Excreta quality and digestive function of singly versus couple caged Sardinian partridges (Alectoris barbara barbara Bonnaterre, 1790) as non-invasive indicators of birds’ coping ability to forced pairing

DOI:10.1111/jpn.13594

(Article begins on next page)

Excreta quality and digestive function of single *vs*. couple caged captive Sardinian partridges (*Alectoris barbara barbara* Bonnaterre, 1790) as valuable indicators of birds' coping ability to permanent forced-pairing

Ahmed F.[1], Pudda, F.[2], Schiavone, A.[3], Varcasia, A.[1], Nery, J.[3], Seidavi, A.[4], Cappai, M.G.[1]

[1]Department of Veterinary Medicine, University of Sassari, via Vienna no. 2, 07100-Sassari, Italy

[2]Rescue Center of the Autonomous Region of Sardinia, FORESTAS, s.s. Olmedo-Sassari, località Tottubella, Italy.

[3]Department of Veterinary Science, University of Turin, Largo Braccini no. CAP, Turin, Italy

[4] Department of Animal Science, Rasht Branch, Islamic Azad University, Rasht, Iran.

ABSTRACT

The Sardinian partridge is a monogamous wild bird and a threatened species of Sardinian fauna, for this reason reared also in captivity. It is well known to bird keepers that the creation of couples is crucial, because forced co-living in cages can lead to female death from male aggression. This study investigated the impact of single *vs*. couple caging of Sardinian partridges on feed intake, excreta composition and nutrient digestibility. A total of 24 couples of breeding partridges (BW, ♀: 384±42.7 g; ♂: 477±33.4) were enrolled for two consequent phases of the investigation (lasting 15 d each). During phase 1, all couples were fed *ad libitum* with a pelleted complete diet (DM, 905 g/kg of diet; CP 225 g/kg; EE 40 g/kg; ash 53 g/kg, as fed). In phase 2, each male from 12 out of 24 couples was moved to an identical cage close to that where the female remained alone. The rest of couples continued like in phase 1. During phase 1 and 2, feed intake and excreta outputs were recorded daily. Pooled excreta of the last three days from couples and single birds were assessed for pH and values of DM, CP and ash. Nutrient digestibility was calculated. No significant differences were noted between single *vs*. couple caging regarding body weight or nutrient digestibility. However, both genders singly housed showed a different feed intake in comparison with birds caged in couple.

INTRODUCTION

Captive game birds are accounted to be an important economic resource of poultry production in different Countries worldwide. In the United Kingdom, for instance, the annual releasing for hunting of ring-necked pheasants (*Phasianus colchicus* Linnaeus, 1758) and red-legged partridges (*Alectoris rufa* Linnaeus, 1758, red-legs hereafter) reaches about 35 million and 6.5 million of specimens, respectively (Raquel Prieto et al., 2018). Rearing of game birds is also carried out for various other purposes, namely for meat production and restocking, as well as for animal biodiversity protection (Dickens & Bentley, 2014). In captivity, breeding birds are randomly assorted to create couples and such practice is used both in partridge and pheasant farming systems. The so called forced-pairing, however, sometimes seems to produce unsatisfactory outcomes. In fact, forced-pairing method often used in commercial partridge breeding farms has been reported to be involved in the increase of agonistic interactions between the male and female in the same cage and a high frequency of 'divorce', where one or both birds become as aggressive as to pose a threat to the survival of each other, more frequently ending with female death (Alonso et al., 2008; Prieto et al., 2012). Typical signs of male aggression over the female range from impaired access to the feeder with consequent reduction or deprivation of feeding sources, to limited movement within the cage and confinement to limited space, often leading to food pad disorders (Cappai et al., 2018), to more severe episodes, like feather pecking or fatal injuries of back and head, often considered as signs of aggressive mating behaviour, out of mating season. The rise in aggression and divorce was also correlated to the size of commercial cages. Alonso and co-workers (2008) pointed out that the bigger the cage the lower the rate of aggressive interactions. This being said, it is noteworthy that partridge females living in larger cages can lay a larger number of eggs if compared to females kept in smaller cages, where less space is available (Gaudioso et al., 2002; Bortolotti et al., 2006). That way, stocking density of bird couples may become an issue too. Extent and effects from stressful co- living of forced-couples in cages is not determined yet (Matheson et al., 2015). Stress in birds involves a cascade of endocrinological and bahavioural pathways (Angelier & Wingfield, 2012). In view of the need to cope with potential discomfort, birds can face the augmented need of readily available energy by the increase of cortisone secretion. As a consequence, the breakdown of muscle protein gives rise to the gluconeogenesis. Plasma glucose levels then increase and concomitantly loose excretory uric acid increase also follows (Puvadolpirod & Thaxton, 2000). This is due to stressing factors of different origin and type, which may also produce systemic effects on a series of other physiological functions and measurable parameters, such as blood pH, electrolyte balance, and nutrient digestibility, together with respective absorption and metabolism, ultimately affecting excreta quality, feed intake and live body weight of animals.

The Sardinian partridge (*Alectoris barbara barbara* Bonnaterre, 1790) is not as known in detail as other bird species and breeds, in spite of its vast geographical distribution and the progressive decreasing number of specimens in nature, for which the Sardinian partridge gained the rare status. Sardinian partridges are monogamous wild birds of the Family of *Phasianidae*, Order of *Galliformes*, mainly found in the Isle of Sardinia, Spain and north and west Africa (Algeria, Morocco and Tunisia) (Cappai et al., 2018). Nurturing of Sardinian partridges in captivity is allowed in accredited private and regional farms due to its rare conservation status (Cappai et al., 2017). It is well known to bird keepers that the creation of couples is crucial, because forced co-living in cages can lead to female death under male aggression. It is a common opinion that

Sardinian partridge is not an exception as to "divorce" or female death, but mortality rate due to force-pairing is not available to date, to the best of our knowledge.

It was hypothesized that different keeping strategies during the non laying period in couples of Sardinian partridges may be useful to explore non invasive measurable parameter (access to feed and digestive function), both in the case of single caged *vs*. coupled birds. For this purpose, this study aimed to evaluate selected indicators like daily feed intake, digestibility of nutrients and quality of excreta for the evaluation of criteria depicting bird adaptation and coping with environment, when single *vs*. couple caging is a factor.

MATERIALS AND METHODS

Housing and Diet

Animal handling complied with the recommendations of European Union Directive 2010/63/EU concerning animal care and further Consolidated Commission Implemented Decisions 2012/707/EU and 2014/11/EU. All practices reported in the investigation are based on conventional good farming practices, and no invasive procedures of animals handling and manipulation were carried out.

A total of 24 couples of adult partridges, with an average body weight (BW) at start of ♀ 384±42.7 g for the females and 477±33.4 g for the males, was enrolled for two consequent phases of the same investigation (lasting 15 days each). During phase 1, all couples were fed *ad libitum* with a pelleted complete diet for partridges and perdix (Table 1).In phase 2, each male from 12 out of 24 couples was moved to an identical cage (30 cm in width x 40 cm in length, with a height of 30 cm) with metal wire flooring (1x2 cm), close to that where the female of the same couple remained alone and continued to be fed with the same identical diet as in phase 1.

Both visual contact and hearing between singly caged birds of the same couple were maintained throughout the trial, in the respect of same environmental conditions. (Figure 1). The rest of couples (no. 12) continued to be kept and fed like in phase 1. Birds were kept in open air with shelter without any artificial ventilation system. Water and feed were offered *ad libitum* During phase 1 and 2 feed intake and amount of excreta produced were recorded on a daily basis, both for singly caged and coupled birds. Feed offer and leftovers were used to calculate feed intake of couples and individual birds. All birds were weighed individually on a digital scale by the end of the trial and clinically inspected. Excreta samples free from debris and foreign substances were collected from a tray placed under each cage. A total of 9 pooled samples per caging system were collected at each sampling time (phase 1 and phase 2) and processed for further laboratory analyses.

2.2-Feed and Excreta analyses

Feed and excreta samples underwent laboratory assessment and different parameters for quality and composition were determined. Pooled excreta samples from all birds (couples *vs*. singly caged female *vs*. singly caged males) during the last three days of phase 1 and phase 2 were analyzed for quality and composition. The quality assessment of excreta consisted of the determination of pH value and moisture content. Immediately after collection, pH value was determined by means of handy pH-meter. For this purpose, one aliquot of each pooled samples was diluted in tubes containing distilled water according to a proportion (g/v) of 1 : 5. Values were determined in duplicated at an environmental temperature of 25 °C. In the lab, the dry matter (DM) of excreta was determined according to AOAC method.

The chemical composition of analyzed nutrients considered of interest for the trial was carried out in feed and excreta to estimate apparent digestibility of crude protein and organic matter.

Samples were therefore processed to determine total nitrogen content according Kieldahl method (AOAC, 1990). Further, total excreted uric acid was determined according to Marquardt (1989) and used for the correction of crude protein of excreta, according to the formula reported by Mansoori and Acamovic (1998), where total uric acid nitrogen expressed in mg is dependent on the total uric acid nitrogen in excreta divided the molecular weight of N (56.7) timed for the molecular mass of uric acid (168.1).

After uric acid correction, crude protein digestibility was calculated. Organic matter digestibility was also calculated following the determination of crude ash, subtracted from one kg DM in feed and excreta.

Statistical Analysis

All data were analyzed following a general linear model procedure (GLM) for the analysis of variance as follows:

$$Y = \mu + D_{j,k} + G_{l,m} + \varepsilon ,$$

where Y is the dependent variable (feed intake, nutrient digestibility, excreta composition), μ is the overall mean, D is the fixed effect of caging system (two level, coupled *vs*. single caging), G is the fixed effect of gender (two levels, females *vs*. male) and ε is the random error. All procedures were carried out using a software package (Minitab statistical software package, Minitab, New York, NY, USA). Effects were studied by pairwise comparison by using the Tukey multiple comparison test. Statistic significance was set for *P*-value <0.05.

RESULTS

Daily intake and body weight changed according to caging system (Table 2).

All animals appeared clinically healthy throughout the trial, during both phase 1 and 2. No significant differences were noted between single *vs*. couple caging regarding body weight and nutrient digestibility. However, singly housed females showed increasing daily feed intake in the first week of single caging and reached a plateau setting at a higher intake than estimated when kept in couple (Figure 2). On the opposite, the singly housed males showed decreasing daily feed intake in the first week of phase 2, to stabilize g in the second week to lower values of daily feed intake estimated when kept in couple. Excreta quality was not affected by caging system.

DISCUSSION

This study aimed to estimate bird behavior during non-laying period in single caged *vs*. coupled Sardinian partridges by evaluating noninvasive parameters from excreta which indirectly shows birds adaptation to deal with stress. Stress depresses immune system and endocrine function which could indirectly impact laying and production(Lara & Rostagno, 2013; Loyau et al., 2014; Farghly et al., 2018a, b), as well as feed conversion ratio, which results in improper digestion and absorption and loose watery droppings (Dunlop et al., 2016). A study conducted Ziaei and co-workers (2007) to evaluate male/female bird differences in retention of DM, CP and feed efficiency pointed out that a higher feed consumption and water intake together with lower DM in excreta was observed in males if compared to females. Such findings are in agreement with what observed in this trial, for which a potential explanation of the different feed intake behaviour between singly caged female and males could be observed. In fact, despite no significant variation could be stated between the caging system as to daily feed intake, in detail, females tended to an immediate increase in feed intake probably because of ease of access to the feeder with no interference from the male. On the contrary, the male tended to decrease the intake of feed in the first days of single caging due to unnecessary predominance on the female attempts to get to the feeder. So, this may be the reason behind the similar outcome between singly housed and couple caged birds. In addition, our results appear similar to those reported by other authors (Alonso et al., 2008) which show how when forced-pairing is adopted, females are frequently attacked by males on the head and back, showing different extents of aggression. This fact is supported by the potential causes of aggression reported in the literature, due in high proportion to competition for feeding/drinking access, space competition, and male undefined behaviour (Craig & Ramos, 1986). Successful breeding relies on appropriate management of social behavior (Swaisgood, 2007), to warrant the welfare of captive game birds. Chronic stress and injuries should be expectedly limited by modulating keeping conditions according to bird behaviour and physiological state, to protect welfare and health of captive game birds (Mourão et al., 2010) like the Sardinian partridge.

CONCLUSION

Caging system of permanent couples of Sardinian partridge plays a pivotal role as a factor to be considered when such captive game birds are kept during the non laying period. In particular, aggressive behaviour due to forced-pairing can seriously affect welfare conditions of birds. This may strongly impact on bird behaviour, kept under chronic stressful conditions in confined space, and in which injuries from aggression may become fatal. Feed intake, excreta quality and digestive function appear to be effective in evaluating the coping ability with caging in birds singly housed, as it demonstrated how in particular feed intake can mirror the free access to the feeder and the expression of feeding behaviour both in the female and the male. In fact, while the female showed to increase the daily feed intake when singly housed, probably due to ease of access to the feeder, the male decreased the daily feed intake, probably because non competing condition for the primary source (feed) with the female is ceased.

References:

Abd El-Wahab, A., Beineke, A., Beyerbach, M., Visscher, C. ., & Kamphues, J. (2011). Effects of Floor Heating and Litter Quality on the Development and Severity of Foot Pad Dermatitis in Young Turkeys. *Avian Diseases Digest*. https://doi.org/10.1637/9802-968411-digest.1

Al-Rawi, B., Craig, J. V., & Adams, A. W. (1976). Agonistic Behavior and Egg Production of Caged Layers: Genetic Strain and Group-Size Effects ,. *Poultry Science*. https://doi.org/10.3382/ps.0550796

Alonso, M. E., Prieto, R., Gaudioso, V. R., Pérez, J. A., Bartolomé, D., & Díez, C. (2008). Influence of the pairing system on the behaviour of farmed red-legged partridge couples (Alectoris rufa). *Applied Animal Behaviour Science*. https://doi.org/10.1016/j.applanim.2008.05.006

Angelier, F., & Wingfield, J. C. (2012). Importance of the glucocorticoid stress response in a changing world: Theory, hypotheses and perspectives. In *General and Comparative Endocrinology*. https://doi.org/10.1016/j.ygcen.2013.05.022

Bortolotti, G. R., Blas, J., Negro, J. J., & Tella, J. L. (2006). A complex plumage pattern as an honest social signal. *Animal Behaviour*. https://doi.org/10.1016/j.anbehav.2006.01.016

Buner, F. D., Browne, S. J., & Aebischer, N. J. (2011). Experimental assessment of release methods for the re-establishment of a red-listed galliform, the grey partridge (Perdix

perdix). *Biological Conservation*. https://doi.org/10.1016/j.biocon.2010.10.017

Cabezas-Díaz, S., Virgós, E., & Villafuerte, R. (2005). Reproductive performance changes with age and laying experience in the Red-legged Partridge Alectoris rufa. *Ibis*. https://doi.org/10.1111/j.1474-919x.2005.00406.x

Cappai, M. G., Abd El-Wahab, A., Arru, G., Muzzeddu, M., & Pinna, W. (2018). Prevalence of foot disorders in captive Sardinian partridges (Alectoris barbara barbara Bonnaterre, 1790) as useful indicators of fitness to natural environment. *Journal of Animal Physiology and Animal Nutrition*. https://doi.org/10.1111/jpn.12847

Cappai, M. G., Arru, G., Manconi, M., Muzzeddu, M., & Pinna, W. (2017). Morphometric traits of gizzard in relation to feeding habits of wild Sardinian partridges (Alectoris barbara barbara, Bonnaterre, 1790) with particular regard to clast selection. *Journal of Animal Physiology and Animal Nutrition*. https://doi.org/10.1111/jpn.12631

Choudhury, S. (1995). Divorce in birds: A review of the hypotheses. *Animal Behaviour*. https://doi.org/10.1006/anbe.1995.0256

Craig, J. V., & Ramos, N. C. (1986). Competitive feeding behavior and social status in multiple-hen cages. *Applied Animal Behaviour Science*. https://doi.org/10.1016/0168-1591(86)90041-9

Dickens, M. J., & Bentley, G. E. (2014). Stress, captivity, and reproduction in a wild bird species. *Hormones and Behavior*. https://doi.org/10.1016/j.yhbeh.2014.09.011

Dunlop, M. W., Moss, A. F., Groves, P. J., Wilkinson, S. J., Stuetz, R. M., & Selle, P. H. (2016). The multidimensional causal factors of “wet litter” in chicken-meat production. In *Science* *of the Total Environment*. https://doi.org/10.1016/j.scitotenv.2016.03.147

Farghly, M. F. A., Alagawany, M., & Abd El-Hack, M. E. (2018). Feeding time can alleviate negative effects of heat stress on performance, meat quality and health status of turkey. *British Poultry Science*. https://doi.org/10.1080/00071668.2017.1413233

Farghly, M. F. A., El-Hack, M. E. A., Alagawany, M., Saadeldin, I. M., & Swelum, A. A. (2018). Wet feed and cold water as heat stress modulators in growing Muscovy ducklings. *Poultry Science*. https://doi.org/10.3382/ps/pey006

Gaudioso, V. R., Alonso, M. E., Robles, R., Garrido, J. A., & Olmedo, J. A. (2002). Effects of housing type and breeding system on the reproductive capacity of the red-legged partridge (Alectoris rufa). *Poultry Science*. https://doi.org/10.1093/ps/81.2.169

Klint, T., & Enquist, M. (1981). Pair formation and reproductive output in domestic pigeons. *Behavioural Processes*. https://doi.org/10.1016/0376-6357(81)90016-4

Krause, J. S., Pérez, J. H., Chmura, H. E., Meddle, S. L., Hunt, K. E., Gough, L., Boelman, N., & Wingfield, J. C. (2018). Weathering the storm: Do arctic blizzards cause repeatable changes in stress physiology and body condition in breeding songbirds? *General and Comparative Endocrinology*. https://doi.org/10.1016/j.ygcen.2018.07.004

Lara, L. J., & Rostagno, M. H. (2013). Impact of heat stress on poultry production. In *Animals*. https://doi.org/10.3390/ani3020356

Li, D., Davis, J. E., Wang, G., Nabi, G., Bishop, V. R., Sun, Y., Meddle, S. L., Wingfield, J. C., & Lei, F. (2020). Coping with extremes: Remarkably blunt adrenocortical responses to acute stress in two sympatric snow finches on the Qinghai-Tibet Plateau during winter relative to other seasons. *General and Comparative Endocrinology*. https://doi.org/10.1016/j.ygcen.2020.113434

Love, A. C., Lovern, M. B., & DuRant, S. E. (2017). Captivity influences immune responses, stress endocrinology, and organ size in house sparrows (Passer domesticus). *General and Comparative Endocrinology*. https://doi.org/10.1016/j.ygcen.2017.07.014

Loyau, T., Bedrani, L., Berri, C., Métayer-Coustard, S., Praud, C., Coustham, V., Mignon-Grasteau, S., Duclos, M. J., Tesseraud, S., Rideau, N., Hennequet-Antier, C., Everaert, N., Yahav, S., & Collin, A. (2014). Cyclic variations in incubation conditions induce adaptive responses to later heat exposure in chickens: A review. *Animal*. https://doi.org/10.1017/S1751731114001931.

Mansoori, B., Acamovic, T. (1998). Real dry matter and nitrogen digestibility: Further correction of true dry matter and nitrogen digestibility of proteins in tube fed birds, using uric acid-corrected nitrogen values, British Poultry Science, 39:sup001, 35-36, DOI: 10.1080/00071669888241

Matheson, S. M., Donbavand, J., Sandilands, V., Pennycott, T., & Turner, S. P. (2015). An ethological approach to determining housing requirements of gamebirds in raised laying units. In *Applied Animal Behaviour Science*. https://doi.org/10.1016/j.applanim.2015.02.001

Mench, J. A., van Tienhoven, A., Marsh, J. A., McCormick, C. C., Cunningham, D. L., & Baker, R. C. (1986). Effects of cage and floor pen management on behavior, production, and physiological stress responses of laying hens. *Poultry Science*. https://doi.org/10.3382/ps.0651058

Mourão, J. L., Barbosa, A. C., Outor-Monteiro, D., & Pinheiro, V. M. (2010). Age affects the laying performance and egg hatchability of red-legged partridges (alectoris rufa) in captivity. *Poultry Science*. https://doi.org/10.3382/ps.2009-00377

Prieto, R., Sánchez-García, C., Alonso, M. E., Rodríguez, P. L., & Gaudioso, V. R. (2012). Do pairing systems improve welfare of captive Red-Legged partridges (Alectoris rufa) in laying cages? *Poultry Science*. https://doi.org/10.3382/ps.2011-01677

Prieto, Raquel, Sánchez-García, C., Tizado, E. J., Alonso, M. E., & Gaudioso, V. R. (2018). Mate choice in red-legged partridges (Alectoris rufa L.) kept in commercial laying cages; does it affect laying output? *Applied Animal Behaviour Science*. https://doi.org/10.1016/j.applanim.2017.10.007

Puvadolpirod, S., & Thaxton, J. P. (2000). Model of physiological stress in chickens 4. Digestion and metabolism. *Poultry Science*. https://doi.org/10.1093/ps/79.3.383

Romero, L. M. (2019). Fight or flight responses. In *Encyclopedia of Animal Behavior*. https://doi.org/10.1016/B978-0-12-809633-8.20760-7

Romero, L. M., & Wingfield, J. C. (2016). Tempests, poxes, predators, and people: Stress in wild animals and how they cope. In *Oxford series in behavioral neuroendocrinology*.

Swaisgood, R. R. (2007). Current status and future directions of applied behavioral research for animal welfare and conservation. *Applied Animal Behaviour Science*. https://doi.org/10.1016/j.applanim.2006.05.027

Ziaei, N., Guy, J. H., Edwards, S. A., Blanchard, P. J., Ward, J., & Feuerstein, D. (2007). Effect of gender on factors affecting excreta dry matter content of broiler chickens. *Journal of Applied Poultry Research*. https://doi.org/10.1093/japr/16.2.226

Table 1- Ingredient and nutrient composition of the pelleted diet fed to captive Sardinian partridges

Ingredient composition	Additives	Chemical composition
Mais	E672 Vitamin A	Moisture 12.00%
Wheat hulls	E671 Vitamin D3	Crude Protein 16.00%
Soybean meal	E1 Iron (Ferrous sulphate, monohydrate)	Crude fat 2.50 %
Wheat straw	E2 Iodine (Calcium iodate, anhydrous)	Cellulose 6.50%
Alfa alfa meal	E3 Cobalt (Cobalt carbonate, monohydrate	Crude ash 8.00%
Mais meal	E4 Copper (Cupric sulphate, pentahydrate)	Calcium 1.10%
Nut hull meal	E5 Manganese (Manganous oxide)	Phosphor 0.80%
Yeasts	E6 Zinc (Zinkoxid)/(Zinc oxide)	Sodium 0.10%
Calcium carbonate	E8 Selenium (Sodium selenite)	Methionine 0.20%
Calcium phosphate	E7 Molybdenum (Ammonium molybdate)	Lysine 0.70%
Sodium chloride	E562 Sepiolite	
	E321 BHT	

Table 2. Non invasive parameters explore in partridges according to the two caging systems, singly housed vs. coupled.

Parameters	Couple	Single	pooled SD	p-value
BW (g, final phase 2)	422	438	60.6	0.380
Feed intake, g d^{-1} (0-15, phase 2)	20.4	21.9	4.76	0.452
Feed intake, g BW^{-1} d^{-1} (0-15, phase 2)	0.05	0.05	0.01	0.267
Excreta output, g DM $intake^{-1}$ d^{-1}	0.44	0.36	0.07	0.318
Quality of excreta (pooled 3days phase 2)				
DM content, g kg^{-1}	263.9	291.7	27.3	0.697
pH	6.53	6.78	0.29	0.250
Digestibility coefficients*				
Crude protein	73.3	74.6	5.88	0.765
Organic matter	59.7	64.0	7.49	0.439

*uric acid corrected

Figure captions

Figure 1. Battery of cages in which breeding partridges are conventionally kept.

Figure 2. Linear Plot of daily feed intake in phase 2 of singly caged females vs. males over time (two weeks).