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Original Research Article

EGG WEIGHT AFFECTS HATCHING RESULTS, BODY WEIGHT AND FEAR-RELATED BEHAVIOR IN JAPANESE QUAILS

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Abstract: This study was conducted to determine effect of egg weight on hatching results, chick performance and tonic immobility duration in Japanese Quails. Eggs were weighed individually and divided by weight into four groups: group 1 = 11.0 - 11.9 g, group 2 = 12.0 - 12.9 g, group 3 = 13.0 - 13.9 g and group 4 = 14.0 - 14.9 g. Egg weight loss during incubation and hatching parameters were recorded. Additionally, hatching weight and body weight were measured until 5 weeks and tonic immobility (TI) test was performed at the end of the experiment. Results revealed that egg weight loss during incubation decreased as egg weight increased. Group 2 was determined as the best suitable egg weight group in terms of fertility and group 2 and 3 in terms of hatchability of incubated eggs and hatchability of fertile eggs. Body weight increased as the egg weight increased and the heaviest chicks were hatched from heaviest egg group. Significant differences in TI duration between birds were noticed, as higher body weight birds had longer TI duration than birds with lower body weight. Thus, birds of high body weight were more fearful than birds of low body weight. It was concluded that the medium to heavy weight eggs are better eggs for hatching results and chick weight. Therefore, we recommend the selection of medium and heavy weight eggs for hatching implementations in Japanese quail.

Key words: body weight; egg weight; fertility; hatchability; tonic immobility

Introduction

Poultry production is the main source of household nutrition and income in the developing countries. Quail meat and eggs are considered a good source of animal protein that is very low in fat and cholesterol as compared to eggs of all other poultry species, which makes it the choice of people who suffer from high blood pressure (1). Egg weight is the most important factor affecting hatch weight in Japanese quail (2) and this is due to the positive correlation between the two traits (3, 4). Additionally, there are strong positive correlations, between poultry egg weights and hatching results (hatchability, embryonic mortality), hatching weights and subsequent performance of chicks (5-8). Egg weight also affects growth performance including the slaughter weight (9, 10). Thus, egg size is an important factor affecting homogeneity and uniformity of the flock.

Egg weight in Japanese quail ranges from 8.31 to 13.00g, as reported by Havenstein et al. (11). Hatching weight of Japanese quail chicks' increases with increased in egg weight. For example, the hatching weight of chicks originating from egg weight groups of 11.0 - 11.9, 12.0 -12.9 and 13.0 -13.9 g were as 6.98, 7.56 and 8.39 g, respectively (12). Other parameters impacted by egg weight include egg weight loss on 18th day

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of incubation, hatchability, and 7 days body weight in broilers (13). Seker et al (14) showed that highest levels of fertility and hatchability of incubated egg and hatchability of fertile egg were found in egg weight group of 10.5-11.5 g, followed by 11.51-12.50 g and then 9.50-10.50 g group. Furthermore, they found that increased egg weight was associated with significant increases in chick weight. Another study demonstrated that the least egg weight loss occurred in large egg size groups at different incubation periods with better fertility and hatchability traits recorded for medium egg weight and the heaviest chick weight was attained from large egg size of broiler breeder hens (15).

Since increases in egg weight positively correlate with higher chick weights, it has direct impact in enhancing all productive performances of chicks. Although different egg weight results in birds with different body weights, the criteria of how high or low body weight quails respond to different stressors is not well characterized. So we tried to investigate the relationship between body weight and fear related behavior of Japanese quails. Fear is widely regarded as a potent stressor that causes damaging effects on a number of performance indicators in poultry. There is mounting evidence for a positive association between fearfulness and adrenocortical activation in birds (16). Fearfulness is multidimensional trait that can be defined as a psychological profile resulting in an individual's consistent reactivity to fear-eliciting situation (17). Indeed, there are many studies showed that fear and fearfulness are associated with some performance indicators such as body weight and growth. Extreme or inappropriate expression of fear-related-behavior has negative effects on productivity and welfare in the domestic fowl (18). Reduction of fearfulness levels improves the bird's economic performance and ability for adaptation to environmental changes (19).

There are many tests to measure fearfulness level for birds. The test used in present study is tonic immobility test (TI). Many invertebrate and vertebrate animals, upon release from brief physical restraint, do not attempt to escape but instead, remain in an immobile state characterized by relative muscular hyper tonicity, intermittent eye closure, and depressed heart rate (20). This phenomenon has been labeled variously as death feigning, animal hypnosis and more recently TI. Fear can be assessed by measuring the duration of TI reaction (21). TI is a variable period of immobility induced by manual restraint. A long duration of TI is indicative of high levels of fearfulness, and a short duration is indicative of low levels of fearfulness (22).

In Japanese quail, fear-related behavior studied for lines divergently long term selected for high or low body weight at four weeks of age. They found that low body weight quail line showed greater avoidance of conspicuous novel objects placed near the home cage and longer tonic immobility fear reactions, which included vocalizing and struggling later and less often during mechanical restraint than did the control quail and those with high body weight (23). Generally, low body weight quails showed intermediate responses and greater plasma corticosterone concentrations following mechanical restraint when compared to control non selected line or high body weight groups. Mills et al. (24) demonstrated that lines of Japanese quail which have been divergently selected for plasma corticosterone levels in response to restraint reared in crush cage showed marked differences in their fear responses. Moreover, birds' lines selected for high response to restraint in relation to plasma corticosterone levels show longer duration of the tonic immobility reaction than did lines selected for low response to restraint.

Therefore, the objectives of the present study were to determine 1) the effects of egg weight on egg weight loss during incubation, hatching results and subsequent Japanese quail performance (body weight), and 2) the relationship between body weight and fear related behavior as measured by the duration of TI reaction.

Materials and methods

The experiment was carried out at department of Animal Husbandry and Animal Wealth Development, Faculty of Veterinary medicine, Alexandria University, Egypt. A total of 1200 fertile eggs of (non-genetically selected) Japanese quails (*Coturnix coturnix japonica*) were purchased from a private farm. Eggs were weighed individually and divided by weight into four groups (300 eggs per group): group 1= 11.0 - 11.9 g, group 2 = 12.0 - 12.9 g, group 3 = 13.0 - 13.9 g and group 4= 14.0 - 14.9 g. Eggs were fumigated and incubated vertically with broad end up in the setting trays in standard incubator at 37.5˚C (dry bulb temperature), with 65% relative humidity. Eggs were turned automatically with turning angle ± 45 degree from vertical position eight times daily using automatic timer. On day 15 of incubation, the eggs were transferred to the hatchery at 36.5˚C (dry bulb temperature), with 75-80% relative humidity. At the time of hatch (18^{th} day of incubation), chicks were wing banded from each group and randomly distributed in rearing rooms. The chicks were brooded at a temperature of 35˚C using automatic gas heaters as a source of heat. Temperature was gradually reduced weekly by 3˚C until room temperature reached 24˚C at the fifth week. Chicks were fed *ad libitum* commercial starter diets containing 22% crude protein and 3100 kcal of metabolizable energy meeting requirements (25). Chicks were allowed free access to water as well. Lighting regime was 24 hours (2.5 foot candle) from first day until day seven, and then reduced to 8 hours (0.5 foot candle) until end of the experiment.

Parameters:

Egg Weight Loss during Incubation

Eggs (60 eggs/ group) were weighed just prior to setting into incubator. At 5, 10 and 15 days of incubation eggs were removed from incubator and weighed using an electronic scale to determine average egg weight and egg weight loss percentages. Weight loss (%) = [(egg weight at setting – egg weight at different days of incubation)/ egg weight at setting].

Hatching Parameters

At the end of the incubation period, the eggs having no chick release (Unhatched) were cracked to determine the fertility, and hatchability of incubated eggs and the hatchability of fertile eggs. Calculations were made as follows:

Fertility (%) = (number of fertilized eggs / total numbers eggs placed into incubator) x 100.

Hatchability of incubated eggs (%) = (number of released chicks / total number of egg placed into incubator) x 100.

Hatchability of fertile eggs (%) = (number of released chicks / number of fertilized eggs placed into incubator) x 100.

Body Weight

A total number of 600 chicks were used with 150 bird/ group. Hatched chicks were wing-banded and weighed at hatch. Body weight was recorded every week until 5^{th} week. At age of three weeks, the sex of each bird was determined and recorded.

Tonic Immobility Test (TI):

A sample of 16 random mixed sex birds with sex ratio 1:1 (8 males and 8 females) from each group with total number 64 birds was induced into tonic immobility at 5 week of age. Each bird was tested individually and once in a separate room. TI was induced by inverting the birds and restraining it for 15 s on a table on their back, raising their necks by a towel warped underneath; their head was dropped down from the edge of the table. One hand rested on the sternum while the other lightly cupped the head. The time elapsed till the bird raised up again and number of inductions did by the bird for rising was recorded using stop watch to record latencies until the bird righted itself. If TI could not be induced after 5 consecutive attempts, the bird was considered to be non-susceptible. If the bird failed to right itself after 10 minutes, a maximum score of 600 s was given.

Statistical Analysis:

Data for egg weight loss, fertility and hatchability were analyzed using one way ANOVA while, data on body weight, tonic immobility test were analyzed by Two-way ANOVA. In case of significant differences (P<0.05), means were compared by Duncan's test using SAS statistical package (26). Statistical model for two way ANOVA: $X_{ijk}=\mu + A_{i} + B_{j} + (AB)_{ij} + e_{ijk}$

Where: X_{ijk} = Value of i^{th} observation (body weight, tonic immobility test) of the i^{th} group; μ = Overall mean; Ai = Effect of i^{th} group (different egg weight group); B_j = Effect of j^{th} sex of birds; (AB) ij = Effect of interaction between i^{th} egg weight and the j^{th} sex of birds; e_{ijk} = random error.

Table 1: Effect of Japanese quail egg weight on egg weight loss during incubation period

Group of Eggs weight	Egg weight at time of incubation (g)	Egg weight loss (%) at different incubation periods *			
		0-5 days	5-10 days	10-15 days	0-15 days
G1 (11.0-11.9g)	11.50± 0.30[d]	2.97±0.73[ab]	3.61±1.23[a]	2.80±1.10	9.37±1.73[a]
G2 (12.0-12.9 g)	12.46±0.24[c]	3.35±1.40[a]	2.74±0.81[b]	3.10±1.24	9.19±2.16[a]
G3 (13.0-13.9 g)	13.39±0.34[b]	3.14±1.05[a]	2.55±1.21[b]	2.94±1.16	8.63±1.92[ab]
G4 (14.0-14.9 g)	14.39±0.36[a]	2.60±0.87[b]	2.74±1.25[b]	2.86±1.08	8.20±2.50[b]
P value	<.0001	0.0068	<.0001	0.6273	0.0301

Means bearing different letters within the same column are significantly different (P<0.05). Data were shown as mean ±SD.*Egg weight loss (%) = [(egg weight at setting – egg weight at different days of incubation)/ egg weight at setting

Table 2: Effect of Japanese quail egg weight on hatching result

Group of Eggs Weight	Fertility (%)[1]	Hatchability of incubated eggs (%)[2]	Hatchability of fertile eggs (%)[3]
G1 (11.0-11.9 g)	87.22 ± 4.24[c]	63.76 ± 14.77[b]	71.91 ± 12.13
G2 (12.0-12.9 g)	96.11 ± 1.78[a]	77.77 ± 7.87[a]	80.89 ± 8.81
G3 (13.0-13.9 g)	91.91 ± 1.67[b]	74.76 ± 4.75[a]	80.60 ± 6.19
G4 (14.0-14.9 g)	93.00 ± 2.47[b]	71.72 ± 6.96[ab]	76.81 ± 7.49
P value	<.0001	0.0212	0.1363

Means bearing different letters within the same column are significantly different (P<0.05). Data were shown as mean ±SD. [1]Number of fertile eggs/ number of eggs set × 100. [2]Number of hatched chicks /number of total eggs set × 100. [3]Number of hatched chicks /number of fertile eggs set × 100

Table 3: Means ±SD of Japanese quail chick's body weight as affected by eggs weight and sex of the bird

Group of Eggs Weight	Sex	Weight (g)/ age					
		Hatch Weight	Week 1	Week 2	Week 3	Week 4	Week 5
G1 (11.0-11.9 g)	F	8.70±0.54[a]	19.12±2.38	39.59±4.70	92.60±7.24[a]	140.45±17.45[a]	183.64±18.94[a]
	M	8.53±0.37[b]	18.57±2.59	39.44±4.75	80.19±7.66[b]	131.23±10.46[b]	161.23±18.46[b]
Overall		8.58 ±0.43[D]	18.73 ± 0.53[D]	39.48 ± 4.72[D]	83.83 ± 9.41[D]	133.93 ±13.52[C]	167.80 ± 21.18[C]
G2 (12.0-12.9 g)	F	9.24±0.39	20.25±2.50	43.06±4.81	88.81±11.53	136.97±15.65	178.88±19.20[a]
	M	9.25±0.30	20.00±2.46	41.94±6.02	85.60±10.94	140.72±10.65	173.31±11.54[b]
Overall		9.24 ±0.34[C]	20.11 ± 2.47[C]	42.44 ± 5.52[C]	87.04 ±11.28[C]	139.05 ± 13.21[B]	175.33 ± 16.40[B]
G3 (13.0-13.9 g)	F	10.01±0.46	21.43±3.01	44.03±7.10	89.82±14.04	138.62±17.97	178.59±22.62
	M	10.00±0.45	21.00±2.45	44.75±6.49	92.33±11.09	140.22±15.00	178.36±17.43
Overall		10.01 0.45[B]	21.27 ± 2.81[B]	44.31 ± 6.86[B]	90.79 ±13.00[B]	139.24 ± 16.85[B]	179.33 ± 33.60[B]
G4 (14.0-14.9 g)	F	10.62±0.46[a]	23.85±2.69 [a]	48.81±5.73 [a]	95.90±12.42 [a]	150.25±18.72 [a]	191.78±18.56 [a]
	M	10.40±0.71[b]	22.85±2.14 [b]	46.60±4.59 [b]	88.90±12.38 [b]	137.72±18.79 [b]	177.07±13.52 [b]
Overall		10.55 0.55[A]	23.55 ± 2.57[A]	48.13 ± 5.48[A]	93.75 ± 2.78[A]	146.41 ± 19.56[A]	186.07 ± 20.82[A]

Means bearing different letters within the same column are significantly different (P<0.05). Data were shown as mean ±SD

Table 4: Effect of eggs weight and sex of the bird on Tonic immobility test (TI) reaction of Japanese quail

Group of Eggs Weight	Sex	No. of inductions	Duration till self-righting (s)
G1 (11.0-11.9 g)	F	1.10+0.15	31.57±1.29[a]
	M	1.11 ± 0.17	26.17 ± 2.07 [b]
Overall		1.11 ± 0.10	33.58 ± 2.84[C]
G2 (12.0-12.9 g)	F	1.15+0.13	48.52±3.97[a]
	M	1.17 ± 0.01	44.37±2.37 [b]
Overall		1.18 ± 0.13	47.07 ± 3.28[B]
G3 (13.0-13.9 g)	F	1.20± 0.19	53.45±4.55[a]
	M	1.24± 0.04	46.02±2.54[b]
Overall		1.24 ± 0.11	51.95 ± 4.21[B]
G4 (14.0-14.9 g)	F	1.27± 0.06	65.54±3.93[a]
	M	1.32± 0.18	57.34±4.82[b]
Overall		1.32 ± 0.18	67.25 ± 6.52[A]

Means bearing different letters within the same column are significantly different (P<0.05). Data were shown as mean ±SD. No = number, s = seconds

Results

Data on egg weight losses during incubation period among different egg weight groups of Japanese quail are presented in Table 1. Significant differences were found during first five days of incubation (P = 0.0068) and from 5th to 10th day of incubation (P<.0001) in egg weight loss of different egg weight groups. The weight loss from 10th to 15th day of incubation was not significant (P = 0.6273). However, the overall percentage of egg weight loss for entire period of incubation was significantly (P = 0.0301) different among egg weight groups.

Data on effects of egg weight on hatching results are presented in Table 2. The effect of egg weight on fertility (P < 0.0001) and hatchability of incubated eggs (P = 0.0212) were significant. However, the hatchability of fertile egg (P = 0.1363) was not significant.

Average body weight within different egg weight groups are presented in Table 3. The effects of egg weight on quail hatching weight, one week, two weeks, three weeks, four weeks, and five weeks body weights were significant (P<.0001). However, the effect of sex on body weight for group 1 was significant at hatch weight, 3rd, 4th and 5th week of age with females being heavier than males. In Group 2, there was no difference in body weight between males and females. In group 3, significant difference in body weight between male and females was observed at age of 5th week only. In group 4, significant difference between males and females was observed from hatch till 5th week with females being heavier than males.

In Table 4, results of effect of egg weight on (TI) are presented. Birds with higher body weight (Group 4) showed more fearfulness than birds with lower body weight in (Group 1) as it need longer TI duration for bird's up-righting. With increased bird weight, TI duration increased as in Groups 2 and 3 (vs Group 1). While, there was no significant difference in number of inductions required to straight between groups. Moreover, sex significantly affects TI duration as it was higher in females than in males in all groups.

Discussion

Percentage of weight loss decreased as egg weight increased. The lowest percentage of weight loss in large eggs can be attributed to the greater percentage of albumen content in large eggs. Egg weight loss that occurs during incubation of eggs is due to water diffusion through the shell (27). Our observation in Japanese quail is similar to observations in Cobb 500 broiler breeder hen (28). Deeming (29) determined that 10–12% weight loss is necessary during incubation in order to get a good incubation result in stored and non-stored eggs.

In the current study, the higher percentage of egg weight loss during the entire period of incubation was recorded for group 1 and 2 (9.37% and 9.19%, respectively, followed by 8.63 and 8.20

% in group 3 and 4 that is lower than the value of 12.94% for quail eggs (30). It is also lower than the values of 24.76% and 20.90% in quail eggs (31, 32). Egg weight loss is an important parameter for incubation and it has been used to estimate vital gas exchange (33). It is also correlated with embryo metabolism and development rates (34). Gonzalez et al. (35) noted that increased egg weight loss is positively correlated with increased egg weight. They attributed the increased loss to surface area of the egg and high demand for energy needed for the embryonic development. It was reported that for every gram of fat burned from the stored fat in the yolk, an almost equal mass of metabolic water is generated.

The higher fertility percentage in this study was observed in group 2 (96.11%) followed by group 4 (93%), group 3 (91.91%) and group 1 (87.22%). The same trend was noticed in hatchability, where the maximum hatchability was recorded in group 2 and 3 followed by group 4 and the lower percentage was recorded in group 1. This finding is similar to that of Rashid et al. (36) who reported that percentage hatchability of medium-sized eggs was higher than those in large sized eggs in chickens. Similarly, Iqbal et al. (15) reported that better fertility and hatching traits were attained in medium egg size in broiler breeder.

In the present study, maximum fertility and hatchability of Japanese quail egg were observed in group 2 (12.0-12.9 g) and heavier group while the minimum values were recorded in group 1 (11.0-11.9 g). This was in agreement with Taskin et al. (37) reported that fertility percentage was affected by quail egg weight categories; the highest value (91%) was recorded for heaviest group (>13.00g). Another study showed that the highest fertility and hatchability of incubated eggs of Japanese quails was observed with the eggs in the weight of 11.6g and greater (38). They also found the highest hatchability of fertile eggs with lighter eggs in the weight of 10.6-11.5g. They determined the lowest level of fertility, hatching and hatchability of fertile eggs in eggs that were in the weight of 9.5g and lower.

With regard to body weight of Japanese quail chicks, significantly higher body weight was recorded for the heavier egg group and this trend continued from hatch weight till 5th week body weight. Our findings are in agreement with Dudusola (39) who reported that the chick weight of Japanese quail increased significantly as a result of the increasing egg size. Similarly, Abiola et al. (40) reported that small chicks were hatched from small eggs while large chicks were hatched from large eggs in broiler breeder. The improved growth performance of chicks from heavier eggs may be attributed to the mass of the residual yolk sac that the chick retains at hatching (41, 42, 43 and 44).

Regarding the effect of sex, our data showed that there was a significant difference between males and females in three groups (1,3 and 4) at 5th week of age. Similar findings documented that females of Japanese quails were heavier than males (2 ,45, 46 and 47). The differences in body weight between males and females may attributed to the fact that females grow faster and yield larger muscles and more abdominal fat than males at the same age (48). Subtle differences in the biology of the two sexes, such as the functions of hormonal and regulatory systems may also differentially impact the growth and development of the two sexes at early ages as it is the case in most species of animals (49).

Fear is considered a strong emotion that exerts a huge inhibition on behavioral patterns generated by all other motivational systems (50, 51). Generally, increasing fear is characterized by increased withdrawal, silence and inactivity (52). Longer tonic immobility reactions and more pronounced silence and inactivity are all due to elevated levels of fearfulness (53, 54 and 55).

In present study, higher body weight quails showed longer tonic immobility duration a trait that is associated with high level of fearfulness (56). This was manifested by longer time for self-righting than in birds with lower body weight. Recoquillay et al. (57) reported that the duration of tonic immobility was positively correlated with weight at 17 and 65 days of age (R = 0.76 and 0.79, respectively) in Japanese quail resulted from second generation crossing of two lines divergently selected for their social reinstatement behavior. In contrast, others observed that fearfulness is higher in quails with lower body weight bird than in higher body weight at 4th week of age (58, 59). Jones et al. (23) also found that low body weight quail lines showed greater avoidance of conspicuous novel objects and were more fearful than quails with high body weight. On the other hand, some studies reported that there is no relationship between body weight and TI duration as body mass did not significantly affect TI duration in Japanese quails (60).

The development of tonic immobility response varies between the sexes. TI duration was longer in females than in males indicating that female quails are more fearful than males. A similar finding was supported by Pittet et al. (61) who stated that female is more fearful than males in quails. Since females have higher body weight than males, this could be responsible for the prolonged TI duration. This trait could also be attributed to estrogen hormone in females while in males, the presence of more activity and reduced fearfulness may attributed to testosterone hormone as evidenced by reduced fearfulness of Japanese quail's chicks produced following injection of testosterone in to the yolk (62).

Conclusion

We found that Group 2 (12-12.9g) was as the most suitable egg weight group in terms of fertility and the group 2 and 3 (13-13.9g) in terms of hatchability of incubated eggs and hatchability of fertile eggs. The heaviest chicks were obtained from the heaviest eggs (weight of 14.0-14.9g). Moreover, birds with higher body weight had longer TI duration and were more fearful than birds with lower body weight. It is concluded that medium to heavy weight eggs are associated with better hatching outcomes and chick growth. Therefore, we recommend the selection of medium and heavy weight eggs for hatching implementations in Japanese quails.

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TEŽA JAJC VPLIVA NA NESNOST, TELESNO TEŽO TER VEDENJE POVEZANO S STRAHOM PRI JAPONSKIH PREPELICAH

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Povzetek: Študija je bila izvedena z namenom določanja vpliva teže jajc na rezultate izvalitve, uspešnost piščancev in trajanje tonične negibnosti pri japonskih prepelicah. Jajca smo posamezno stehtali in jih po teži razdelili v štiri skupine: skupina 1 = 11,0 - 11,9 g, skupina 2 = 12,0 - 12,9 g, skupina 3 = 13,0 - 13,9 g in skupina 4 = 14,0 - 14,9 g. V času valjenja so bile zabeležene izgube teže jajc ter parametri valjenja. Izmerili smo tudi težo valilne mase in telesno maso do 5 tednov starosti, na koncu poskusa pa izvedli test tonične negibnosti (TI). Rezultati so pokazali, da se je izguba teže jajca med inkubacijo zmanjševala s povečanjem teže jajc. Skupina 2 je bila kot najbolj primerna skupina jajc glede plodnosti, skupini 2 in 3 pa glede na valilnost inkubiranih jajc in valilnost plodnih jajc. Telesna teža se je povečevala, ko se je povečala teža jajc in najtežji piščanci so se izvalili iz najtežje skupine jajc. Opažene so bile pomembne razlike v trajanju TI med pticami, saj so imele ptice z višjo telesno težo daljše trajanje TI kot ptice z nižjo telesno težo. Tako je bilo ptice z veliko telesno težo bolj strah kot ptice z nizko telesno težo. Ugotovljeno je bilo, da so jajca srednje do višje teže boljša jajca glede valjenja in teže piščancev. Zato priporočamo izbiro jajc srednje in višje teže za valjenje japonskih prepelic.

Ključne besede: telesna teža; teža jajc; plodnost; valilnost; tonična negibnost