

Effect of the number of incubated eggs and nurturing squabs on the behaviour and performance of breeding pigeons

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ABSTRACT

The objective of this study was to evaluate the incubated egg and nurturing squab numbers on breeding pigeon behaviour and performance. Fifteen pairs of mature Egyptian Baladi Pigeons (12-18 months) were divided into 3 groups (one pair x 5 replicates for each group). Group1 (G1): each pair incubated one egg and reared one squab, Group2 (G2): parents incubated 2 eggs and reared 2 squabs and Group3 (G3): incubated 3 eggs and reared 3 squabs. Maintenance and reproductive behaviour were recorded for each pair of parents using a digital camera and then analysed. In addition, the performance of adults and brooded squabs was evaluated. Results showed that the frequency of foraging and walking behaviour of females was significantly higher in G3. On the other hand, the other comfort behaviour was significantly higher for both parents in G1 compared to other groups indicating more welfare they had. No a significant impact was observed on other maintenance behaviour of breeding pigeons. Nest defence behaviour and prolactin level were increased with increasing the reared chicks or incubated eggs, while nest building was significantly decreased. Besides the prolonged incubation period & egg laying intervals in G3, the body weight (at 7 days old) and body weight gain (BWG) of squab at 7 and 14 days old declined significantly. A significant increase in body weight loss of female and male with increasing the number of reared chicks, moreover, raising the prolactin level in G3 compared to G1 and G2. It was concluded that, neither adult behaviour nor squab performance were adversely affected by increasing nurturing squab or incubated egg up to 3 squabs or 3 eggs which could be recommended practice for raising the economic profit in addition to feed supplements for a breeding pigeon to alleviate the loss in body mass at brooding period.

Introduction

Pigeon is the popular name for a member of the enormous Columbidae family, which is found worldwide in temperate and tropical climates and belongs to the Order *Columbiformes* (Akter *et al.*, 2020). Pigeons are one of the most important commercial poultry species as well as being ornamental birds. This is due to their tasty meat which is rich in nutrients like proteins (19-20%) (Apata *et al.*, 2015; Muraduzzaman *et al.*, 2023) in addition to minerals and vitamins. They begin to mate and forming couples at about 5-7 months of age so, their populations typically contain an equal number of males and females (Ding *et al.*, 2020; Shariar, 2020). Both are responsible for building the nest and egg incubation and brooding the baby squabs. Therefore, it is crucial to comprehend how the parents behave and their requirements. Murrell (2020) stated that breeding males took the day shift sitting on the incubated eggs in intervals (from 9.30 a.m. to 1.30 p.m. and 3.30 p.m. to 5.30 p.m.), while females primarily carried out incubated them from late afternoon to mid-morning (about 5.30 p.m. to 9.30 a.m.). Pigeon eggs need about 17-18 days to hatch. When the baby squabs are about 15 days old, the female parent may start laying new eggs for the next breeding cycle so, feeding the squabs is mostly handled by the male parent (Adawy and Abdel-Wareth, 2023). Generally, breeding pigeons construct double nests to raise their squabs, and they alternately use each nest to raise the newly hatched squabs and to incubate their eggs of the consecutive cycle. Pigeons are altricial birds, which means that after hatching, they are unable to feed themselves and must instead rely solely on the milk supplied by the parent pigeon's crop (crop milk), which is given to the squab mouth-to-mouth by both male and female (Mohamed *et al.*, 2016; Mahdy, 2021; Jin *et al.*, 2023) so, both male and female sharing in nurturing the squab. Lactation

process and reproductive behaviour (such as nest defence, egg incubation and brooding squabs are associated with the Prolactin level (Gillespie *et al.*, 2012). Prolactin promotes the sitting of the parent in the nest, thus enhancing the parental feeding invitations (squab-oriented bill opening) (Angelier *et al.*, 2016; Skrade *et al.*, 2017). Due to crop milk's crucial role in squab growth, there is a great attention of nutritionists and research into its composition and nutrient content has persisted (Jin *et al.*, 2023). According to studies, crop milk's chemical composition primarily consists of protein and fat, as well as some minerals, fatty acids, and amino acids. However, varied breeding pigeon diets and squab stages result in various crop milk compositions.

Typically in one breeding cycle, a pair of breeding pigeons can feed up to two squabs, which is insufficient to satisfy the rising market demand, commercial production uses manual incubation technologies to increase production effectiveness so that a pair of breeding pigeons can feed more than 2 squabs for greater economic advantages (Zhang *et al.*, 2023). Moreover, Tang *et al.* (2019) showed that the survival rate of 28-day-old squabs, the egg-laying interval, and the parent pigeon body mass changes were all significantly impacted by the number of reared squabs. The objective of this work was to investigate the effect of different numbers of incubated eggs and nurturing squabs on the behaviour and productive performance of breeding pigeons.

Materials and methods

Birds and management

This study was done in the experimental chambers belonging to Zagazig University, Egypt, to investigate the effect of different nurturing

squabs or incubated eggs practices on the behaviour and performance of pigeons. The study protocol was approved by the Institutional Animal Care and Use Committee at Zagazig University (ZU-IACUC/2/F/381/2022).

A total of 30 mature Egyptian Baladi pigeons (15 males and 15 females) aged 1-1.5 years were used in this work. Birds were kept in pens that allowed for enrichment and a variety of behaviour, including flying if possible. Each pair was kept in wooden open-type cages that were externally hanged to walls at a level of 150 cm from the ground. Boxes were divided into partitions not increased than 4 cages in height and 4 cages in width, each battery was provided with a barrier 3cm height on the edge to prevent slipping of the nest, and in the opposite wall it hanged perch's sites with 120 cm height. Two nests were provided to each pair of pigeons for rearing their squabs, and both nests were alternately used for egg incubation and raising the hatchings, thus ensuring a regular breeding cycle (Maity *et al.*, 2020). Experimental house and all the equipment (feeders, waters, nest bowl) were fumigated according to Jabbar *et al.*, (2020). Sexing of the pairs was determined according to pelvic bone test (Abdel Fattah *et al.*, 2019) and phenotype of adult pigeon (Kabir, 2014). Adult pairs were marked by nontoxic permanent colors and by different fabrics colors tied at their wings. Leg bands (leg rings) were used to identify squabs. according to Abdel Fattah *et al.* (2019). In each nest, eggs were carefully marked with a non-toxic marker within each nest (Dijkstra *et al.*, 2010). Nests are marked with numbers to identify each pair's nest site.

The lighting system was 16L:8D lighting schedule (Xie *et al.*, 2017), with a light intensity of 2.5 w/m (Amir *et al.*, 2016). Artificial light was provided by neon lamb (30w). The mean daily temperature was $23 \pm 2^\circ\text{C}$, and relative humidity (RH) was 60 to 75%. Pigeons have unrestricted access to food and water. Water from a clean source should be changed twice daily. Refilling of feeders was done twice daily (at morning: 8 am and at evening: 6 pm). Extra midday feeding was required for up to seven days after hatching. Pigeons were fed on commercial pellets diet obtained from El -HAYANI factory and its chemical composition was crude protein(17%), fat (2.95%) and metabolizable energy not less than 2750 k.cal/kg crude fibre (3.74%) while its ingredients as follow: Yellow corn, soybean, sorghums, wheat bran, corn gluten, CaHPO₄, calcium carbonate lime powder, sodium chloride NaCl, a mixture of mineral, salts and vitamins, Methionine (D-L) and choline chloride as mentioned in the recommendation

sheet by the company.

Additional grit was supplied day after day. It consists of a mix of different and safe granite gravels, a mix of minerals and trace elements (MN-ZN-FE-CU-I-SE-CO-CA-PH-NA), a mix of vitamins (AD3E, K3, B1, B2, B6, B12, Biotin, Pantothenic acid, Folic acid, and nicotinic acid).

A vaccination and prevention programs were carried out in accordance with Marlier and Vindevogel (2006).

Experimental design

Fifteen pairs of adult pigeons were randomly divided into 3 experimental groups (each group had 5 replicates of one pair of pigeons). Group1(G1) consists of 5 females and 5 males and each couple incubated only 1 egg and reared 1 hatched squabs. In G2, each couple incubated 2 egg and reared 2 hatched squabs (as a control), while in G3, each pair incubated 3 eggs and 3 hatched squabs. The additional eggs were always the same age as one of the original eggs in the nest. Regular candling was carried out at days 5 and 10 of incubation to identify any unviable egg according to Ernst *et al.* (2004) and Hassanien (2015). Shortly following the predicted hatching date, check the nests to see if all the eggs have hatched. When possible, change the brood in G3 if some eggs were delayed in hatch so that there were three younger chicks at the same age.

The experimental duration included an incubation period of (17-20 d), a lactation period of 28 d and a period to another egg-laying cycle (recovery period) of 8-12d. The recovery period can occur before the end of the lactation period so the female can build a nest and put eggs during the lactating period (Łukasiewicz *et al.*, 2014; Tang *et al.*, 2019).

Data collection

Behavioural observation

Video cameras (DVR) that connected to a computer, located in a separate room away from the birds, were fixed in pens for recording birds' activities. A focal sample technique was used to record behavioural patterns (Altmann, 1974) of each group. The first month of the experimental period was given for adaptation of pigeons for place, feed, pair formation

Table 1. The observed behavioural patterns of pigeon.

Behaviour	Description
Maintenance behaviour	
Ingestive behaviour	Feeding, foraging and drinking.
Comfort behaviour	Feather Preening and Other comfort behaviour (Wings and legs stretch, Wing flapping, Wing ruffling, Wing and tail shaking, Head shaking and Foot pecking).
Resting behaviour	Crouching, huddling and perching (Alert immobility).
Kinetic behaviour	Standing, walk and fly.
Aggressive behaviour	Attack and Fight.
Reproductive behaviour	
Male sexual activity	Head bowing- Driving-Attacking- Mounting and displacement preening.
Female sexual activity	Begging - pushing male - being driven.
	Collection of nest material.
	Nest building.
Male and Female sexual activity.	Nest demonstration.
	Reciprocal preening (mutual preening).
	Nest defense.
Egg sitting	Brooding or natural incubated of the eggs.
Egg turning	Turn the eggs on each side by parent's beak or legs through the incubation period.
Brooding behaviour (young sitting)	The act of giving young squabs heat by their parents and alternating between female and male care.
Feeding squabs	Mouth to mouth feeding between parent and squabs through periods of observation in the day.

and ensuring their ability for production and reproduction. Observations were performed twice daily in the morning between 8-10 A.M. and in the afternoon between 2-4 P.M. Each couple were inspected for 20 minutes per day during egg incubation till the weaning of young at 28 days of age. The recorded behaviour was according to Goodwin (1956); Spudeit *et al.*, (2013); EL Shoukary *et al.*, (2018a;b); Yan and Ton (2017) and EL Shoukary and Mousa (2018), the ethogram presented in Table 1.

Reproductive and productive performance

Parental performance

Incubation period (days): Days the parent spent sitting on egg until hatching).

Egg laying interval: The number of days between the first egg laid and the first egg of the following clutch as described by Stock and Haag-Wackernagel (2016).

Egg number: number of eggs produced per pair of parents in the cycle.

Embryonic death % as described by Hetmański and Barkowska (2007)

Egg weight (g) before incubation.

Hatching egg weight: Weight of egg on day 18, according to (Lukanov *et al.*, 2020).

Hatching success: The ratio of the number of new-borns hatched to the number of eggs laid.

Brooding Period: Days that male and female parent look after and protect the young squabs between 2 cycles.

Body weight of parents (g): at the beginning of hatching period and at the end of rearing period.

Body weight loss of parents during nursing squabs: Weight at beginning of rearing cycle –Weight at end of this period, according to Zhang *et al.* (2023).

Squab performance

Live body weight (BW): Recorded weekly weight and was performed in early morning (7 a.m.) before feeding and drinking.

Nestling Growth Rate: The increase of squabs' weight in the first 4 weeks of life, the squab weight was collected at the age of 1, 7, 14, 21 and 28 respectively. Nestling's weights were performed only when the squabs were not being brooded during the day to prevent desertion by parents and avoid disturbance to nestlings (to avoid premature fledging) (Pawlina and Borys, 2009; Hassanien, 2015; Islam *et al.*, 2021).

Body weight gain (BWG): Determination of the growth gains of squabs by fasting for 12 h, then weighed in the morning on days 0, 7, 14, 21, and 28 during the experimental periods according to Zhang *et al.* (2023).

(the body weight at end of one week) – (the body weight at the beginning of this week).

Hatching weight (day old squab): weight of squabs at day of hatching, just before being fed by its parent (Darwati *et al.*, 2010).

Squabs' mortality (%)= Number of dead squabs/total number of hatched squabs \times 100.

Average feed intake (AFI): In our study, it was measured mainly for crop milk feeding by Weight of squabs after being fed by the parent - Weight the squabs before feed supplementation (Islam *et al.*, 2021).

Survival rate: Each pair of squabs was assigned a survival rate of 100% if both were still alive, 50% if one died during the experiment, and 0% if both died (Islam *et al.*, 2021).

Fledging Age: age at which baby squabs can fly and leave the nest.

Weaning age: age at which the parent stop feeding squabs, and they depend on their own feeding.

Fledging success: the ratio of the fledged young to the hatched nestlings.

The relative growth rate (RGR): was calculated using the following equation (Islam *et al.*, 2021): $1/2 \text{Final weight} - \text{initial weight} / \text{Final weight} + \text{initial weight} \times 100$.

Biochemical parameters

Blood Sampling

Blood samples were collected in tubes without anticoagulant from the wing vein on the 8th day of incubation (Dong *et al.*, 2013), blood samples were processed for separation of serum that was used for measuring serum prolactin levels. Procedures and precautions were done in accordance with EL Shoukary *et al.* (2018a).

Crop milk contents

Crop milk was obtained from mixed-sex newly hatched squabs about 1-3 days old in the morning after parent feeding by manual pipette. because as the squabs become older, crop milk is gradually replaced by grains soaked in the parents' crops (Jin *et al.*, 2023). Samples were collected via a pipette (disinfected with alcohol and lubricated with Vaseline) with the narrow end that was applied through peak and inserted down to the oesophagus until the crop then aspirate the crop milk then transfer sample to Eppendorf tube rapped by aluminium foil and freeze at -18:20 until processed for further analysis. Total protein %, total fat %, Total fiber %, moisture, and total ash % of crop milk samples were determined according to AOAC (2000) methods (Hu *et al.*, 2016). Methods used in measurement of these contents are certified by ISO IEC 17025-2017 with stander specification ES:5465-1/2006 for protein, EN26.2L54/37 volum.25/2009 for fats, EN26.2L54/40 Volum. 52/2009 for fibre, ES:5462/2006 for moisture, ES:5464/2006 for ash.

Statistical analysis

Results were reported as mean \pm SEM (Standard Error of Mean). In order to assess the influence of the three treatment groups on the different behavioural patterns and performance parameters, one-way analysis of variance (ANOVA) by Duncan multiple test as post hoc test and independent T-test were used. The value of $P < 0.05$ was used to indicate statistical significance. ALL Analyses and charts were done using Statistical Package for Social Sciences version 24.0 (SPSS, IBM Corp., Armonk, NY) and Graph Pad prism 8.0.2 (Graph Pad Software, Inc).

Results and Discussion

Frequencies of some maintenance behaviour of breeding pigeon during incubation and brooding period with different number incubating eggs and nurturing squabs were evaluated. The results (Tables 2 and 3) showed no significance difference of ingestive (feeding, drinking, kinetic behaviour, resting (crouching, perching) and preening activity frequencies. On other hand, foraging and walking frequencies in 10 min observation period was significantly ($P < 0.05$) higher for females incubated 3 eggs and brood 3 chicks (G3) in comparison with other groups. This may be attributed to the same housing and managerial conditions and the influence of the study treatment that did not reach to the level of serious change in birds' welfare. However, other comfort behaviour frequencies were statistically higher in both male & female of G1 (parents nursed one squab and incubated only one egg) than G2 and G3. Aggression was slightly higher in males of G3 than of other groups without significance level. When parents believe their eggs are in danger, they become hostile and aggressive so, they engage in nest protection behaviours such wing and feather erection (Wang *et al.*, 2023).

Regarding reproductive behaviour, it includes courtship activities, egg sitting, egg turning, (brooding) young sitting and feeding squabs. The data in Table 4, revealed that no significant influence of various numbers of the brooded eggs and feeding squabs on most of reproductive activities of male and female pigeon. The results in consistency with Scott (1971) who observed that the normal breeding pigeon had no qualitative

Table 2. Effect of number of incubated eggs and squabs on frequency (times/10 min) of some maintenance behaviour of female breeding pigeons.

Item	G1	G2	G3	P-value
Feeding	0.50±0.17	0.5476±0.22	0.98±0.32	0.32
Drinking	0.60±0.18	0.55±0.27	0.81±0.22	0.68
Foraging	5.19±1.04 ^b	4.93±1.14 ^b	11.86±1.88 ^a	0.00
Standing	10.40±1.60	11.74±1.77	14.21±1.97	0.31
Walking	6.45±1.24 ^b	5.10±1.13 ^b	11.31±1.61 ^a	0.00
Flying	1.095±0.23	1.095±0.29	0.67±0.19	0.35
Crouching	2.48±0.67	3.31±0.87	1.33±0.48	0.13
Perching	3.40±0.84	3.40±0.73	2.45±0.51	0.55
Preening	13.31±1.11	12.14±1.10	12.17±1.15	0.70
*Other Comfort	9.00±0.82 ^a	6.36±0.49 ^b	6.55±0.55 ^b	0.01

^{a,b,c} Means within the same row carrying different superscripts are sig. different at P < 0.05 based on Duncan multiple Significant Difference test. G1: Parents incubated 1egg and/or reared 1squab, G2: Parents incubated 2 eggs and/or reared 2 squabs, G3: Parents incubated 3 eggs and/or reared 3 squabs. *Other comfort behaviour including Wings and legs stretch, Wing flapping, Wing ruffling, Wing and tail shacking, Head shaking and Foot pecking).

Table 3. Effect of number of incubated eggs and squabs on frequency (times/10 min) of some maintenance behaviour of male breeding pigeons.

Item	G1	G2	G3	P-value
Feeding	2.19±0.62	2.26±.62	1.07±.32	0.22
Drinking	0.36±0.11	0.74±.18	0.50±.16	0.20
Foraging	8.24±1.23	8.05±1.31	7.48±1.23	0.91
Walking	9.67±1.66	12.83±1.97	16.79±4.41	0.24
Standing	12.52±1.94	15.95±1.95	15.33±1.87	0.41
Flying	1.71±0.30 ^b	3.69±.80 ^a	2.62±0.78 ^{ab}	0.12
Crouching	1.90±0.60	1.17±.38	2.00±0.57	0.47
Perching	4.19±0.79	5.38±.99	5.40±0.87	0.54
Preening	9.95±1.03	11.33±1.33	8.71±1.09	0.28
*Other comfort Frequency	9.48 ±.94 ^a	6.52 ±.57 ^b	6.83 ±0.64 ^b	0.01
Aggression	0.05±.0.05	0.11±.0.07	0.26±.0.16	0.36

^{a,b,c} Means within the same row carrying different superscripts are sig. different at P < 0.05 based on Duncan multiple Significant Difference test. G1: Parents incubated 1egg and/or reared 1squab, G2: Parents incubated 2 eggs and/or reared 2 squabs, G3: Parents incubated 3 eggs and/or reared 3 squabs. *Other comfort behaviour including Wings and legs stretch, Wing flapping, Wing ruffling, Wing and tail shacking, Head shaking and Foot pecking).

Table 4. Frequency of reproductive behaviour of pigeons under different number of incubated eggs and nursing squabs on (times/10 min).

Item	Groups			P-value
	G1	G2	G3	
Male sexual behaviour				
Courtship behaviour				
Head bowing	00.00±.00.000	0.07±.05	0.07±.05	0.40
Driving	0.071±.071	0.26±.26	0.095 ±.057	0.66
Attack	0.43±.0.27	0.45±0.17	1.17±0.5	0.25
Mount	00.00±00.00	0.048±.048	0.09±0.07.	0.42
Displacement preening	00.00±00.000	00.00±00.000	0.12±0.08	0.14
Follow female	0.14±0.14	0.048±.048	0.07± 0.053	0.75
Circling female	0.02±0.02	0.09±0.05	0.33±0.175	0.11
Collection on nest material	0.62±.38	0.15±0.05	0.19±.19	0.24
Nest building	2.17±.67	1.64±.39	0.79±.28	0.12
Mutual preening	0.38±.25	0.02±.02	0.14±.09	0.26
Nest defense pecking	0.10±0.05	0.24±0.14	0.29±.120	0.45
Nest defense attack	0.45±0.17	0.43±.27	1.17±.52	0.25
Egg sitting	9.38±1.97	8.24±1.79	6.74±1.90	0.61
Egg turning	0.57±0.17	0.42±0.12	0.79±0.28	0.45
Brooding (young sitting)	3.02±1.16	2.26±1.19	2.29±.89	0.85
Feeding squabs	2.095±.63	2.02±0.52	0.79±0.28	0.12
Female sexual behaviour				
Courtship behaviour				
Begging	0.05±0.05	0.88±0.44	0.50±0.24	0.13
Pushing	0.05 ±0.03	00.00±00.00	00.00±00.00	0.13
Being driven	0.12±0.07	0.12±0.08	0.07±0.05	0.86
Collection on nest material	0.07±0.07	0.05±0.05	0.047±0.03	0.94
Nest building	5.02±1.08 ^a	4.29±0.77 ^a	1.05±0.39 ^b	0.00
Mutual preening	0.88±0.44	0.05±0.05	0.50±0.24	0.13
Nest defense pecking	0.07±0.05	0.17±0.12	0.19±0.08	0.61
Nest defense attack	00.00±00.000 ^b	0.023±0.02 ^b	0.17±0.083 ^a	0.04
Egg sitting	12.095±2.08	11.79±2.19	7.93±1.96	0.29
Egg turning	0.57±0.15	0.67±0.21	0.76±0.25	0.81
Brooding (young sitting)	4.38±1.43	3.17±1.18	4.93±1.53	0.66
Feeding squabs	0.31±0.18 ^b	0.36±0.15 ^b	1.71±0.60 ^a	0.01

^{a,b,c} Means (Mean ±SE) within the same row carrying different superscripts are sig. different at P < 0.05 based on Duncan multiple Significant Difference test. G1: Parents incubated 1egg and/or reared 1 squab, G2: Parents incubated 2 eggs and/or reared 2 squabs, G3: Parents incubated 3 eggs and/or reared 3 squabs.

variation in their daily cycle behaviour. Notably, nesting behaviour (nest defence pecking and attack) was higher for males and female in G3 compared to G2 and G3 this may be due to the more danger feeling about their eggs and chicks as by increased their number needed more care. The frequency of feeding squabs was significantly higher for females in G3. This may be due to low feeding intake amount with increase the litter size and they still hungry and significant increase of prolactin in females with increasing the squabs nursed (our results). This finding agrees with aforementioned by Gillespie *et al.* (2012) who stated that lactation in birds is controlled by prolactin. Similarly, Frequency of nest building action by females was significantly ($P=0.001$) lower in G3.

As shown in Table 5, The impact of managerial practices on the performance of pigeon during the pigeon's incubation and brooding periods revealed that the incubation period was significantly ($P \leq 0.000$) increased with increasing the number of incubated egg. This may be attributed to changing of incubation temperature with increasing the number of covered eggs. Also, the egg laying intervals were prolonged significantly ($P=0.034$) in group of rearing 3 squab and incubating 3 eggs (G3) compared to G1 and G2. Our findings were supported by Tang *et al.* (2019) who recorded that pigeon strains that raising 4 and 3 squabs had significantly longer laying intervals than others raising 2 and 1 squab. Additionally, Hetmański (2005) explained that the egg-laying interval was shorter

when there was only one baby in the nest than when there were two.

Eggs produced by tested breeding pigeon were fertile, had no deformities or cracks, and natural incubated by the parents. Weight of eggs in all group had minor differences due to individual variation. This may be attributed to the same managerial conditions and feeding of the females. However, the average of brooding time of squabs increased in G1 12.00 days than G2 and G3 (10.67 and 10.00 days, respectively) this may be attributed to the fact that, the squabs became older, their parents began to leave them for varied lengths of time to feed, drink, groom themselves, or gather nesting materials instead of constantly watching over them. At this time, the adults often nurse the squabs without sitting on them, approaching to the nest and pecking at the squabs' bills (Lehrman, 1955) thus by reducing the number of squabs, the parents will have more room in the nest to sit or rest with the squabs for an extended period of time.

No significance difference in average crop milk intake among groups were observed although it was slightly lower in G3. This may be due to distribution of produced milk on the litters nursed.

Hatching percent was 100 % in both G1 and G2 while G3 recorded 77.7%. All hatched chicks were healthy, live squabs and reached to the fledging and weaning age except in G3 one of squabs died at end of the first week with weight of (21 g) due to insufficient food and care supplied by parents.

Table 5. Effect of number of incubated eggs and squabs on the performance of pigeon during the pigeon's incubation and brooding periods

Items	Groups			P- value
	G1	G2	G3	
Incubation period(days)	16.67±0.33 ^c	17.50±0.22 ^b	19.11±0.20 ^a	0
Egg laying interval(days)	31.67±.33 ^b	33.67±0.33 ^b	43.00±4.16 ^a	0.03
Average brooding time (days)	12.00±0.00 ^a	10.67±0.67 ^{ab}	10.00±0.58 ^c	0.08
Average crop milk intake (g)	7.00±0.58	7.00±0.00	6.67±0.33	0.79
Embryo death (%)	0.00±0.00	0.00±0.00	11.10±11.10	0.42
Egg weight (g)	16.33±0.33 ^b	17.83±0.31 ^a	16.777±0.22 ^b	0.01
Hatching egg weight (g)	14.66±0.33 ^b	16.00±0.26 ^a	15.00±0.235 ^b	0.02
Hatching success (%)	100.00±0.00	100.00±0.00	77.77±22.23	0.42
Body weight loss of male during brooding period (g)	21.33±0.88 ^c	35.67±1.86 ^b	45.67±2.96 ^a	0.00
Body weight loss of female during brooding period (g)	10.33±1.45 ^b	19.33±1.45 ^b	32.67±5.49 ^a	0.01

^{a,b,c} Means (Mean ±SE) within the same row carrying different superscripts are sig. different at $P < 0.05$ based on Duncan multiple Significant Difference test. G1: Parents incubated 1 egg and/or reared 1 squab, G2: Parents incubated 2 eggs and/or reared 2 squabs, G3: Parents incubated 3 eggs and/or reared 3 squabs.

Table 6. Effect of number of incubated eggs and squabs on the performance of squabs

Parameters	Groups			P-value
	G1	G2	G3	
Hatching weight of squab (g)	13.33±0.33 ^b	14.17±0.31 ^a	13.3±0.17 ^b	0.05
Body weight (g)				
After 24hrs	24.00±1.15	23.83±0.48	24.11±1.06	0.98
At 7 day	188.33±3.76 ^a	136.17±3.40 ^b	103.78±13.96 ^b	0.00
At 14 days	266.00±12.34 ^a	241.50±5.86 ^{ab}	172.67±24.50 ^b	0.03
At 21 days	310.33±7.22 ^a	281.17±6.75 ^a	214.00±28.89 ^a	0.06
At 28 days	347.67±4.48 ^a	289.17±6.11 ^{ab}	229.00±29.75 ^b	0.04
Body weight gain (BWG)				
At 7 day	175.00±3.79 ^a	122.00±3.65 ^b	92.00±12.63 ^b	0.00
At 14 days	105.33±5.14 ^a	77.67±11.86 ^a	68.89±13.32 ^a	0.11
At 21 days	44.33±8.84 ^a	39.67±4.72 ^a	41.33±6.67 ^a	0.93
At 28 days	37.33±3.18 ^a	12.83±6.90 ^a	15.44±5.67 ^a	0.10
Weaning age	32.67±0.67 ^a	36.00±0.26 ^a	33.89±4.25 ^a	0.86
Relative weight of squab at one day age	90.96±2.15	88.11±0.35	88.53±1.86	0.64
Squab mortality (%)	0.00±0.00	00.00±00.00	11.10±11.1	0.42
Fledge age	29.67±0.33	27.17±1.82	23.89±3.12	0.46

^{a,b,c} Means (Mean ±SE) within the same row carrying different superscripts are sig. different at $P < 0.05$ based on Duncan multiple Significant Difference test. G1: Parents incubated 1 egg and/or reared 1 squab, G2: Parents incubated 2 eggs and/or reared 2 squabs, G3: Parents incubated 3 eggs and/or reared 3 squabs.

There was a linearly increase of the body weight loss of male and female during brooding period with increasing the reared chicks. It was observed that the loss in body weight of males was higher than that of females. This changes in weight of parents because the most of their food supply went for squab feeding. These results were in agreement with Xie et al. (2017) and Zhang et al. (2023).

Results in Table 6 show the effect of rearing system on the performance of squabs. The hatching weight of squabs and relative growth weight of squab at one day old did not had an obvious difference among groups. Squabs' weights after 24hrs were 24.00, 23.83 and 24.11g for G1, G2 and G3 respectively, which increased about 10 gm within 24hr after hatching. This result was agreed with Majewska and Drenikowski (2016) who observed that chicks' body weight became nearly the double after 48hrs of hatching. Results showed that number of incubated egg and squabs had no significant effect on the body weight of squabs at 24 hrs and 21 days after hatching. On other hand, mean of squab's weight at 7,14 and 28 days (188.33, 266.0 and 347.6 respectively) was significantly higher for group 1 in which the parents incubated one egg and brooded one squab than G2 and G3. In contrast, our results did not agree with Pawlina and Borys (2009) who reported that pigeon chicks from single clutches were insignificantly heavier until the 21st day of life but were similar by day 28. The day-old pigeon's weight ranged from 10.9 to 16.2 g, according to Darwati et al. (2010) then from week 0 to week 4, the squab weight rose which may be the highest rate through the pigeon life (Xu et al., 2020); however, in week 5, it fell. The growth rate peaked at week 1, then declined from weeks 2 to 5, with the fifth week seeing a negative growth rate. The significant decline of growth rate with age may be due to high level of grains mixed with crop milk (Shetty et al., 1992) indicating that the crop milk has an important role in the rapid early growth of squabs (Wang et al., 2023). Also, when the squabs grew older, the parents' concern turned to the upcoming egg cycle (Adawy and Abdel-Wareth, 2023). On other side, Kabir (2013) recorded that squabs grow slower during the first week and faster in third week of age. In the same line, A significant increase in body weight gain (BWG) of squabs at 7 days old was observed with decreasing the number of nursed squabs. The lowest mean of body squab weight was recorded for pigeon that brood 3 young and incubate 3 eggs. This confirmed by Blockstein (1989) who discovered that growth rates for 3 chicks were much lower than of the usual nursed (2 chicks). On other hand, BWG at 14, 21 and 28 after hatching, was not statistically differ between groups. It was observed that the body weight gain decreased with increasing the squab age. Our finding supported by Zhang et al. (2023) who assessed the laying, hatching, and nursing of multiple squabs and found that increasing litter size was associated with a decline in the pigeon egg hatching rate and the squabs' average daily weight gain at 28 days of age. We stated the weaning age of squabs was differ among experimental groups but didn't reach the significance level (32.67, 36.00, 33.89 days in G1, G2 and G3, respectively).

Squabs produced from parents that incubated 3 eggs and nursed 3 squabs were fledged more earlier (at 23.89 days) than others produced from parents brooded 2 and Incubated 2 eggs and parent incubated one egg and brooded one squab. These results were in consistency with our finding of body weight gain. The locomotor behaviour and movement increase after fledging from the nest so loss more weight than non-fledging squabs resting in the nest.

Interestingly, the amount of prolactin hormone produced during a pigeon's breeding cycle is a crucial for healthy egg incubation, squab hatching and rearing (Mohamed et al., 2016). Our results presented in Figure 1, shows that the prolactin was significantly increased in female pigeon with increasing the number of incubated eggs. The visual and tactile stimulation supplied by the eggs and nest during egg incubation could be the cause of difference between groups (Schmid et al., 2011; Mohamed et al., 2013; Mohamed et al., 2016). Prolactin often increases shortly after egg laying starts. This increase is crucial to keep contact with the eggs while brooding (Smiley, 2019). Nevertheless, there was no sig-

nificance difference in prolactin levels for males among groups. Xie et al. (2018) measured level of prolactin in both sex of pigeon during the egg incubation and squab rearing, and found that the prolactin concentration in the period of second day of incubation to the 14th day ranged between 0.29 to 0.47 ng/mL and 0.46 to 0.57 ng/mL in female and male pigeons, respectively. In this context, Mohamed et al. (2016) confirmed the previous finding where they reported that there was a variation in circulating prolactin during the breeding cycle and the highest level was recorded at egg incubation stage.

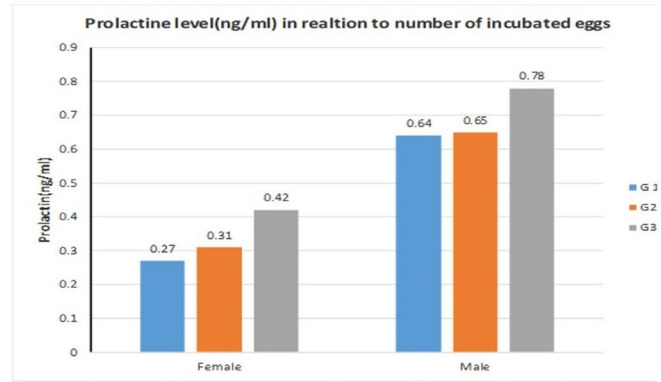


Fig. 1. Serum Prolactin level (ng/ml) in relation to the incubated eggs. G1: parents incubated 1 egg, G2: parents incubated 2 eggs, G3: parents incubated 3 eggs.

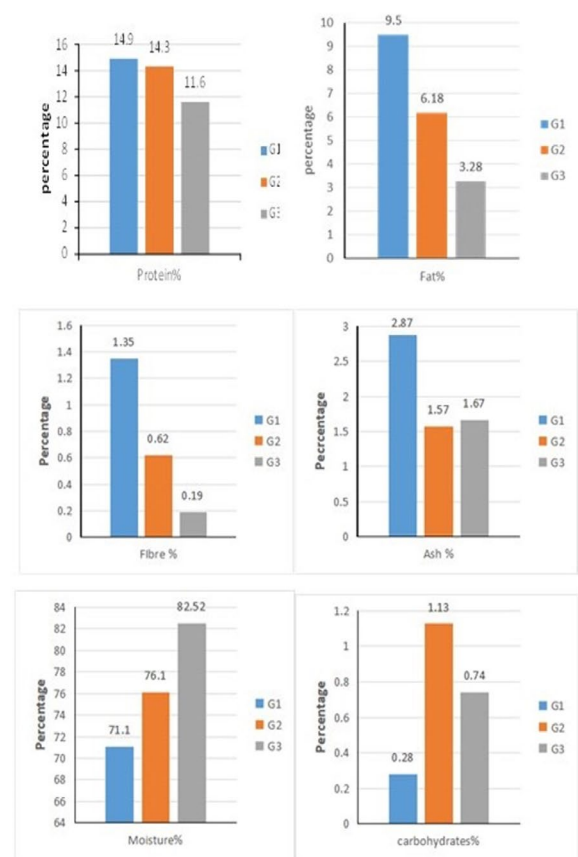


Fig. 2. Chemical analysis of crop milk ingredients in relation to number of nurturing squabs. G1: Parent's nursed 1 squab; G2: parents nursed 2 squabs; G3: parents nursed 3 squabs.

Figure 2 shows the chemical analysis of crop milk in the experimental groups. Due to very small amount aspirated from squabs' crop, only one sample could be totally collected from all replicates of each tested group so, we couldn't statistical analysis the results. The highest nutrients content were recorded in crop milk of squabs reared by parent brooded only one squab. Furthermore, the moisture content increased with increase the number of nursed squabs. As proven by many scientists, young

squabs totally rely on the crop milk for feeding especially in first 3 days of age then after that crop milk is mixed with grains gradually till replaced with grains (Sales and Janssens, 2003; Jin et al., 2023; Wang et al., 2023). There have been various research on the constituents of crop milk, however the findings are conflicting, which may be due to the variety of diets consumed by parent pigeons and sampling techniques. (Sales and Janssens, 2003)

Xie et al. (2013) stated that concentrations of fat and protein in crop milk were 9–11% and 9–13%, respectively in the period of 1-5 days of age. Previous studies reported that pigeon milk had no carbohydrates. On the other line, our findings are in line with those of Shetty et al. (1992), who discovered that pigeon milk contains roughly 0.9-1.5% carbs.

Conclusion

The current study revealed that increasing the number of incubated eggs or reared squabs by pigeons than usual (2 eggs or 2 squabs/pair) has no adverse effect on the behaviour of adults or the performance of squabs, indicating the ability to increase the economic profit of using breeding pigeons under certain circumstances with a recommendation to feed supplements to alleviate the loss in body mass at the brooding period.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Abdel Fattah, A.F., Roushdy, E.-S.M., Tukur, H.A., Saadeldin, I.M., Kishawy, A.T., 2019. Comparing the effect of different management and rearing systems on pigeon squab welfare and performance after the loss of one or both parents. *Animals* 9, 165.

Adawy, A., Abdel-Wareth, A., 2023. Productive performance and Nutritional of domesticated pigeons. Present status and future concerns. *SU-Inter. J. Agricul. Sci.* 5, 160-167.

Akter, M., Sarder, M., Islam, M., Islam, M., Parvez, N., Alam, M., 2020. Evaluation of the productive and reproductive performance of pigeon in selected districts of Bangladesh. *Clin. Surg.* 4, 1-4.

Altmann, J., 1974. Observational study of behavior: sampling methods. *Behaviour* 49, 227-266.

Amir, A., Shama, T., Abas, W., 2016. Early weaning of pigeon squabs. *Egypt. Poult. Sci.* 36, 205-232.

Angelier, F., Parenteau, C., Ruault, S., Angelier, N., 2016. Endocrine consequences of an acute stress under different thermal conditions: A study of corticosterone, prolactin, and thyroid hormones in the pigeon (*Columba livia*). *Comp. Biochem. Physiol. A. Mol. Integ. Physiol.* 196, 38-45.

Apata, S.E., Koleoso, I.M., Tijani, L.A., Obi, O.O., Okere, I.A., 2015. Effect of sex on meat quality attributes of pigeon birds (*Columba livia*) in Abeokuta metropolis Ebonulawa.

Blockstein, D.E., 1989. Crop milk and clutch size in mourning doves. *Wilson Bull.* 11, 20-23.

Darwati, S., Martojo, H., Sumantri, C., Sihombing, D.T.H., Mardiasuti, A., 2010. Productivity, Repeatability of Productive and Reproductive Traits of Local Pigeon. *J. Indon. Trop. Anim. Agricul.* 35.

Dijkstra, C., Riedstra, B., Dekker, A., Goerlich, V.C., Daan, S., Groothuis, T.G., 2010. An adaptive annual rhythm in the sex of first pigeon eggs. *Behav. Ecol. Sociobiol.* 64, 1393-1402.

Ding, J., Liao, N., Zheng, Y., Yang, L., Zhou, H., Xu, K., Tang, C., 2020. The composition and function of pigeon milk microbiota transmitted from parent pigeons to squabs. *Front. Microbiol.* 11, 1789.

Dong, X., Zhang, M., Jia, Y., Zou, X., 2013. Physiological and hormonal aspects in female domestic pigeons (*Columba livia*) associated with breeding stage and experience. *J. Anim. Physiol. Anim. Nutr.* 97, 861-867.

EL Shoukary, R.D., Abdel-Raheem, G.S., Osman, A.S., 2018a. Impact of Heat Stress on Reproductive Behavior, Performance and Biochemical Parameters of Pigeon: A Trial to Alleviate Heat Stress By Propolis or Wheat Diets. *Alex. J. Vet. Sci.* 56.

EL Shoukary, R.D., Mousa, M.A., 2018. The impact of some feed additives on behavior, welfare and performance of heat-stressed pigeon squabs. *Inter. Open J. Appl. Sci.* 1, 15-29.

EL Shoukary, R.D., Sayed, R.K., Hassan, R.I., 2018b. Behavioral, hepato-morphological, and biochemical studies on the possible protective effect of black seed and water bath against change-mediated heat stress on pigeon. *J. Basic Appl. Zool.* 79, 1-11.

Ernst, R., Bradley, F., Abbott, U., Craig, R., 2004. Egg candling and breakout analysis. *ANR Publication* 8134.

Gillespie, M.J., Stanley, D., Chen, H., Donald, J.A., Nicholas, K.R., Moore, R.J., Crowley, T.M., 2012. Functional similarities between pigeon 'milk' and mammalian milk: induction of immune gene expression and modification of the microbiota. *PLoS One* 7, e48363.

Goodwin, D., 1956. The significance of some behaviour patterns of pigeons. *Bird Study*, 3, 25-37.

Hassani, A.F.A., 2015. Parental Care During Incubation, Brooding and Growth Rates of Egyptian Baladi Pigeon Nestlings. *Bri. J. Poult. Sci.* 4, 29-33.

Hetmański, T., 2005. The effect of environmental factors and nesting conditions on clutch overlap in the Feral Pigeon *Columba livia f. urbana* (Gm.). *Polish J. Ecol.* 53, 105-111.

Hetmański, T., Barkowska, M., 2007. Density and age of breeding pairs influence feral pigeon, *Columba livia* reproduction. *Folia Zool.* 56, 71-83.

Hu, X.-C., Gao, C.-Q., Wang, X.-H., Yan, H.-C., Chen, Z.-S., Wang, X.-Q., 2016. Crop milk protein is synthesised following activation of the IRS1/Akt/TOR signalling pathway in the domestic pigeon (*Columba livia*). *Br Poult. Sci.* 57, 855-862.

Islam, O., Khatun, S., Famous, M., Uddin, M.N., 2021. Comparative studies on squab growth performance and egg morphometrical attributes of different pigeon breeds. *Anim. Husb. Dairy Vet. Sci.* 5.

Jabbar, A., Yousaf, A., Hameed, A., Riaz, A., Ditta, Y., 2020. Influence of fumigation strength on hatchery parameters and later life of chicks. *South Asian J. Life Sci.* 8, 6-10.

Jin, C., He, Y., Jiang, S., Wang, X., Yan, H., Tan, H., Gao, C., 2023. Chemical composition of pigeon crop milk and factors affecting its production: a review. *Poult. Sci.* 102681.

Kabir, M.A., 2013. Productivity of crossed indigenous pigeon in semi intensive system. *Basic Res. J. Agricul. Sci. Rev.* 2, 1-4.

Kabir, M.A., 2014. Sexing in pigeons by phenotypic method. *Inter. J. Adva. Res. Biol. Sci.* 1, 33-38.

Lehrman, D.S., 1955. The Physiological Basis of Parental Feeding Behavior in the Ring Dove (*Streptopelia risoria*). *Behaviour*, 7, 241-286.

Lukanov, H., Pavlova, I., Genchev, A., 2020. Effect of the quail's productive type on the incubation characteristics of domestic quail eggs (*Coturnix japonica domestica*). *Bulg. J. Agricul. Sci.* 26.

Lukasiewicz, M., Wnęk, K., Boruc, K., 2014. Biology of embryo development in pigeon *Columba livia domestica* in conditions of artificial incubation. *Adv. Anim. Vet. Sci.* 2, 401-406.

Mahdy, M.A., 2021. Comparative morphological study of the oropharyngeal floor of squabs and adult domestic pigeons (*Columba livia domestica*). *Micro. Res. Tech.* 84, 499-511.

Maity, B., Das, T.K., Ganguly, B., Pradhan, K., 2020. Pigeon rearing-an investment analysis for secondary income generation to farm women, landless, marginal and small farmers. *Asian J. Agricul. Exten. Econom. Soc.* 38, 1-6.

Majewska, D., Drenikowski, T., 2016. Analysis of reproduction and growth in fancy pigeons. *Acta Scient. Polon. Zootechnica* 15, 1.

Marlier, D., Vindevoel, H., 2006. Viral infections in pigeons. *Vet. J.* 172, 40-51.

Mohamed, R.A., Shukry, M., Mousa-Balabel, T.M., Elbassiouny, A.A., 2016. Assessment of plasma prolactin and nest defense behaviour during breeding cycle of pigeon (*Columba livia domestica*). *J. Environ. Agricul. Sci.* 7, 19-22.

Mohamed, R.A., Mousa-Balabel, T., Elbassiouny, A., 2013. Evaluation of some management procedures for controlling broodiness in turkey and muscovy duck. *J. Adv. Vet. Res.* 3, 161-166.

Muraduzzaman, M., Ahammed, M., Habib, M., Azad, M.A.K., Hashem, M.A., Ali, M.S., 2023. Comparison of meat yield and quality characteristics between pigeon and quail. *Meat Res.* 3.

Murrell, K., 2020. The effect of environmental enrichment on abnormal conspecific aggressive behaviours of the Racing pigeon (*Columba livia domestica*) within a loft. Degree of BSc (Hons) Animal Science Writtle University College. March 2020.

Pawlina, E., Borys, K., 2009. Growth of Wrocław Meat breed pigeons in relation to the number of birds in the nest. *Roczniki Naukowe Polskiego Towarzystwa Zootechnicznego*, 5.

Sales, J., Janssens, G., 2003. Nutrition of the domestic pigeon (*Columba livia domestica*). *World's Poult. Sci. J.* 59, 221-232.

Scott, F.O., 1971. Effects of estrogen on the differentiation of some reproductive Behaviours In Male Pigeons (*Columba livia*). *Animal Behav.* 19, 277-286.

Schmid, B., Chastel, O., Jenni, L., 2011. The prolactin response to an acute stressor in relation to parental care and corticosterone in a short lived bird, the Eurasian hoopoe. *Gen. Comp. Endocr.* 174, 22-29.

Shariar, S., 2020. A production report on Management system of backyard pigeon farming in the town of Mymensingh: Chattogram Veterinary and Animal Sciences University.

Shetty, S., Bharathi, L., Shenoy, K.B., Hegde, S.N., 1992. Biochemical properties of pigeon milk and its effect on growth. *J. Comp. Physiol.* 162, 632-636.

Skrade, P.D., Dinsmore, S.J., Vleck, C.M., 2017. Testosterone and prolactin levels in incubating Mountain Plovers (*Charadrius montanus*). *Wilson J. Ornithol.* 129, 176-181.

Smiley, K.O., 2019. Prolactin and avian parental care: new insights and unanswered questions. *Hormones behavior* 111, 114-130.

Spudeit, W.A., Sulzbach, N.S., Bittencourt, M.D.A., Duarte, A.M.C., Liang, H., Lino-de-Oliveira, C., Marino-Neto, J., 2013. The behavioral satiety sequence in pigeons (*Columba livia*). Description and development of a method for quantitative analysis. *Physiol. Behav.* 122, 62-71.

Stock, B., Haag-Wackernagel, D., 2016. Food shortage affects reproduction of Feral Pigeons *Columba livia* at rearing of nestlings. *Ibis* 158, 776-783.

Tang, Q., Mu, C., Qu, Z., Zhang, R., Bu, Z., 2019. Effects of Different Breeds, Number of Squabs and Temperature on Production Performance of Pigeons. *Asian Agricul. Res.* 10, 76-79.

Wang, L., Zhu, J., Xie, P., Gong, D., 2023. Pigeon during the Breeding Cycle: Behaviors, Composition and Formation of Crop Milk, and Physiological Adaptation. *Life* 13, 1866.

Xie, P., Wan, X., Bu, Z., Diao, E., Gong, D., Zou, X., 2018. Changes in hormone profiles, growth factors, and mRNA expression of the related receptors in crop tissue, relative organ weight, and serum biochemical parameters in the domestic pigeon (*Columba livia*) during incubation and chick-rearing periods under artificial farming conditions. *Poult. Sci.* 97, 2189-2202.

Xie, P., Wang, X.-p., Bu, Z., Zou, X.-t. 2017. Differential expression of fatty acid transporters and fatty acid synthesis-related genes in crop tissues of male and female pigeons (*Columba livia domestica*) during incubation and chick rearing. *Br. Poult. Sci.* 58, 594-602.

Xie, P., Wang, Y., Wang, C., Yuan, C., Zou, X., 2013. Effect of different fat sources in parental diets on growth performance, villus morphology, digestive enzymes and colorectal microbiota in pigeon squabs. *Arch. Anim. Nutr.* 67, 147-160.

Xu, Q., Li, H., Zhou, W., Zou, X., Dong, X., 2020. Age-related changes in serum lipid levels, hepatic morphology, antioxidant status, lipid metabolism related gene expression and enzyme activities of domestic pigeon squabs (*Columba livia*). *Animals* 10, 1121.

Yan, B., Ton, G., 2017. Organizing effects of adverse early life condition on body mass, compensatory growth and reproduction: experimental studies in rock pigeons. *J. Avian Biol.* 48.

Zhang, S., Zhang, Y., Mo, W., Yang, M., Huang, W., Gao, H., Peng, J., 2023. Metabolomics analysis of the effects of different litter size on reproductive metabolism and oxidative stress in breeding pigeon (*Columba livia*). *Heliyon* 9, e14491.