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Individual cage housing affects feed intake and induces sex-specific effects on body weight in Japanese quails

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SUMMARY

Individual cage housing in poultry production could be a potentially stressful environment for the birds that can alter feed intake, consequently induce negative effects on performance. Previous studies used individual bird-based experiments to extract the detailed molecular, physiological, and fitness outcomes of treatments. Understanding sex-specific effects of isolation on social birds such as Japanese quails (Coturnix japonica) may reveal important considerations. To understand this phenomenon, birds were kept in groups of 10 for one week and they were kept in individual cages for another week. Daily feed intake (DFI) was measured each day and body weight was measured at the beginning, middle, and end of group rearing and at the beginning and end of individual rearing. It was found that both males and females showed a reduction DFI in response to individual rearing ($p<0.0001$). Females consumed on average 29.38% higher amount of feed than males. Additionally, females showed a pronounced reduction in body weight after isolation, while the effect on males was not significant. Similarly, females had on average 17.61% higher body weight than males. The body weight to DFI ratio was higher in males than females. The finding of this research revealed an important implication of isolation and sex differences.

Keywords: *Daily feed intake; group rearing; individual rearing; sex difference; Coturnix japonica*

INTRODUCTION

Isolation in poultry farms has been identified as a significant welfare concern (Health et al., 2023). Poultry may respond negatively to social isolation with increased stress, increased or decreased feed consumption, and impaired growth or reproduction (Feltenstein et al., 2002). Japanese quails are naturally social birds that often live in groups and on farms are kept in flocks (Guzmán et al., 2021; Schweitzer and Arnould, 2010). Pair formation is a typical trait of the social behavior of domestic quails, where males show a relatively close relationship with a preferred female during reproduction season, though they can copulate with multiple females (Schmid and Wechsler, 1997). A study on high and low-density groups of conspecific quails (*Coturnix japonica*) revealed that birds are better suited for rearing in high-density conditions. This would involve a trade-off among several behavioral characteristics such as head injuries due to aggressive pecking and head-banging, fearfulness, and territoriality, among others (Guzmán et al., 2013). When quails are separated from their group, they express increased fearfulness and stress, while becoming less active and more responsive to environmental stimuli (Mills et al., 1993). Similarly, social isolation in broiler chickens results in reduced activity, intake and feed conversion ratio (Ogbonna et al., 2022). This can affect the overall growth and reproduction explained by reduced intake, vocalizations, egg production and body weight in both female and male birds.

The response to social isolation could also have a sex-specific effect on organisms. A study on chickens kept in naturalist conditions reported that social isolation is sex dependent: females showed more reactive behavioral responses than males (Weldon et al., 2016). Japanese quails are sexually dimorphic birds, where females have higher feed intake and heavier body weight (Balthazart et al., 1983; Reda et al., under review; Sezer et al., 2006). In the first three weeks post hatching, both female and male quails grow at a similar pace. However, males reach sexual maturation one to two weeks earlier than females and then sharply reduce growth to the minimum level, while females continued growing and reach sexual maturity at the age of six to eight weeks (Du Preez and Sales, 1997; Rezvannejad et al., 2013; Sezer et al., 2006; Sezer and Tarhan, 2005). Behaviorally, Japanese quails show distinct differences between males and females; males show aggressive and fearful behavior, while females are calmer (Balthazart et al., 1983; Chang et al., 2009). The response to social isolation could also have sex-specific effect on Japanese quails.

Therefore, this study aims to explore the sex-specific effects of individual cage rearing on feed intake and body weight of Japanese quails. The results may be applicable in determining the necessary acclimation period for individual cage-based experiments and could provide insights into how isolation may affect the efficiency of nutritional allocation in male and female quails. It is hypothesizing that isolation leads to a decrease in both

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feed intake and body weight among birds. Additionally, it is anticipating that the effect is more pronounced in females compared to males.

MATERIALS AND METHODS

The study received ethical approval from the University of Debrecen, Hungary's Ethical Committee for Animal Use, under Protocol No. 5/2021/DEMAB, and adhered to both institutional and national regulations.

Animals, housing and feeding

Four-weeks-old Japanese quail chicks were obtained from Budai Fürjészet, a quail breeder in Hungary and housed at the Animal House of the Institute of Animal Science, Biotechnology, and Nature Conservation of the University of Debrecen (Hungary). Chicks were reared for an additional two weeks in four grower cages (18.5 cm in length × 21 cm in width × 18.5 cm in height) at a density of 25 quails/cage, with males and females caged together. At 42 days (week 6) of age, all birds were relocated to 20 breeder cages (18.5 cm in length × 21 cm in width × 18.5 cm in height). The birds were housed in groups of 10 birds at a density of 38.85 m^2 per bird, with males and females separated, and were provided *ad libitum* feed without any measurements taken. The experimental room was kept at a temperature of 24±3 °C, with a relative humidity ranging between 60% and 75%, and following a daily photoperiod cycle of 12 hours of light and 12 hours of darkness.

At the age of 49 days, daily feed intake measurements started for the group-reared birds, continuing until they reached 56 days of age. Individual daily feed intake (DFI) was calculated as the difference between the amount of feed offered and the feed remaining, divided by the number of birds in the group. Feeders were modified to minimize spillage. At the age of 56 days, 64 birds were selected with similar sex-specific body weight (using a median ± 3.9% for females and a median ± 4% for males) and housed in individual cages for 7 days. Individual DFI was calculated every morning as soon as the experimental room was opened and the lights were turned on. Feed was offered *ad libitum* at a rate of 50 g per bird every morning. Body weight was measured at the ages of 49 days, 52 days, 56 days, and 62 days using a digital balance to 0.1 g accuracy. Birds received a standardized breeder quail ration formulated on a corn, soybean, and wheat basis. Other supplementary nutrients such as DL-methionine, sunflower oil, limestone, monocalcium phosphate (MCP), salt, and vitamin and mineral premix were added based on the nutrient requirements of quails (NRC, 1994).

Statistical analysis

All data were analyzed using R v. 4.2.2 "Innocent and Trusting" (R Core Team, 2022). To analyze the effect of isolation and sex on DFI across the two-week period, generalized linear-quadratic mixed-effect models was fitted incorporating the quadratic time pattern of DFI. The model was fitted to capture nonlinear trends across days (Zhang et al., 2022). Days birds reared in group or individual cages, sex and their interaction were considered fixed factors, while cage number of groups and birds' identity were considered random factors. A linear mixed-effect model was used to analyze how isolation and sex affected body weight and the body weight to intake ratio across days. The 'lme4' package (Bates et al., 2015) was used for model fitting and employed version 3.1-3 of the 'lmerTest' package (Kuznetsova et al., 2017) to calculate Satterthwaite's denominator degrees of freedom and associated p-values. Statistical significances were analyzed using three-way ANOVA. For comparing means, Tukey post hoc test was employed at the $p < 0.05$ significance level, and means were represented with bars set at ± SEM.

RESULTS AND DISCUSSION

Effect of Isolation on feed intake in Japanese quails

Sex and isolation period affected daily feed intake (DFI), while their interaction was not significant (sex: $F_{1,62.56} = 132.91$, $p < 0.0001$, poly(day, 2): $F_{2,355.48} = 27.45$, $p < 0.0001$, sex × poly(day, 2): $F_{2,355.48} = 0.02$, $p = 0.985$). At all-time points, females consumed (on average, 29.38%) more feed compared to males (*Figure 1*), which aligns with findings from other studies (Abou-Kassem et al., 2019; Kaya Başar and Narinç, 2023). Domestic Japanese quails are continuous egg-laying birds if their eggs are removed daily (Lukanov et al., 2018). They are larger and invest a significant amount of resources into egg production and quality, which could be a reason to consume more feed than males (Retes et al., 2019). Furthermore, isolation imposed a significant effect on DFI in both male and female groups. The first day after isolation, birds sharply reduced their intake, while they started to increase it after one day. However, they do not reach the level of intake they consumed during group rearing until the 7th day of the acclimation period. Their average consumption was approximately 12.66% lower (females: 12.64%, males: 12.69% lower). This indicates that social isolation could permanently affect intake, or birds may need more days to acclimate into the individual cage system. Contrary to our study, broiler chickens showed increased feed intake in response to two weeks of frequent daily isolation (Ogbonna et al., 2022).

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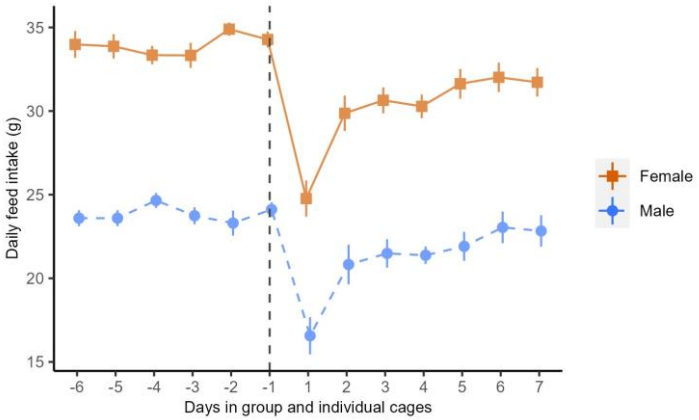
Figure 1. **Daily feed intake of Japanese quails during group and individual rearing conditions**



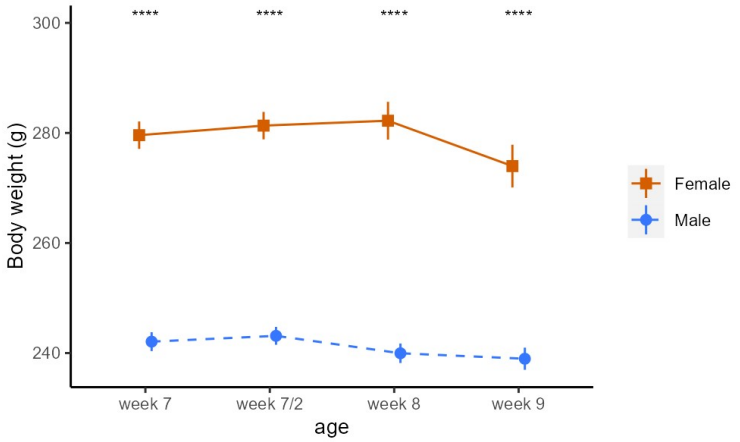
Data were collected on 1 to 6 days before the beginning of isolation (negative scales represent group rearing), and day 1 to 7 after isolation (positive scales). The left from the dashed vertical line indicates the group raring period while the right from the dashed vertical line indicates individual rearing periods.

Body weight is affected by sex and isolation

Body weight was affected by sex and isolation (sex: $F_{1,61.99} = 193.13$, $p < 0.0001$, age: $F_{3,186.52} = 4.48$, $p < 0.0001$, sex × age: $F_{3,186.52} = 0.73$, $p = 0.537$). Females scored significantly higher body weight (on average 17.61%) than males at all measured segments of age of the birds (*Figure 2*). Japanese quails are size-dimorphic birds after sexual maturity, where males mature and stop growth earlier than females, while females sexually mature one to two weeks later and show heavier body weight (Rathert et al., 2017; Sezer et al., 2006). The effect of isolation was higher in females than in males. In females, body weight on week 9 was significantly reduced compared to weight on week 8 ($p = 0.018$) and the middle of week 7 ($p = 0.044$). In contrast, isolation had no significant effect on males, indicating that isolation has a severe effect on females (*Figure 3*).

The nutritional costs for males are associated with mounting and attraction efforts, sperm and foam production (a foam discharged by male quails from their foam gland after sexual maturation), fighting and vocalization (Adkins-Regan, 2014; Hiyama et al., 2018; Seiwert and Adkins-Regan, 1998). Most of which are eliminated during individual rearing, consequently reducing the cost of reproduction. In support of our finding, a study on chickens showed that females exhibited higher behavioral and physiological changes than males in response to social isolation (Weldon et al., 2016). On other species, adult female rats also showed a pronounced reduction in body weight gain compared to males in response to isolation stress treatment (Weintraub et al., 2010). Our findings contradict a study conducted on broiler chickens, in which social isolation led to an increase in body weight. This increase was attributed to the reduced activity of the isolated birds, resulting in reduced energy expenditure (Ogbonna et al., 2022).

Figure 2. **Body weight of Japanese quails as affected by sex and isolation**



Females showed significantly higher body weight than males, while isolation significantly affected only females. Data were taken on Week 7, the fourth day after week 7, week 8 and week 9 age of the birds. Asterisks (****) indicates significant difference at $p<0.001$.

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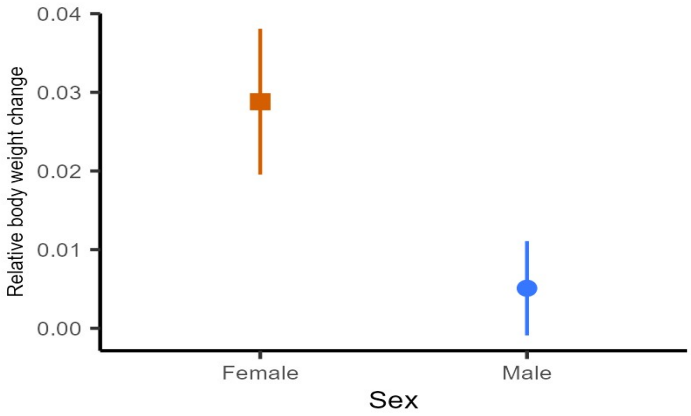
Figure 3. **Change in relative body weight from week 8 to week 9 of isolation period**

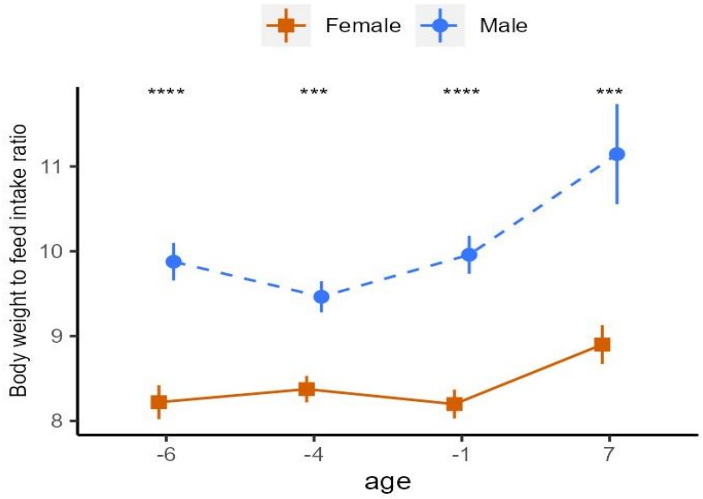


Relative body weght change was calculated as: (individual body weight during week 8 minus individual body weight during week 9) divided by average body weight of week 8 and week 9 of individual birds.

Body weight to intake ratio is higher on males

The body weight to feed intake ratio was calculated to give insights into birds' efficiency in converting feed into body mass. There was significant sexual variation in body weight to feed intake ratio across four time points (sex: $F_{1,120} = 34.08$, $p < 0.0001$, day: $F_{1,120} = 7.73$, $p = 0.004$, sex × day: $F_{1,120} = 4.83$, $p = 0.229$). The body weight relative to feed intake was significantly higher in males than in females at all-time points. The difference is magnified at the isolation period (*Figure 4*). The variation might be attributed to females' higher level of reproductive investment, which may be traded off against their body weight (Marn et al., 2022). Both females and males invest in reaching sexual maturity; however, after maturation, the dynamics change: while males invest in sexual attraction, mounting, and foam production, which is less costly, females invest heavily in both attracting males and egg laying (Horváthová et al., 2012; Marn et al., 2022). The growth and reproduction cumulative feed conversion ratio could definitely be higher in females than males. We did not measure egg parameters during the acclimation period, which will be a limitation of this study. Interestingly, the weight to intake ratio increased when birds were isolated in individual cages in both sexes, despite the fact that body weight and intake were reduced in response to isolation.

Figure 4. **Body weight to feed intake ratio during 6, 4, and 1 days before isolation and 7 days after isolation**



Body weight to feed intake ratio was calculated as: body weight of individual bird divided by the amount of feed that individual bird consumed that day. Asterisks (***) indicates significant difference at p<0.001.

CONCLUSIONS

Overall, isolating birds in individual cages has a significant impact on DFI for both females and males, although the effect is severe at the beginning. However, only females' body weight was significantly affected by isolation, explaining the trade-off for nutrition between body weight and egg production, while males

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under individual cages have less reproduction cost. Females consume a higher amount of feed and show more than 17.61% higher body weight than males. This indicates the side-dimorphic nature of Japanese quails. Under both group and individual rearing conditions, the body weight to DFI ratio was higher in males than in females, which again confirms the notion of a higher reproductive cost for females. Altogether, this study provides relevant output on the importance of acclimation and sex-specific responses to individual cage rearing.

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