

Formation of the Pecking Order during Small-Scale Floor Feeding in Helmeted Guinea Fowl (*Numida meleagris*)

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Helmeted guinea fowl are social animals and only males form a hierarchy in the wild. Non-cage husbandry systems benefit the reproductive health of guinea fowl; however, there are concerns that the feeding duration of subordinate individuals is insufficient. Here, the pecking orders formed during small-scale floor feeding were investigated. There were three experimental categories: male-only (four males), female-only (four females), and mixed category (two males, two females). Each experimental category was set up three times and included different individuals. Behaviors were recorded for 130 h 52 min, 89 h 11 min, and 98 h 46 min in the male, female, and mixed categories, respectively. Male helmeted guinea fowls pecked other males, whereas females exhibited little pecking behavior. Male pecking behavior was not homogeneous within each experimental group. It has been suggested that males form a pecking order, whereas females have no hierarchy under small-scale floor-feeding conditions, as observed in the wild. In most cases, on the first day the number of pecking behaviors was low 20 min after the start of the experiment. The three subordinate individuals in the mixed category had little time to feed, whereas the other birds in the mixed category and all helmeted guinea fowl in the male-and female-only categories had longer feeding durations. We suggest that helmeted guinea fowl may be reared under small-scale floor feeding, and that the health of males should be managed. However, rearing females and males under small-scale floor feeding conditions should be avoided. This study contributes to improving the welfare of helmeted guinea fowl reared under small-scale floor feeding.

Key words: flocking type, Numida meleagris, pecking behavior, pecking order

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Introduction

In terms of animal welfare, poultry housing systems are shifting from cage-based to non-cage husbandry. Conventional caging environments accelerate the risk of bone loss owing to a relative lack of exercise[1]; therefore, a non-cage husbandry system is promoted to improve poultry health. However, the non-cage husbandry system is imperfect because diseases and parasites spread easily and problematic behaviors, such as pecking, may occur[2,3]. Agonistic behaviors increase in smaller group sizes in female domestic chickens[4]; therefore, whether there is a problem when animals are reared in a non-cage husbandry system must be known to ensure their health.

Some animals, such as domestic chickens (*Gallus gallus do-mesticus*)[5], ring-necked pheasants (*Phasianus colchicus*)[6,7], turkeys (*Meleagris gallopavo*)[8], and Japanese quail (*Coturnix japonica*)[9] form a dominant hierarchy. Individuals in a high-ranking hierarchy access resources, such as food[10]. The hierarchy decided by the pecking behavior, or "pecking order" is commonly linear, and individuals are ranked from most dominant to most subordinate[5]. This agonistic behavior also determines the hierarchy of ring-necked pheasants[7]. Hierarchy affects feeding behavior in female domestic chickens and the feeding duration at the feeder of dominant females is significantly longer than that of individuals in other hierarchies[11]. This previous study indicates that the feeding duration of subordinate individuals was insufficient.

The family Numididae is raised globally for gamey flesh and

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eggs[12]. Non-cage husbandry systems are commonly used to rear guinea fowls, whereas cage systems are more effective for artificial insemination. However, the ovary weight and number of large yellow follicles of guinea fowl reared in a non-cage husbandry system are much greater than those of fowl reared in a cage system, suggesting that the non-cage husbandry system improves the reproductive and physiological performance of guinea fowl[13]. This suggests that non-cage husbandry systems are superior to cage systems in terms of animal welfare and improved reproductive abilities.

Helmeted guinea fowl (Numida meleagris) were originally distributed across tropical and subtropical areas of Sub-Saharan Africa[14]. In the wild, helmeted guinea fowl form a group that remains together for at least one breeding season[15], and they have a monogamous breeding system[16]. Although a non-cage husbandry system is commonly used to rear these animals, it is unclear what problems may arise, such as a decrease in feeding duration. Two conditions must be considered in non-cage husbandry systems for breeding purposes. The first kept the sexes in separate rooms to facilitate artificial insemination. In the second condition, males and females were kept together in the same room, allowing breeding and the production of fertilized eggs. In the wild, helmeted guinea fowl males form a hierarchy, whereas females have no hierarchy[15]; therefore, it is possible that some individuals, especially males forming hierarchies, may be unable to adequately feed because of the pecking order that forms when helmeted guinea fowls are reared under these conditions. However, to the best of our knowledge, no study has examined the inter-individual relationships during small-scale floor feeding to determine whether any issues arise from rearing these birds under such conditions.

We hypothesized that helmeted guinea fowl reared under small-scale floor feeding would exhibit pecking behaviors and that subordinate individuals would not have enough feeding time. Social relationships were investigated in groups comprising four helmeted guinea fowls raised under small-scale floor feeding conditions, focusing on the hierarchy determined by pecking behavior. Whether helmeted guinea fowl could be reared in groups comprising male, female, or mixed categories was assessed.

Materials and Methods

Ethics

These experiments were conducted following the license for experimental animals of the Tokyo University of Agriculture (No. 160464) and were conducted according to the ethical code. If the condition of an individual participant was unsatisfactory during the observation period, the experiment was terminated.

Individual information

Seventeen helmeted guinea fowls (ten males and seven females) were used for these experiments at the Atsugi campus of the Tokyo University of Agriculture. Table 1 shows information on the individuals, including their ID, sex, and weight. The mean \pm the standard deviation (SD) of weight was 2.61 \pm 0.34 kg and 2.63 \pm 0.67 kg in males and females, respectively. Most helmet-

on September 26, 2018.						
ID*	Weight (kg)					
M1	2.30					
M2	2.95					
M3	2.16					
M4	3.40					
M5	2.45					
M6	2.73					
M7	2.59					
M8	2.44					
M9	2.46					
M10	2.60					
F1	2.55					
F2	3.39					
F3	3.69					
F4	2.05					
F5	ND					
F6	2.00					
F7	2.09					

Table 1. The information of the individuals, which includes ID, sex, and weight. All individuals were weighed

* M: male, F: female

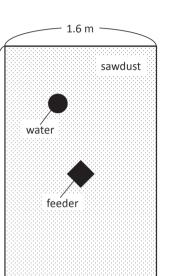
ed guinea fowls were adults over 117 weeks old, except for F7, which was 29 weeks old at the start of the observations. All individuals were weighed on September 26, 2018.

Observation

Observations were conducted from May to October 2018, and each observation lasted for 3-7 d. The experimental room was 1.6×2.7 m and the temperature was adjusted to 20-25 °C (Fig. 1). Sawdust was spread over a few centimeters on the concrete floor of the room. Light was automatically controlled to provide 14 h of light (6:00–20:00) and 10 h of darkness. Food and water were continuously provided and added as needed. The feeder had a feeding spot with a size of 50×100 mm, which prevented multiple helmeted guinea fowls from feeding simultaneously. A 3 L plastic poultry waterer (27 cm diameter) that allowed simultaneous access was used. A video camera (Mothertool Co., Ltd., Japan, MT-WC M300) was set on the side of the room; the angle of view covered the room and the light time (14 h) was recorded (Fig. 1).

Experimental category and group

Three experimental categories were used: the first category included four males (male-only category), the second category included four females (female-only category), and the final category included two males and two females (mixed category). Information on each observation is shown in Table 2, including the group ID, weight with SD, date, starting time, and total observation time. The total observation time was not the same because data was excluded from the analysis in cases where the video failed (e.g., when helmeted guinea fowl huddled in front of the



video camera

Fig. 1. The experimental room. The sawdust was spread a few centimeters thick on the concrete floor of the room. The feeding spot had a size of 50×100 mm. The feed and water were continuously available.

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camera or when the camera fell over) and when the memory media capacity was low. All individuals were reared in separate-layer cages in the same room prior to the experiment. The transfer sequence was random and each group was composed of as many different individuals as possible. When it was unavoidable to use the same individuals, an experimental interval was set over four weeks. Individual identification was performed using a colored tag placed at the base of the wing.

Behavioral analysis

2.7 m

The pecking and feeding behaviors were recorded from video data using an event sampling method[17]. Pecking behaviors were counted and feeding behavior was recorded as the duration based on continuous recording. Pecking behavior was divided into two types: gentle feather pecking, which does not damage feathers, and aggressive feather pecking, which causes serious damage to feathers, including broken or missing feathers[18]. According to Dong[19], aggressive feather pecking is defined as a forceful pecking behavior usually directed at the head or neck of other guinea fowls. In this study, pecking behavior was defined as aggressive feather pecking (attacking a part of another individual's body with its beak). The pecked individuals and their sexes were recorded. The presence of a pecking order was confirmed when the number or rate of pecking behaviors was biased toward certain individuals in each group. The feeding duration was defined as the time from the start of feeding until the individual moved away from the feeder.

Statistical analysis

To compare the rates of pecking behavior between the experimental groups, the "pecking rate" was defined as the number of pecking behaviors per experimental duration (h) per individual. Pecking rates were compared between the sexes and temporal changes based on the day were investigated. Sex differences in the pecking rates were compared using the Wilcoxon rank-sum test. If a male or female helmeted guinea fowl pecked other individuals a few times, it was excluded from the temporal change analysis. "Pecked rate" was defined as the number of given pecking behaviors per experimental duration and individual.

The correlation between the pecked rate and feeding duration was also investigated. Helmeted guinea fowl were divided into two groups based on their pecked rates. Helmeted guinea fowls that were pecked more than other individuals were grouped as "pecked-much", whereas others were grouped as "pecked-few" in each group and sex. When the experimental category was male- or female-only, the first and second individuals with low pecked rates were grouped as pecked-few, and the others were grouped as pecked-much. If the pecked rates of the second individual with few pecked rates were equal to those of the third individual, the group was excluded from the analysis. When the experimental category was mixed, the first individual with few pecked rates was grouped as pecked-few, and the other was grouped as pecked-much for each sex. If the pecked rates of the first individual with few pecked rates were equal to those of the

 Table 2.
 The information of group ID, date, start time, total examination time duration, experimental category, individual ID and weight with SD.

Group ID	Experiment duration	Start time	Duration	Experimental category	Individual ID	SD
А	5/2-5/5	15:47	32 h 49 m	mixed	M5, M9, F3, F5	0.58
В	5/9-5/13	18:27	46 h 33 m	mixed	M1, M2, F1, F2	0.41
С	5/23-5/25	18:53	19 h 24 m	mixed	M3, M4, F3, F2	0.59
D	6/7-6/13	13:00	56 h 50 m	male-only	M5, M6, M7, M8	0.12
Е	7/11-7/16	12:10	28 h 01 m	male-only	M1, M4, M7, M9	0.42
F	10/3-10/8	17:04	46 h 01 m	male-only	M4, M5, M7, M10	0.37
G	6/20-6/24	16:52	34 h 43 m	female-only	F2, F3, F4, F6	0.77
Н	9/26-9/29	11:05	35 h 09 m	female-only	F1, F2, F3, F7	0.64
Ι	10/17-10/23	14:00	19 h 19 m	female-only	F2, F3, F4, F5	0.71

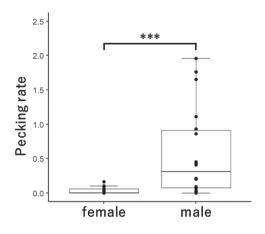


Fig. 2. Box plots of the pecking rate. There were significant differences between the sexes (Wilcoxon rank-sum test: W = 287.5; p < 0.001).

second individual, the group was excluded from the analysis.

All statistical analyses were conducted using R software. The Wilcoxon rank-sum test was used for the coin package in R software.

Results

The total recording duration was 130 h 52 min, 89 h 11 min, and 98 h 46 min for the male-, female-only, and mixed categories, respectively. The pecking rates in males were significantly higher than those in females (Wilcoxon rank-sum test: W = 287.5, p <0.001; Fig. 2). In most cases, males pecked males (85.3%). The number of pecking behaviors of males was not homogeneous in each experimental group (Table S1). The pecking rate was high on the first experimental day, whereas the pecking rates were low on the other days for all experimental groups (Fig. 3). On the first day, the number of pecking behaviors at 20 min from the start of the experiment was high, whereas the time thereafter was low, and the number of pecking behaviors after 100 min was low, except in one group (group D in Table 2 and Fig. 4). All helmeted guinea fowls in the male- and female-only categories had long feeding durations (Fig. 5). One male grouped as pecked-few in each group in the mixed category had a long feeding duration, whereas another male grouped as pecked-much in each group had a shorter feeding duration (Fig. 5). Females were compared with males in the same group, because females were pecked only a few times by other individuals (Fig. 5). Three individuals in the mixed category were frequently pecked by another male and huddled in a corner of the room. Mating behavior was observed once in group A and once in group C; however, no eggs were fertilized.

Discussion

In helmeted guinea fowl, pecking behaviors and hierarchies occurred in males, and subordinate males did not have a long

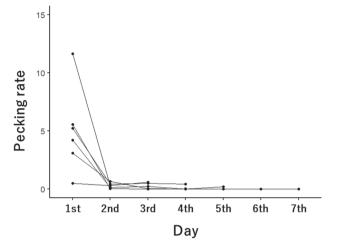


Fig. 3. Changes in the pecking rate by day. Rates were calculated by the number of pecking behaviors per male individual and hour. The data of male helmeted guinea fowls in six experimental groups of male-only and mixed category are shown.

enough feeding duration in the mixed category. We suggest that it is possible to rear only males under small-scale floor feeding; however, it is necessary to monitor their health. It is better to avoid rearing females and males together under small-scale floor feeding because male subordinates have insufficient time for feeding. The number of pecking behaviors was low in females and they had sufficient feeding durations in the female-only and mixed categories. We propose that at least four female helmeted guinea fowl may be reared under small-scale floor feeding.

Ring-necked pheasants[6] and domestic chickens[20] peck on other individuals of both sexes. However, male helmeted guinea fowls showed a significant trend in most cases where males pecked at other males rather than females. The number of agonistic behaviors in wild male helmeted guinea fowl is greater than that in females (males: 21 times, females: 2 times)[15]. The number of pecking behaviors is biased in some individuals in the mixed and male-only categories, as observed in domestic chickens[5] and ring-necked pheasants[6]. These data suggested that male helmeted guinea fowls formed a domestic hierarchy under small-scale floor feeding, whereas females had no hierarchy.

The pecking rate decreased 20 min after the start of the experiment on the first day. The same trend has been observed in domestic chickens; the number of attacks in domestic chickens significantly decreases after a few days[5]. The duration of the decrease in pecking behavior of helmeted guinea fowl was shorter than that of domestic chickens. This might be caused by the group composition of helmeted guinea fowls. Helmeted guinea fowl form a group composed of 7–10 individuals in the wild[15]; therefore, prolonged conflict within a group is regarded as less beneficial to the group as a whole. The occurrence of aggressive behavior is low in the wild, indicating that aggressive interactions are limited to ensure cohesion[15]. These data imply that

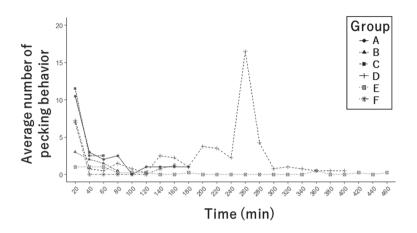


Fig. 4. Changes of the average pecking numbers at 20 min on the first day. These numbers were calculated using the number of pecking behaviors per male individual. The data of male helmeted guinea fowls in six experimental groups of male-only and mixed category are shown.

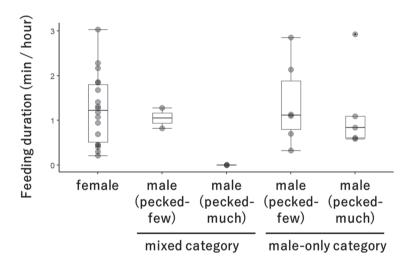


Fig. 5. Box plots of the feeding duration. Males that were pecked more than other males were grouped as "pecked-much", whereas others were grouped as "pecked-few" in each group.

the duration for deciding the pecking order was short to maintain group cohesion. The number of pecking behaviors in one group (group D in Fig. 4) increased 100 min after the start of the experiments. This might be due to differences in weight. The SD of group D was smaller than those of the other groups (Table 2). The hierarchy decision might be prolonged when the weight differences were small because the aggressiveness of the individuals was approximately the same. Thus, the effect of weight differences should be investigated before conducting experiments on helmeted guinea fowl.

Feather pecking behavior occurs in the afternoon rather than

in the forenoons in female domestic chickens[21]. The current experiments began in the afternoon, except in group H. If there is a bias in pecking behaviors depending on the time period, future studies should arrange the starting time for rearing helmeted guinea fowls to reduce pecking behaviors. Additionally, genetic lines affect the number of agonistic behaviors in males[22]. Future studies should change the number of rearing males and females, feeders, and sex ratio and investigate the effect of genetic lines to understand the conditions in which pecking behaviors occur.

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Author contribution

Tomoyoshi Terada conceived the ideas and designed the methodology, analyzed the data, and wrote the manuscript; Mitsuki Shimoda conceived the ideas, designed the methodology, collected the data, and wrote the manuscript; Daisuke Waku conceived the ideas, designed the methodology, and wrote the manuscript; Hiroshi Ogawa conceived the ideas, designed the methodology, and wrote the manuscript. All authors contributed critically to drafts and approved the final manuscript for publication.

Conflicts of Interest

The authors declare no conflict of interest.

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