control treatment given fresh water with no supplement. The hatchability percentage and some embryonic traits were determined. At the end of the experimental period, birds were weighed and slaughtered, autopsied and body internal organs were weighed. Some blood constituents were determined for quail progeny.

The results showed that Progeny of old females, whether mated with young or old males (M4 and M2) exhibited higher LBW, BWG and FC in comparison with the other mating systems (M1 and M3), and the effect was more pronounced with AgNps administration. It could be concluded from the present study that, it is possible to improve the productive and healthy status of the progeny if the old age breeder is mated with younger ones. The magnitude of this effect could be achieved through the administration of AgNps in the breeder's drinking water.

Key words: Age at mating, silver nanoparticles, productive performance, offspring ,blood constituents, Japanese Quail.

Introduction

The main purpose of poultry breeder farms is to produce an optimum number of fertile eggs. Both male and female are important for achieving reproductive success. Reproductive performance of breeder flocks is influenced by various physiological and environmental factors[1].

For improving and maximizing the potentiality of reproduction of breeder flocks, manipulating the physiological and environmental factors is necessary. Among the various physiological factors that necessary for production and reproduction are age, body weight, hormonal make up and sex ratio[2,3,4].

The quail breeder age and /or age at mating are important factors affecting fertility, hatching characteristics, chick weight, chick mortality at early ages and quail performance during growing period[5].

[6]Fertility is proved to be significantly higher ($P < 0.01$) with increasing quail breeder males and females ages up to the age of 18 WOA and declined thereafter**.**

[7]**W**ater supplemented with silver nanoparticles (AgNPs) improved the stomach internal environment for aged birds. Approximately 80% of the silver in AgNPs is in the metallic form while the remaining silver is in ionic form. Ionic silver become silver chloride in the stomach or blood stream. However, only metallic particles can resist high acidity stomach of birds and remain effective inside the blood stream. Silver has also been known for its medicinal properties, especially as an antimicrobial agent, but it may be toxic when it is in ionic state. However, the toxicity of Ag can be eliminated, when used in nanoparticle form.

Nanoparticles are structures ranged from 1 to 100 nm. Due to their small size, the total surface area of the particles exposed in solution is maximized, resulting in the highest activity per unit of weight.

Since AgNPs is composed of tiny particles of silver, (so tiny they are smaller than a virus, less than 2 nm in diameter) that can kill bacteria on contact 8. Several studies illusterated that AgNPs showed strong antimicrobial properties against *Salmonella Enteritidis* (SE) and *Escherichia coli (E.coli)*. It is hypothesized that Ag-NPs when used as an antimicrobial agent can stimulate development as well as immunity of chicken [9]**.**

In quail, AgNPs affects performance, intestinal microbial flora and morphology of enterocytes of duodenal villi[10,11,7]**.**

Studies have been done to evaluate the impact of AgNPs as an immunmodulators in birds. The effects exerted by AgNPs administration on the overall reproductive performances of poultry have not been fully investigated.

Apparently, studies concerning the interactions between age at mating and silver nanoparticles administration are scanty.

Therefore the purpose of the present study was to elucidate the interactions between AgNPs in drinking water and age of quail breeder males and females at mating on Fertility and Hatchability, progeny performance, Concentration of blood biochemical constituents of progeny.

Materials and methods

1.Experimental procedures and design:

A total of 64 males and 192 females from Japanese quail breeders flock (*Coturnix coturnix japonica*) represent two equal groups of different ages (old birds; 52 weeks of age (WOA) and young birds; 10 WOA) were used in this study. Breeders of each age were randomly subdivided into 4 subgroups resulting in 8 subgroups each of 8 males and 24 females. Birds were kept in an individual wire cage system with a sloping floor for egg collection. These 8 subgroups were used in a series of four natural mating groups, each of 2 replicates, in a regular and reciprocal manner. In the 1^{st} and 2^{nd} mating groups, old males were paired with young and old females, respectively. While for the 3^{rd} and the 4^{th} groups, young males were paired with young and old females, respectively. For all mating groups, the sex ratio was 1 ♂: 3 ♀, thus each male was caged with each female for a period of 24 h.

Birds of one replicate within each of the four mating groups were offered drinking water supplemented with 20 ppm silver nanoparticles (AgNPs). Silver nanoparticles were obtained from International Care American Company. While the other replicate within each mating was used as control treatments given fresh water with no AgNPs supplement.

All birds were kept in a windowed house, fed *ad libitum* on a diet which was formulated to meet the nutrient requirements of Japanese quail breeders according to [12]**.** Drinking water was available all the time. Birds were exposed to a light cycle of 16 h light and 8h dark throughout the experimental period.

2.Fertility, Hatchability and Embryonic mortality:

A total of 760 eggs were collected from breeder hens of all treatments. Eggs from each treatment group were taken and incubated in a forced draft laboratory incubator at 37.5°C and relative humidity (RH) between 55 to 66% [13]. Turning of eggs was automatically every four hours until the 14th day of incubation.

At 14 d of incubation, eggs were transferred to the hatcher. At hatching, eggs that failed to hatch were broken to determine the percentages of infertility and early and late embryonic mortality. After hatching; percentages of fertility and hatchability (as a percent of fertile eggs) were recorded. Early and late embryonic mortality percentages were calculated based on the number of fertile eggs.

3. Embryological traits:

3.1. Embryos and yolk sac weight:

At the end of the 14th day of incubation, 15 eggs from each treatment group were weighed, immediately broken and the residual yolk sac wet weights were recorded. Embryos were excised from the extra embryonic membranes and weighed to the nearest two decimal points. The weight of residual yolk sac and embryos were proportionated to both the initial egg weight and to the egg weight at 14 days of incubation.

3.2. Egg weight loss:

The egg weight loss was calculated by subtracting the weight of an egg at 14 days of incubation from its initial weight before incubation.

4. Productive performance parameters:

Chicks were individually weighed at hatch and then biweekly till six wk. Their live body weights (LBW) were recorded to the nearest 0.1 g. The average body weight gain (BWG) was calculated by subtracting the average of initial body weight of the birds from the final one. Feed consumption (g/bird/week) was calculated by subtracting residual feed from the offered feed. Feed conversion ratio (FCR) was calculated as feed consumption (g) to body weight gain (g).

5. Carcass characteristics:

A total of 40 unsexed quail birds at 6 WOA were randomly taken, weighed and slaughtered by slitting the jugular vein, then scalded and defeathered. Carcass weight and weights of liver, gizzard, heart, spleen and bursa.

6. Physiological and biochemical parameters:

A total of 40 blood samples were collected from the birds at the end of the experimental period, during their exanguination into heparinized tubes. The tubes were centrifuged at the speed of 4000 r.p.m. for 15 min. Plasma samples were decanted into Ependorf tubes and stored at -20ºC until analysis.

Plasma total proteins (g/dl) were determined according to the method described by [14]**.** the determination of plasma albumin (g/dl) based on a colorimetric method described by [15]**.** Globulin was calculated by subtraction of plasma albumin from plasma total protein, and then A/G ratio was calculated.

Total Plasma lipids (mg/dl) were determined according to the method of [16]**.** Triglycerides (mg/dl) were determined by the method of [17]**.** Cholesterol (mg/dl) and high density lipoprotein (mg/dl) were determined according to the method of [18]**.** Low density lipoprotein was calculated by the following equation:

LDL (mg/dl) = Total Cholesterol – Cholesterol in the supernatant

The determination of plasma calcium (mg/dL) was measured, based on a colorimetric method as described by method[19]. Phosphorus (mg/dl) was determined according to the method[14].

The activities of AST and ALT enzymes (U/L) were calorimetrically measured using commercial kits purchased from Spectrum Diagnostics, and determined according to [20]**.**

7. Statistical analysis:

Data were subjected to a two-way analysis of variance with age at mating and drinking water sliver nanoparticles treatment (N) as the main effects using the General Linear Models (GLM) procedure of **SAS User's guide (1998)**. Duncan's multiple range test[21] was used to separate difference among treatment means when separation was relevant.

Results and discussion

1. Fertility and hatchability %:

Fertility and hatchability percentages (%)as influenced by age at mating and silver nanoparticles treatments are illustrated in Table 1 . It is clear from results that fertility % was significantly higher for the mating groups with young males (M3 and M4) regardless of female's age. The effect of AgNps treatments was not significant.

Statistical analysis of data revealed significant effects of the interaction between AgNps and age at mating on the fertility %.

Table 1 . Effect of age at mating and administration of silver nanoparticles of Japanese quail breeder hens on fertility, hatchability and chicks weight at hatch.

Nanosilver (N)	*Mating system (M)*				*Overall mean*	Significance		
	1	2	3	4		M	N	M*N
Fertility%								
N_0	85.97±1.53	84.86±2.64	87.22±2.78	81.25±1.25	**84.82**			
N_1	83.47±0.97	81.11±1.11	90.69±1.80	90.45±0.45	**86.43**			
Overall mean	**84.72^b**	**82.98^b**	**88.95^a**	**85.85^{ab}**		*	NS	*
Hatchability (% of fertile eggs)								
N_0	91.88±2.41	88.80±3.09	91.89±0.22	79.67±0.88	**88.06^B**			
N_1	89.99±2.11	91.26±0.63	98.65±1.35	88.19±0.69	**92.02^A**			
Overall mean	**90.93^b**	**90.03^b**	**95.27^a**	**83.93^c**		**	*	NS

1, (old ♂x young♀); 2, (old ♂x old ♀); 3, (young ♂ x young ♀); 4, (young ♂x old ♀).N0, (Zero nanosilver); N1, (20ppm nanosliver).
* P≤ 0.05, ** P≤ 0.01, NS= non-significant**.**
Mean within a column (having capital letters) or row (having small letters) are significantly different.

Hatchability as % of fertile eggs was significantly (P≤0.01) changed with age at mating. Where the highest hatchability % values were recorded for eggs of M3 (95.27%) followed by M1and M2. While the lowest value was recorded for eggs from M4 . The lower hatchability (%) of the M4 in spite of the because of the high fertility (%) recorded for this group. This may be because this group produced heavier eggs than the other groups which may affect their hatchability.

In this respect, [22] reported high hatchability of Japanese quail on basis of fertile eggs (71.2%). They postulated that the lower hatchability occurred in a flock, if embryonic mortality is higher and fertility is lower.

Results show also that AgNps administration significantly increased hatchability. However, the interaction between age at mating and AgNps treatments on hatchability was not significant. This results are in close agreement with those reported by[23,22,24,25,26,27] concluded that age does not adversely affect reproduction and fertility in broiler breeders but hens undergo some physiological changes as they aged, and this affect their ability to be fertilized.

As reported in previous studies on broiler breeders[28] declared that the age of the quail breeder significantly reduced the overall hatchability of the total eggs. The eggs obtained from young breeders produced more chicks.

2. Embryological traits:

Table 2, illustrates the effect of age at mating and silver nanoparticles on egg weight loss, yolk sac and embryo weight. There was insignificant effect of AgNps on egg weight loss, yolk sac and embryo weight.

The current data showed that the mating regimen M4 recorded significantly the highest egg weight loss (7.97) followed by the M2 regimen, however the later mating did not significantly differ from M1 and M3 regimens. This result may interpret the achieved reduction in the hatchability percentages for M2 and M4 regimens (Table 1).

These results might be ascribed to higher egg weight that could be parallel with lower egg shell strength and thickness, consequently higher egg shell conductance which may reveal the presence of imbalance in gas exchange and egg weight loss. It is known that as hens aged, egg weight increases [29] shell thickness decreases [30] and the proportion of yolk increases at the expense of albumen and eggshell[31]. The proportions of components of the hatching egg are also affected by egg size.

Table 2 .Effect of age at mating and silver nanoparticles administration of Japanese quail breeder hens on some embryonic traits.

Nanosilver (N)	*Mating system (M)*				*Overall mean*	Significance		
	1	2	3	4		M	N	M*N
			Egg weight loss (%)					
N_0	6.74±0.09	7.65±0.34	7.15±0.22	8.28±0.39	**7.39**			
N_1	7.46±0.18	6.84±0.35	7.10±0.39	7.70±0.31	**7.33**			
Overall mean	**7.05^b**	**7.35^b**	**7.13^b**	**7.97^a**	-	*	NS	NS
			yolk sac (% of embryo weight)					
N_0	34.40±0.84	30.36±1.85	33.13±0.82	34.52±1.48	**32.96**			
N_1	33.96±1.85	32.31±2.43	33.73±0.91	35.99±1.30	**34.08**			
Overall mean	**34.16^{ab}**	**30.62^b**	**33.43^{ab}**	**35.17^a**	-	*	NS	NS
			Embryo weight (% of egg weight)					
N_0	70.61±2.16	73.16±1.86	73.84±0.75	74.52 ±2.19	**72.95**			
N_1	70.68±1.40	74.05±1.37	73.59±0.81	72.63±1.51	**72.67**			
Overall mean	**70.64**	**73.55**	**73.7**	**73.47**	-	NS	NS	NS

1, (old ♂x young♀); 2, (old ♂x old ♀); 3, (young ♂ x young ♀); 4, (young ♂x old ♀).N0, (Zero nanosilver); N1, (20ppm nanosliver).
* P≤ 0.05, ** P≤ 0.01, NS= non-significant.
Mean within a column (having capital letters) or row (having small letters) are significantly different.

Small eggs have a greater proportion of yolk than large eggs from the same flock age [32]. The weights of embryonic yolk sac as a percentage of the initial egg weight were significantly differed between treatments, where M4 recorded the highest relative residual yolk sac weight followed M1.

For instance, fertile eggs from 25 and 41-week-old broiler-breeder stock, yolk lipid absorption by embryos from young hens is much lower than that of embryos from older breeders [33].

It clearly noted from the present results that neither age at mating nor AgNps had and their interaction exerted insignificant effects on embryo weight.

3. Productive Performance:

Results presented in Table (3) showed highly significant effect of different the ages at mating and the administration of AgNps, on productive performance of growing quails.

It is clear from the results that the highest live body weight (LBW) values were recorded for quails that hatched from old females, regardless males age (i.e. M4 and M2 groups). This may be related to egg weight of parental breeders. It is worth noting that egg weight of breeder hens in the present data was significantly heavier for old females. In this respect [34] stated that eggs heavier than 11.50 g were most suitable for successful hatching and subsequent growth performance. However, some contradictory results were reported by[25], they found that parental age was not important for chick weight at hatch. Quails received 20 ppm AgNps in drinking water had significantly higher live body weight (LBW) at hatch than the control (received zero AgNps).

Statistical analysis of data reflect highly significant (P< 0.01) effects of the interaction between age at mating and the administration of AgNps on LBW of quails at growing period. These results may be due to the antibacterial properties of nanosilver affecting microbial populations without inducing resistance and increasing anabolicactivity that may lead to the stimulation of development and growth of animals and increase the rate of metabolism, so, this may lead to the improvement of the growth of broiler [35,36].

This is in close agreement with the findings of [31] who found that chick weight at hatch was significantly affected by parent age.

In domestic poultry species, including Japanese quail as the bird grows older during production can have significant positive effects on hatchling body weights [37,38].

Likewise [32] reported smaller eggs produced by young (25 wk of age) broiler breeder hens have been found to yield smaller chicks with larger residual yolk sacs than breeders at 41 wk.

It is clearly noted from the present results that body weight gain of quails, followed similar aforementioned trend for LBW through the growing period.

Table 3 . Effect of age at mating and silver nanoparticles administration of quail breeder on productive performance of their progeny.

Age (days)	Nanosilver (N)	Mating system (M)				*Overall mean*	Significance		
		1	2	3	4		M	N	M*N
Live body weight (g)									
Hatch	N_0	8.94± 0.06	9.55±0.10	9.26± 0.09	8.33± 0.06	**9.055^B**			
	N_1	9.10± 0.12	9.64± 0.14	9.08± 0.09	9.45±0.08	**9.305^A**			
	Overall mean	**9.024^{bc}**	**9.597^a**	**9.168^b**	**8.945^c**	-	**	**	**
42	N_0	201.27±2.26	197.28± 2.06	185.98±3.23	204.24±2.01	**197.029**			
	N_1	190.44±3.01	200.61±3.20	188.25±3.21	215.41±5.61	**198.530**			
	Overall mean	**196.352^b**	**199.187^b**	**187.072^c**	**209.561^a**	-	**	NS	**
Body weight gain(g)									
0-42	N_0	192.16±2.29	187.69± 1.96	176.87±3.23	194.74±1.97	**187.727^B**			
	N_1	181.50±3.04	191.05±3.23	179.00±3.22	207.08±4.59	**189.478^A**			
	Overall mean	**187.318^b**	**189.614^b**	**177.894^c**	**200.622^a**	-	**	*	**
Feed intake (g)									
0-42	N_0	930.48±9.52	878.28±9.57	900.00±10.00	1087.97±12.92	**949.18^A**			
	N_1	871.28±9.34	953.66±3.46	855.29±6.90	888.74±8.52	**892.24^B**			
	Overall mean	**900.88^b**	**915.97^b**	**877.65^b**	**988.35^a**	-	*	*	**
Feed conversion ratio									
0-42	N_0	5.13± 0.24	4.60±0.30	5.05±0.15	5.21±0.23	**5.00**			
	N_1	4.59±0.20	4.96±0.07	4.73±0.04	4.55±0.30	**4.71**			
	Overall mean	**4.86**	**4.78**	**4.89**	4.88	-	NS	NS	NS

1, (old ♂x young♀); 2, (old ♂x old ♀); 3, (young ♂ x young ♀); 4, (young ♂x old ♀).N0, (Zero nanosilver); N1, (20ppm nanosliver).

* P≤ 0.05, ** P≤ 0.01, NS= non-significant.

Mean within a column (having capital letters) or row (having small letters) are significantly different.

Silver nanoparticles treatment had a highly significant positive effect on weight gain at growing periods from 0-42 DOA than the control group. The cumulative weight gain of quails showed significant increase in both M4 followed by M2 groups.

This finding could be related to the preferable healthy status of the AgNps parent stocks, which might be reflected on to productive performance of the progenies. The interaction between age at mating and AgNps administration was significant during the growing period.

Our results are in close agreement with those reported by [39] who reported that egg weight was increased significantly with age between 35, 50 and 62 wk. Despite these differences in egg weight, there were only numerical increases in hatching chick weight with increased breeder age. Small eggs from commercial meat parent genotypes at 26 and 28 WOA produced smaller offspring at 28 and 48 d of growing period compared to those from larger eggs [40]. Also [41] found that embryos from young breeder hens had slower growth rate than those from older breeders. As shown in Table 3, feed consumption of quails hatched from mating system (M4) had significantly consumed more feed than the other treatments. The cumulative feed consumption from hatch to 42 DOA exhibited differences among treatments where AgNps chicks consumed significantly less feed than the control group.

On the other hand, the interaction between both ages at mating and AgNps was significant for feed consumption which may reflect the beneficial effects of both treatments. It appears that feed consumption is related to the body weight of growing quails which were heavier for M4 and M1 groups, respectively.

This is in close agreement with the findings of [42] who reported an increase in feed consumption of broilers hatched from eggs that had the cuticle removed and that were laid by hens at old age 52 WOA.

They added that the performance of chicks from hens at 52 wk was evidently influenced by the increase in water loss and gaseous exchange in eggs that had the cuticle removed.

Similarly, [43] studied the effect of the age of the parental broiler breeders on feed intake of their progeny up to 21 DOA. They observed that feed intake of progeny from young breeders was reduced significantly compared with old breeder.

The feed conversion ratio was insignificantly improved during the whole experimental period compared to control group. It is appears that the combined effect of both the age at mating and AgNps administration that the later had no impact on feed conversion ratio of quails during the whole period (0-42 DOA). [44] found that 25 and 50ppm of nanosilver used in broiler starter diet had no significant effect on feed intake and FCR compared with control. A contradictory results were reported by [45] who found that feed conversion ratio (FCR) was improved for the progeny of young breeders and not for the progeny of old breeders.

4. Slaughter Traits:

4.1. Carcass and Giblets:

Data in Table 4 , illustrated that carcass and the relative weight of liver and gizzared of the quails of M2 significantly increased than the progeny of the other groups. On the other hand, the heart relative weight was not significantly increased differs between progeny group.

Similarly, there was no significant effect of AgNps on relative weights of carcass, liver and heart. But it was significant for only gizzard relative weight. Moreover, there were significant effects of the interaction between age at mating and nanoparticles silver on all traits.

These results are in disagreement with [46] who reported that there were no significant effects of maternal body weight on carcass composition of their offspring.

4.2. Lymphoid organs relative weights:

Relative weights of lymphoid organs (spleen and bursa of Fabricius) in growing quails as influenced by their parental quail breeder age and AgNps treatment are presented in Table 5.

Statistical analysis of the data revealed highly significant effects of the mating system and AgNps administration on the relative weights of the lymphoid organs. Thus, a significant increase was noticed in the relative weight of bursa in quails that were hatched from the mating system M4 comparable to the chicks of the other mating systems.

Table 4.Effect of age at mating and silver nanoparticles administration of quail breeder on dressing percentage and giblets relative weights of their progeny.

Nanosilver (N)	*Mating system (M)*				*Overall mean*	Significance		
	1	2	3	4		M	N	M*N
			Carcass (%)					
N_0	67.04±0.766	72.39±0.959	67.17± 1.238	61.42± 2.519	**67.00**			
N_1	68.03± 1.900	70.35± 0.528	67.40±1.070	67.62 ± 1.242	**68.35**			
Overall mean	**67.53^b**	**71.37^a**	**67.28^b**	**64.52^b**	-	**	NS	*
			Liver (%)					
N_0	2.02± 0.102	2.76±0.145	2.21±0.130	2.27± 0.064	**2.33**			
N_1	2.64± 0.270	2.63±0.082	2.47±0.104	2.22±0.096	**2.50**			
Overall mean	**2.33^b**	**2.70^a**	**2.34^b**	**2.25^b**	-	**	NS	*
			Gizzard (%)					
N_0	1.59±0.038	1.85±0.108	1.69±0.093	1.55± 0.038	**1.68^B**			
N_1	2.02±0.034	1.91±0.055	1.51± 0.144	1.78±0.065	**1.83^A**			
Overall mean	**1.83^{ab}**	**1.87^a**	**1.60^c**	**1.68^{bc}**	-	**	*	**
			Heart (%)					
N_0	0.81± 0.048	0.83±0.037	0.81± 0.058	0.97±0.025	**0.85**			
N_1	0.78±0.052	0.78±0.040	0.88±0.030	0.78± 0.037	**0.80**			
Overall mean	**0.80**	**0.81**	**0.85**	**0.86**	-	NS	NS	NS

1, (old ♂x young ♀); 2, (old ♂x old ♀); 3, (young ♂ x young ♀); 4, (young ♂x old ♀).N0, (Zero nanosilver); N1, (20ppm nanosliver).
* P≤ 0.05, ** P≤ 0.01, NS= non-significant.
Mean within a column (having capital letters) or row (having small letters) are significantly different.

This trend was not observed for spleen (%) where the chicks produced from the M3 mating group showed the highest value. Moreover, AgNps administration increased both spleen and bursa relative weights.

Table 5. Effect of age at mating and silver nanoparticles administration of quail breeder on some immune measurements of their progeny.

Nanosilver (N)	*Mating system (M)*				*Overall mean*	Significance		
	1	2	3	4		M	N	M*N
			Bursa of Fabricius (%)					
N_0	0.055±0.002	0.076±0.003	0.040±0.004	0.065±0.002	**0.058^B**			
N1	0.074±0.005	0.058±0.003	0.082± 0.006	0.095±0.005	**0.076^A**			
Overall mean	**0.065^b**	**0.065^b**	**0.061^b**	**0.080^a**	-	**	**	**
			Spleen (%)					
N_0	0.042±0.004	0.047±0.002	0.046± 0.003	0.055±0.002	**0.048^B**			
N_1	0.050±0.004	0.066±0.004	0.073±0.006	0.053±0.003	**0.064^A**			
Overall mean	**0.046^c**	**0.057^b**	**0.070^a**	**0.054^{bc}**	-	**	**	**

1, (old ♂x young ♀); 2, (old ♂x old ♀); 3, (young ♂ x young ♀); 4, (young ♂x old ♀).N0, (Zero nanosilver); N1, (20ppm nanosliver
* P≤ 0.05, ** P≤ 0.01, NS= non-significant.
Mean within a column (having capital letters) or row (having small letters) are significantly different.

5. Blood Constituents:

5.1. Plasma Total Proteins, Albumin, Globulin and A/ G ratio:

Results in Table 6 showed the plasma protein level and its fractions of hatched quail chicks as influenced by the mating system between their parents and AgNps administration in drinking water.

The presented values of total plasma proteins showed insignificant differences among chicks of all mating groups. However the lowest values were recorded for chicks derived from the old ages either males or females.

In accordance with these results [47] observed a negative correlation between total proteins (TP) values and age of Japanese quail.

A similar trend was observed for the effect of AgNps administration. However, plasma albumin was significantly increased in quails hatched from the older females regardless of male ages compared with chicks hatched from the other groups.

Table 6 .Effect of age at mating and silver nanoparticles administration of quail breeder on plasma total protein, albumin, globulin and A/ G ratio of their progeny.

Nanosilver (N)	*Mating system (M)*				*Overall mean*	Significance		
	1	2	3	4		M	N	M*N
Total proteins (g/dl)								
N_0	3.45±0.185	4.41±0.326	4.47±0.142	3.60±0.393	**4.02**			
N_1	4.92±0.315	3.86±0.282	4.31±0.446	4.38±0.347	**4.31**			
Overall mean	**4.19**	**4.13**	**4.40**	**3.99**	-	NS	NS	NS
Albumin (g/dl)								
N_0	2.41±0.148	3.03±0.211	2.78±0.106	2.78±0.137	**2.75^A**			
N_1	1.79±0.112	2.14±0.097	2.24±0.193	2.51±0.135	**2.14^B**			
Overall mean	**2.10^b**	**2.58^a**	**2.51^a**	**2.65^a**	-	**	**	NS
Globulin (g/dl)								
N_0	1.38±0.194	1.49±0.203	1.63±0.205	0.837± 0.021	**1.33^B**			
N_1	3.21±0.077	1.74±0.176	2.08± 0.223	1.84±0.097	**2.13^A**			
Overall mean	**2.30^a**	**1.61^b**	**1.86^{ab}**	**1.33^b**	-	**	**	*
A/G ratio								
N_0	1.55±0.214	2.01±0.218	1.90±0.119	3.72±0.268	**2.37^A**			
N_1	0.56±0.061	1.23± 0.090	1.06±0.114	1.51± 0.121	**1.13^B**			
Overall mean	**0.91^b**	**1.62^b**	**1.48^b**	**2.62^a**	-	**	**	NS

1, (old ♂x young ♀); 2, (old ♂x old ♀); 3, (young ♂ x young ♀); 4, (young ♂x old ♀).N0, (Zero nanosilver); N1, (20ppm nanosliver).
* P≤ 0.05, ** P≤ 0.01, NS= non-significant.
Mean within a column (having capital letters) or row (having small letters) are significantly different.

Plasma globulin level was higher in chicks hatched from M1 which may indicate better immune response of quails. The effect of age at mating on decreasing A/ G ratio was significant (P≤0.01) indicating low levels of plasma albumin and higher globulin concentrations.

Moreover, it is clear from the present results that A/ G ratio was lower in birds hatched from breeders receiving AgNps in drinking water which indicate possible role of disease resistance and viability of quails.

It is postulated that plasma protein profile of a given bird is a reflection of the metabolic activities related to protein synthesis and (or) degradation.

A contradictory results was reported by [48] who reported a negative stimulating effect on total proteins (TP), albumin and gamma-globulin in response to the addition of different AgNps levels; 20, 40, and 60 ppm AgNps /kg diet on broiler chicks.

5.2. Plasma Total Lipids, Triglycerides, Plasma Total Cholesterols, High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL):

Concerning plasma concentration of total lipids and triglycerides, the current findings revealed significant increase in total lipids and its fraction in birds hatched from M4 group as compared to the other mating groups as presented in Table 7.

plasma LDL concentration was significantly lower in the chicks group which hatched from M3 followed by M4.

These results may be attributed to the significant increase in feed intake and consequently increase energy, since the excess of energy is diverted to fat, this confirmed the rises in total lipids.

Data indicated that plasma concentration of total lipids was lower in birds hatched from breeders receiving AgNps in drinking water. While, the plasma triglycerides concentration was significantly higher in chicks hatched from breeders receiving AgNps. The interaction between age at mating and AgNps was highly significant ($P \leq 0.01$).

Results clearly show that quails hatched from the breeder females that received AgNps in drinking water had significantly decreased chicks' plasma cholesterol concentration at marketing age (6wks).

However HDL concentration was insignificantly elevated in chicks hatched from M2. Silver nanoparticles administration in drinking water of breeder birds did not affect HDL concentration of their progeny.

Table 7 .Effect of age at mating and silver nanoparticles administration of quail breeder on plasma total lipids and its fraction of their progeny.

Nanosilver (N)	*Mating system (M)*				*Overall mean*	Significance		
	1	2	3	4		M	N	M*N
Total lipids (mg/dl)								
N_0	687.5±52.45	823.1±70.0	841.0± 62.07	1368.9±108.60	**914.86^A**			
N_1	653.7±48.27	773.9± 88.28	687.5±51.08	747.4± 69.77	**722.63^B**			
Overall mean	**668.25^b**	**798.56^b**	**764.27^b**	**980.48^a**	-	**	**	**
Triglycerides (mg/dl)								
N_0	64.88±2.14	85.24±3.87	72.14± 3.31	107.50±3.11	**82.44^B**			
N_1	95.47± 1.37	100.59±2.85	69.52±0.52	87.73± 2.81	**88.33^A**			
Overall mean	**80.17^b**	**92.91^a**	**70.83^c**	**97.61^a**	-	**	**	**
Cholesterols (mg/dl)								
N_0	170.11±38.26	160.00±10.54	201.14±33.07	149.42±16.57	**190.80^A**			
N_1	157.93±11.50	151.72±11.60	156.32±11.49	168.10±20.07	**158.62^B**			
Overall mean	**162.50^b**	**156.32^b**	**228.74^a**	**160.10^b**	-	**	**	**
HDL (mg/dl)								
N_0	57.06±6.875	81.58±5.891	59.81±2.062	70.81±8.288	**69.09**			
N_1	78.83±6.076	68.75±10.524	57.06±6.187	64.96±2.406	**68.68**			
Overall mean	**70.12**	**75.16**	**58.43**	**68.47**	-	NS	NS	NS
LDL (mg/dl)								
N_0	93.00±50.90	126.53±23.39	105.04±7.15	76.09±20.80	**115.95**			
N_1	111.44±2.72	100.82±11.00	74.43±5.50	119.05±11.81	**98.20**			
Overall mean	**102.22^b**	**163.68^a**	**80.80^c**	**97.57^b**	-	*	NS	*

1, (old ♂x young ♀); 2, (old ♂x old ♀); 3, (young ♂ x young ♀); 4, (young ♂x old ♀).N0, (Zero nanosilver); N1, (20ppm nanosliver).
* P≤ 0.05, ** P≤ 0.01, NS= non-significant.
Mean within a column (having capital letters) or row (having small letters) are significantly different.

5.3. Liver enzymes activities

Data presented in Table 8, illustrate the combined effect of mating system and AgNps in drinking water of Japanese quail breeder on the hepatic enzymes activities of their progenies.

The present results show that AST activity was significantly influenced by mating system, but was not significant for the water by AgNps treatment. The higher AST values (240.73, and 195.37 u/L) were noted for chicks hatched from M4 and those of M3, respectively. However, the interaction between age at mating and AgNps was highly significant ($P \leq 0.01$).

Results show that there are significant differences in ALT activity related to AgNps administration.

Table 8 .Effect of age at mating and Silver nanoparticles administration of quail breeder on the activity of AST and ALT (µ/L) of their progeny.

Nanosilver (N)	*Mating system (M)*				*Overall mean*	Significance		
	1	2	3	4		M	N	M*N
				AST(U/ L)				
N_0	136.85±5.675	183.07±7.435	218.02±10.956	213.89±5.699	**193.56**			
N_1	161.61±3.433	196.87±6.499	178.38±13.387	227.55±14.552	**199.36**			
Overall mean	**151.71^c**	**191.35^b**	**195.37^b**	**240.73^a**	-	**	NS	**
				ALT(U/ L)				
N_0	17.61±0.638	16.77±1.263	16.61±1.311	16.87±1.275	**16.98^A**			
N_1	12.39±0.707	14.25±1.642	16.51±0.726	17.38±0.839	**15.59^B**			
Overall mean	**15.65**	**15.82**	**16.55**	**17.13**	-	NS	*	NS

1, (old ♂x young ♀); 2, (old ♂x old ♀); 3, (young ♂ x young ♀); 4, (young ♂x old ♀).N0, (Zero nanosilver); N1, (20ppm nanosliver).
* P≤ 0.05, ** P≤ 0.01, NS= non-significant.
Mean within a column (having capital letters) or row (having small letters) are significantly different.

It appears that both ALT and AST enzymes, as indicators of liver function, are changed according to the physiological status of birds. Where, Aspartate aminotransferase (AST) is not specific for hepatocellular damage, although it is highly sensitive in detecting liver damage. Alanine aminotransferase (ALT) is found in hepatocyte cytosol as well as in muscles and other tissues of birds. ALT has poor specificity for liver disease, and the clinical relevance of an increased ALT value is decreased [49].

5.4. Plasma calcium (Ca) and inorganic phosphorus (P)

The concentration of plasma Ca and P at 6 wk old Japanese quail chicks hatched from quail breeder of different ages at mating and receiving AgNps in drinking water are presented in Table 9.

Table 9 . Effect of age at mating and Silver nanoparticles administration of quail breeder on plasma calcium (Ca) and phosphorus (P) of their progeny.

Nanosilver (N)	*Mating system (M)*				*Overall mean*	Significance		
	1	2	3	4		M	N	M*N
				Calcium (mg/ dl)				
N_0	12.52±1.050	14.75±1.151	15.43±1.073	14.80±1.274	**14.54**			
N_1	12.92±1.601	13.13±1.794	12.36±0.877	15.24±1.691	**13.33**			
Overall mean	**12.76**	**13.90**	**13.67**	**15.02**	-	NS	NS	NS
				Phosphorus (mg/ dl)				
N_0	2.03±0.191	1.90± 0.153	2.59±0.265	2.92±0.230	**2.34^B**			
N_1	2.22± 0.190	3.00±0.303	1.83± 0.153	4.51±0.225	**2.79^A**			
Overall mean	**2.10^b**	**2.45^b**	**2.13^b**	**3.55^a**	-	**	**	**

1, (old ♂x young ♀); 2, (old ♂x old ♀); 3, (young ♂ x young ♀); 4, (young ♂x old ♀).N0, (Zero nanosilver); N1, (20ppm nanosliver).
* P≤ 0.05, ** P≤ 0.01, NS= non-significant.

Mean within a column (having capital letters) or row (having small letters) are significantly different.

It is clearly noted that there were no significant difference for the plasma concentration of Ca among quail chicks. These results indicated that mating groups and AgNps had no effect on the blood level of Ca of the progenies.

However, the data indicated that plasma P level was significantly higher in chicks hatched from M4 than the other mating groups. Also chicks from hens receiving AgNps in drinking water showed significantly higher plasma P levels.

Conclusion

It could be concluded from the present study that, it is possible to improve the productive and healthy status of the progeny if the old age breeder is mated with younger ones. The magnitude of this effect could be achieved through the administration of AgNps in the breeder's drinking water.

Acknowledgments

This research was supported by National Research Centre, Egypt, Grant 10120509

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