Diet and gizzard muscularity as control factors of grit use in domestic chickens (#46973)

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Diet and gizzard muscularity as control factors of grit use in domestic chickens

Ryuji Takasaki Corresp., [1, 2], **Yoshitsugu Kobayashi** [3]

[1] Department of Natural History Sciences, Hokkaido University, Sapporo, Japan

[2] Faculty of Biosphere-Geosphere Science, Okayama University of Science, Okayama, Japan

[3] Hokkaido University Museum, Sapporo, Japan

Corresponding Author: Ryuji Takasaki
Email address: rtakasaki@big.ous.ac.jp

The gizzard is the only gastrointestinal organ for mechanical processing in birds. Many birds utilize grit in the gizzard to enhance mechanical processing efficiency. We conducted an experiment to test the factors that affect chicken grit use by using 68 male layer chicks of *Gallus gallus domesticus*, which were divided into two different groups in gizzard muscularity (muscular and less-muscular gizzard). Each muscularity group was fed two different types of diet (herbivory and non-herbivory) to test whether diet and gizzard muscularity of chicks affect grit characteristics such as amount, size, and shape (circularity, roundness, and solidity) of different stages (ingested grit, grit in gizzard, and excreted grit). Amount of ingested grit and grit in gizzard were larger in herbivorous groups, whereas the amount of excreted grit was smaller in herbivorous groups. Larger grit was selectively ingested, especially in the herbivorous groups. Grit in gizzard was larger in herbivorous groups, while the size of excreted grit was nearly equal or smaller in herbivorous groups. Ingested grit was sharper (low circularity and solidity) than the offered grit. Grit in gizzard was much less-sharp than the offered and ingested grit, especially in the herbivorous, muscular gizzard groups. Excreted grit was less-sharp than the offered grit. These results show that diet affects the characteristics of ingested grit, grit in gizzard, and excreted grit, whereas gizzard muscularity affects the characteristics of grit in gizzard and excreted grit. The use of large size and amount of grit in gizzard in herbivorous groups may be a response upon needs of digesting hard, coarse materials. Flexibility on grit use might reflect the omnivorous nature of *Gallus gallus domesticus* and may aid their smooth diet shifts. The results also show that shapes of grit in gizzard do not reflect the shapes of ingested grit, in contrast to previously published concepts, but the shape of grit in the gizzard reflects diet and gizzard muscularity of chicks.

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Ryuji Takasaki[1, 2], Yoshitsugu Kobayashi[3]

[1]Department of Natural History Sciences, Hokkaido University, Sapporo, Hokkaido, Japan

[2]Faculty of Biosphere-Geosphere Science, Okayama University of Science, Ridaicho, Kita-ku, Okayama city, Okayama, Japan

[3]Hokkaido University Museum, Hokkaido University, Sapporo, Hokkaido, Japan

Corresponding Author

Ryuji Takasaki[1]

Ridaicho Kitaku Okayama 700-0005 Japan

Email address: rtakasaki@big.ous.ac.jp

ABSTRACT

The gizzard is the only gastrointestinal organ for mechanical processing in birds. Many birds utilize grit in the gizzard to enhance mechanical processing efficiency. We conducted an experiment to test the factors that affect chicken grit use by using 68 male layer chicks of *Gallus gallus domesticus*, which were divided into two different groups in gizzard muscularity (muscular and less-muscular gizzard). Each muscularity group was fed two different types of diet (herbivory and non-herbivory) to test whether diet and gizzard muscularity of chicks affect grit characteristics such as amount, size, and shape (circularity, roundness, and solidity) of different stages (ingested grit, grit in gizzard, and excreted grit). Amount of ingested grit and grit in gizzard were larger in herbivorous groups, whereas the amount of excreted grit was smaller in herbivorous groups. Larger grit was selectively ingested, especially in the herbivorous groups. Grit in gizzard was larger in herbivorous groups, while the size of excreted grit was nearly equal or smaller in herbivorous groups. Ingested grit was sharper (low circularity and solidity) than the offered grit. Grit in gizzard was much less-sharp than the offered and ingested grit, especially in the herbivorous, muscular gizzard groups. Excreted grit was less-sharp than the offered grit. These results show that diet affects the characteristics of ingested grit, grit in gizzard, and excreted grit, whereas gizzard muscularity affects the characteristics of grit in gizzard and excreted grit. The use of large size and amount of grit in gizzard in herbivorous groups may be a response upon needs of digesting hard, coarse materials. Flexibility on grit use might reflect the omnivorous nature of *Gallus gallus domesticus* and may aid their smooth diet shifts. The results also show that shapes of grit in gizzard do not reflect the shapes of ingested grit, in contrast to previously published concepts, but the shape of grit in the gizzard reflects diet and gizzard muscularity of chicks.

INTRODUCTION

Digestion, or food processing, is a key phase of animal feeding (Montuelle & Kane, 2019). In order to perform efficient digestion, animals have established morphological and physiological adaptations during their evolutionary history. Multiple specialized gastrointestinal organs have been evolved in birds, including a crop for temporal storage of food (Proctor & Lynch, 1993), caeca for conservation of water and/or nitrogen recycling (DeGolier et al., 1999; Karasawa, 1989), and a gizzard for mechanical processing of ingesta (Moore, 1999). In a gizzard, strong compressing and translational stress is put on ingesta for mechanical processing (Moore, 1998a). Large food particles are selectively retained in a gizzard until these are ground to small size (Hetland et al., 2003; Moore, 1999). For better digestion efficiency, birds ingest and retain grit in a gizzard to break down food particles as efficiently as non-ruminant mammals do with their teeth (Fritz et al., 2011). Some birds even travel a long distance to obtain grit in cases where there is not enough sands or gravel close by (McIlhenny, 1932). Although multiple non-digestive functions, such as parasite destruction, relief of hunger, and ballast, have been proposed for grit use in vertebrates (Wings, 2007), grit use is especially common in herbivorous and granivorous birds (Gionfriddo & Best, 1999).

Benefits of grit use in domestic chickens have been investigated in field of poultry. While grit use is not mandatory, previous works generally agree that grit improve digestion efficiency of chickens, especially of the ones that are fed coarse, less-nutritional food (Fritz, 1937; Hetland et al., 2003; Smith & MacIntyre, 1959). Other than digestive function, previous works attempted to identify the best form and size of limestone to provide as grit for maximizing egg productivity and quality by layer hens (e.g., Guinotte & Nys, 1991; Skřivan et al., 2016). Multiple studies also investigated how different grit (e.g., size, amount, shape) improve nutritional/commercial efficiency in domestic chickens (e.g., Balloun & Phillips, 1956; Cooney, 1941). On the other hand, factors that affect grit use behaviors are less understood. This is partly because previous studies are based primarily on grit collected from gizzards (e.g., Best & Gionfriddo, 1991; Gionfriddo & Best, 1996) although grit characteristics can be strongly modified in a gizzard through abrasions (Buckner et al., 1926; Wings & Sander, 2007).

Here we report an experiment which tests if different diet and gizzard muscularity changes amount, size, and shape of grit used, in order to understand what affect grit use behavior in domestic chickens. In addition to diet, our experiment also test effects of gizzard muscularity difference on grit use behavior. This is done because gizzard with a higher muscularity should put larger stress to ingesta therefore expected to affect chicken grit use. The factors that may affect chicken grit use are tested upon grit ingestion, retention in a gizzard, and excretion in order to understand the thorough process of chicken grit uses. This study will provide insights into how domestic chicks benefit from changing their grit use behaviors upon demands.

MATERIALS AND METHODS

Ethics statement: An experiment in this study was approved by Hokkaido University (Permission number: 16-0023) in Sapporo, Japan, and followed the rules specified on Hokkaido University manual for implementing animal experimentation.

Experimental design and managements

A total of 68 one-day-old male layer chicks (*Gallus gallus domesticus*), purchased from a local feed manufacturer, were used in this experiment. This sample size was set based on the Hokkaido University regulation, space availability, and several prior experiments conducted on domestic chickens (Hetland et al., 2003; Van der Meulen et al., 2008). Prior to the experiment, the chicks were raised for three weeks to produce a difference in initial gizzard muscularity (evaluated as relative weight of a gizzard and the body mass of the chick). The duration was determined based on Amerah et al. (2007), which demonstrated a significant gizzard muscularity difference in three weeks. During the three weeks, all chicks were fed commercial starter pellet (Yamaichi Shiryo Co. Ltd.). Development of a gizzard muscularity (Fig. S1) was enhanced on half of the chicks (34 individuals) by feeding larger amount of insoluble fiber (Sacranie et al., 2012) through feeding 30 wt% of rice hull (Honda Co. Ltd.) mixed to the starter pellet. Rice hull was ground to < 5 mm using food processor prior to feed. The chicks had *ad libitum* access to feed and water.

This experiment was conducted for one week on four groups (17 individuals each): non-herbivorous diet with a muscular gizzard (nH-M), herbivorous diet with a muscular gizzard (H-M), non-herbivorous diet with a less-muscular gizzard (nH-lM), and herbivorous diet with a less-muscular gizzard (H-lM). Herbivorous groups (H-M and H-lM) fed on a mixture of Poaceae (*Medicag sativa*) and Fabaceae (*Phleum pratense*) grasses, both purchased from Mapet Corp. Non-herbivorous groups (nH-M and nH-lM) fed on dried fish (*Engraulis japonicas*), purchased from Sakamoto Corp. The chicks were fed either 100% grass or 100% fish to test effect on grit use under extreme diet difference. Grass and dried fish were ground to < 5 mm using food processor and provided as meal.

During the experiment, all chicks were raised in individual cages with wire mesh floor. Room temperature was maintained between 28 °C to 30 °C. Lighting was controlled as 12 hours in light and 12 hours in dark. All of the chicks were given *ad libitum* access to total of 24 grams of grit per chicks, which were provided separate from feed. Feces on the last day of the experiment were collected to evaluate characteristics of excreted grit. All chicks were weighed and then euthanized by cervical dislocation at the end of the experiment, following the Hokkaido University regulations. Gizzards were removed from all of the carcasses and weighed after removing stomach contents.

Terminology

Offered grit refers to the stones which were given *ad libitum* access to each chick (Fig. 1A). Ingested grit refers to the stones which were swallowed by the chicks out of the offered grit during the experiment. Uningested grit refers to the stones which were not ingested by the chicks out of the offered grit by the end of the experiment. Grit in gizzard refer to the stones remained in the gizzards of the chicks after euthanization. Excreted grit refers to the stones excreted with the feces on the last day of the experiment. Initial gizzard muscularity refers to the gizzard muscularity of chicks at the start of the experiment. “Sharp” is used to describe grit with relatively low circularity, roundness, and/or solidity and “less-sharp” is used to describe grit with relatively high shape index.

Grit characteristics

All of the grit used in the experiment were silicastic stones. The amount, size, and shape of grit (offered grit, uningested grit, grit in gizzard, and excreted grit) were evaluated. The amount of grit was evaluated in weights (grams). Size and shape were evaluated quantitatively using the menu command Analyze > Analyze particles of ImageJ (Schneider et al., 2012). To obtain the images for the analyses, grit was manually separated from each other and was lighted from the background to obtain clear outlines (Fig. 1B, Fig. S2). All images were taken manually. A minor axis was employed as a grit size index (in millimeters). Circularity, roundness, and solidity were employed as grit shape indexes (Schneider et al., 2012): circularity was calculated as four times the product of π and area, divided by square of perimeter; roundness was an inverse of an aspect ratio; and solidity was calculated as an area of a grit divided by an area of convex hull (Fig. 1C).

Offered grit

The amount, size, and shape of ingested grit was inferred by comparing the characteristics of offered and uningested grit. To test size preference by the chicks, the size distribution of offered grit was controlled in advance. Grit was classified into six different size classes by dry sieving using sieves of Sanpo Corp. (0.5–1.0 mm, 1.0–1.4 mm, 1.4–1.7 mm, 1.7–2.0 mm, 2.0–2.8 mm, 2.8–3.35 mm). Four grams of grit from each size classes were supplied in mixture. Prior to the experiment, minor axis, circularity, roundness, and solidity of 500 randomly chosen grit from each size classes were evaluated by ImageJ (Fig. S3). After the experiment, uningested grit was collected and sieved into the six size classes. Five-hundred uningested grit were randomly sampled from each size classes and their size and shape indexes were evaluated (Fig. S3). To test size preferences, amount of ingested grit in each size classes was evaluated by subtracting the weights of the uningested grit from weights of the offered grit by each size classes (4 grams each). Average values of the shape indexes of the uningested grit were compared with those of the offered grit to test if there was any shape preference on the ingested grit. The amount, size, and shape of the uningested grit was then compared among different diet and gizzard muscularity groups.

Grit in gizzard

Grit in gizzard was separated from other stomach contents by a floatation method (decantation), modified from Itani (2015). Stomach contents were soaked with water in a beaker over one night. Gizzard digesta was stirred then low-density floating food particles were gently disposed. This procedure was repeated until only grit was remained in the beaker. Grit smaller than 0.5 mm were collected as much as possible, but removed from the analyses. Total amount was weighed, then size and shape of all grit in gizzard were analyzed using ImageJ (Fig. S4). The amount, size, and shape of the gizzard grit was compared among different diet and gizzard muscularity groups.

Excreted grit

Excreted grit was separated from fecal particles using the same method as separating gizzard grit. Grit smaller than 0.5 mm were collected as much as possible, but removed from the analyses. Total amount is weighed and size and shape of all excreted grit were analyzed using ImageJ (Fig. S5). The amount, size, and shape of the excreted grit was evaluated and compared with those of the gizzard grit to test selection upon excretion. Grit characteristics were also compared among different diet and gizzard muscularity groups. Since excreted grit were collected only on the last day of the experiment, and since they were collected per group instead of per individual, amount and size of excreted grit per individual are unavailable in present study.

Statistical analyses

All statistical analyses were conducted using software R (R Core Team, 2019). Since multiple datasets do not have normal distribution, non-parametric analyses were conducted. Ordinal logistic regressions were conducted as non-parametric equivalent of two-way ANOVA to test effects of gizzard muscularity, diet, and their interactions for body mass, gizzard mass, and grit features (size, amount, and shape), using R package MASS (Venables & Ripley, 2013). Steel-Dwass method was used for post-hoc tests. Correlations between shape indexes of grit in gizzard and gizzard muscularity at the end of the experiment are tested using Spearman’s rank correlation. Grit amount is corrected by chick body mass and grit size is corrected by cubic root of chick body mass upon analyses. Chicks, euthanized prior to the end of the experiment following the Hokkaido University rules, were excluded from the analyses. The data analyzed are provided as Supplemental Information (Data S1-S6).

RESULTS

During the experiment, two chicks from non-herbivorous, muscular gizzard group (nH-M), one chick from the non-herbivorous, less-muscular gizzard group (nH-lM), five chicks from the herbivorous, muscular gizzard group (H-M), and two chicks from the herbivorous, less-muscular gizzard group (H-lM) were euthanized due to sudden drop of body mass, following the Hokkaido University regulations before the end of the experiment. Therefore, all of the analyses were performed on total of 58 chicks.

The average body mass of the chicks at the end of the experiment were affected by both diet and initial gizzard muscularity, as well as their interaction (Table S1). Body mass were higher in the non-herbivorous groups than in the herbivorous groups (nH-M > H-M, nH-lM > H-lM; Fig. 2, Table 1, Table S2). This difference in body mass was significant only between the groups with high initial gizzard muscularity. Average gizzard muscularity at the end of the experiment was affected by diet and initial gizzard muscularity (Table S1). The average gizzard muscularity was significantly higher in herbivorous groups than in non-herbivorous groups (H-M > nH-M, H-lM > nH-lM; Fig. 2B, Table S2). While the differences in initial gizzard muscularity were maintained significant in between the herbivorous groups at the end of the experiment (H-M > H-lM), the differences were insignificant in between the non-herbivorous groups (nH-M and nH-lM). The result is likely to reflect a rapid change in gizzard muscularity associated with diet change.

Grit amount

The experiment demonstrated that amount of ingested grit per chick were approximately 3 g in average (1.7% of body mass), whereas the amount of grit in gizzard were approximately 1 g in average (0.5 % of body mass), suggesting more than half of the ingested stones were excreted during the experiment (Table 1). Amount of the excreted grit on the last day of the experiment was 2.52 g in total (0.045 g per chick in average). Diet affected the average amount of ingested grit and grit in gizzard, both in total and relative to the body mass (Table S1). The post-hoc tests show that herbivorous groups ingested significantly more grit relative to their body mass (H-M > nH-M, H-lM > nH-lM; Fig. 2, Table S2). Amount of grit in gizzard relative to body mass were also greater in herbivorous groups (H-M > nH-M, H-lM > nH-lM; Table S2, Fig. 2). No significant difference in amount of ingested grit and grit in gizzard was detected by difference in initial gizzard muscularity. Amount of excreted grit at the last day of the experiment were larger in non-herbivorous groups than in herbivorous groups in total weights (nH-M: 0.83 g, H-M: 0.26 g, nH-lM: 1.34 g, H-lM: 0.09 g).

Grit size

Large grit (>2.8 mm) were generally ingested more than smaller grit (Fig. 3, Table S3). The average size of grit in gizzard was about 1.84 mm and that of the excreted grit was 1.09 mm (Table 1). Diet primarily affected ingestion of grit larger than 1.4 mm (Table S4). Post-hoc tests show that the difference was significant between herbivorous and non-herbivorous groups of the less-muscular groups (H-lM > nH-lM; Table S5). Diet and interaction of gizzard muscularity + diet affected size of grit in gizzard relative to body mass (Table S1). The average sizes of grit in gizzard were significantly larger in herbivorous groups (H-M > nH-M, H-lM > nH-lM; Table

S2). Within non-herbivorous groups, the less-muscular gizzard group contained larger grit in gizzard in respect to their body mass than the muscular gizzard group (nH-lM > nH-M; Table S2). Average size of excreted grit is affected by diet and interaction of diet + gizzard muscularity (Table S1). Excreted grit is larger in non-herbivorous group than in herbivorous group within less initial gizzard muscularity group (nH-lM > H-lM; Table S2), as well as in non-herbivorous, less-muscular group than in non-herbivorous, muscular group (nH-lM > nH-M, Table S2).

Grit shape

Circularity, roundness, and solidity of the uningested grit were higher than those of the offered grit (Fig. 4A, Table 2), suggesting that ingested grit had low circularity, roundness, and solidity. This trend is generally significant in circularity and solidity for grit larger than 1.4 mm (Table S6). Grit in gizzard have higher circularity, roundness, and solidity than those of offered and uningested grit (Table 2). This trend is significant in circularity and solidity for all size classes and is generally significant in solidity for herbivorous groups (Table S7). Circularity and solidity of excreted grit was larger than those of grit in gizzard (Table 2), although the trend is significant only in solidity (Table S8). The shape indexes of excreted grit was higher than those of the offered grit (significant in circularity and roundness; Table S8).

Neither diet and gizzard muscularity strongly affected shape indexes of the uningested grit (Table S9, S10). Diet and gizzard muscularity affect circularity of the grit in gizzard of nearly all size classes, while diet, gizzard muscularity, and their interaction affect solidity of grit in gizzard (Fig. 4B, Table S11). On the other hand, roundness of grit in gizzard is affected only by diet in size classes 1.0 – 2.0 mm. Post-hoc tests show that grit in gizzard of herbivorous groups was significantly high in circularity and solidity for most size classes (H-M > nH-M, H-lM > nH-lM; Fig. 4B, Table S12). Circularity and solidity of grit in gizzard were also higher in herbivorous, muscular gizzard group than in the herbivorous, less-muscular gizzard group (H-M > H-lM; Table S12). Circularity, roundness, and solidity were correlated with gizzard muscularity at the end of the experiment ($p < 0.05$). Solidity of excreted grit is inferred to be affected by diet and interaction of gizzard muscularity and diet (Table S13), although the difference was undetected in post-hoc tests (Table S14).

DISCUSSION

Grit amount

The larger amounts of ingested grit and grit in gizzard in herbivorous groups (H-lM > nH-lM; Fig. 2, Tables S2, S3) were concordant with previous studies (comprehensive review done in Gionfriddo and Best, 1999). The larger amounts of excreted grit in non-herbivorous groups (nH-M > H-M, nH-lM > H-lM) suggests that the large amounts of grit in gizzard in herbivorous groups were affected by both large amount of grit ingestions and limited grit excretions. Retaining larger amount of grit in gizzard is likely to benefit herbivorous groups to supply higher digestion ability for breaking down tough grass fibers, since larger amount of grit in gizzard improves digestive performance in domestic chickens (Bale-Therik et al., 2012) as long as the amount is not excessive (Moore, 1998b).

From the amount of ingested grit and grit in gizzard, total amount of the excreted grit can be calculated as 20.99 g, 30.46 g, 17.58 g, and 53.22 g in groups nH-M, H-M, nH-lM, and H-lM, respectively. These are much more than the expected amount of grit excreted, assuming that amounts of the excreted grit were same every day as the last day of the experiment. Reasonable explanations are that the chicks excreted much more stones on other days, and that large amounts of excreted grit were less than 0.5 mm in size therefore undetected. While both are likely, the latter suggest that up to 20.36 g, 28.64 g, 8.20 g, and 47.42 g of grit in groups nH-M, H-M, nH-lM, and H-lM, respectively, were abraded to less than 0.5 mm. The larger amount of lost grit in herbivorous groups (H-M > nH-M, H-lM > nH-lM) may reflect rigorous grit use in the herbivorous groups, although a more specific experiment with specific records on amount of excreted grit is mandatory to make any further assumptions.

Grit size

Since the size of grit in gizzard is unlikely to affect digestion efficiency in domestic chickens (Smith, 1960), or larger grit may even have lower digestion efficiency (Moore, 1998c), selective ingestion of large grit in all groups (Table S3) may simply be due to ease to pick large grit. The smaller size of excreted grit than grit in gizzard (Table S8) in all groups suggest that size is one of the primary factors that determine which grit to be excreted in domestic chickens. While the excretion of small grit is concordant with a trend in domestic chicken (Smith, 1960), it is controversial with this trend in house sparrow (Gionfriddo & Best, 1995). Therefore, the responses in size of excreted grit may vary taxonomically. The larger sizes of ingested grit and grit in gizzard of herbivorous groups (Fig. 3, Tables 1, S2, S5) were concordant with previous works (Gionfriddo & Best, 1999; Hoskin et al., 1970; May & Braun, 1973; Norris et al., 1975; Soler et al., 1993; Thomas et al., 1977). The large size of excreted grit in non-herbivorous, less-muscular gizzard group (nH-lM > H-lM, nH-lM > nH-M; Table 1) suggests that this group had low ability to retain grit in gizzard, which is concordant with large amount of excreted grit from the group.

Grit shape and abrasions

Ingested grit being sharp (having higher shape indexes) than offered grit (Fig. 4A, Table S6) is consistent with previous knowledge in domestic chickens (Smith, 1960) as well as in

House Sparrows and Northern Bobwhite (Best & Gionfriddo, 1994). Since sharp grit in gizzard function as “blades”, this selection would increase digestion efficiency (Moore, 1998c). The active ingestions of sharp grit in all groups were likely to be a congenital behavior unlike the amount of ingested or excreted grit which were likely to be controlled upon demands (see above). The grit in gizzard being less-sharp than the offered grit (Table S7) contradicts with selective ingestion of sharp grit. Since the excreted grit was also less-sharp than the offered grit (Table S8), it is most likely that the grit in gizzard were severely abraded inside of the gizzards upon mechanical digestion of ingesta (Wings & Sander, 2007). The severe grit abrasion and associated grit size reduction is concordant with large amount of lost grit (see above). Severe grit abrasion within one week contrasts with the trend in ostrich, in which requires as much as four weeks to make severe change in grit shape (Wings & Sander, 2007). The difference may suggest time required for grit abrasion vary taxonomically and/or by body size.

The dominance of less-sharp grit in gizzard of herbivorous groups (H-M > nH-M, H-lM > nH-lM; Fig. 4B, Table S12), as well as in herbivorous, muscular gizzard group (H-M > H-lM), strongly suggests that diet and gizzard muscularity affect the degree of abrasions on grit in gizzard. Since dietary structures largely affect gizzard muscularity in birds including domestic chickens (Dekinga et al., 2001; Hetland et al., 2003; Sacranie et al., 2012), gizzard muscularity may be a primary factor which determines the degree of abrasion of grit in gizzard. Correlations between gizzard muscularity and shape indexes of grit in gizzard further support this assumption. Therefore, the shapes of grit in gizzard were unlikely to reflect grit selection patterns in domestic chickens, in contrast to previously published concepts (Best & Gionfriddo, 1991; Gionfriddo & Best, 1996). Instead, our experiment suggests that the differences in shapes of grit in gizzard reflect differences in diets and gizzard muscularities, although investigations in broader taxonomic sets are essential to test the assumption.

Chick grit use behaviors

This study is the first attempt to examine whether diet and gizzard muscularity affect chicken grit use behaviors throughout ingestion, retention, and excretion. This experiment strongly suggests that chick grit use behaviors were primarily affected by a diet and secondarily by a gizzard muscularity (Fig. 5, Table 1, 2). The flexible grit use upon needs of digesting tuff, coarse ingesta may be reflecting omnivorous nature of *Gallus gallus domesticus* and might have benefited for their shifts between herbivorous and carnivorous diets. Since numerous birds are known for omnivory and seasonal diet shifts (e.g., del Hoyo et al., 2005), flexibility in use of grit in gizzard may not be limited to domestic chickens but might had been a key importance for wide diet range of omnivorous birds, together with the gizzard phenotypic flexibility (Dekinga et al., 2001; Starck, 1999; van Gils et al., 2005). Further studies on other birds are mandatory to test the hypothesis.

CONCLUSION

This experiment on chick grit use behaviors demonstrated that chicks had a selection on size, amount, and the shape of ingested and excreted grit. It also revealed that grit in gizzard was greatly modified through abrasion; therefore, grit did not retain their original size and shapes upon ingestion. Instead, gizzard grit shapes reflected diets and gizzard muscularities of chicks. Ingestion of sharp grit regardless of diet and gizzard muscularity is likely to be congenial to chicks and facilitates better digestion efficiency. On the other hand, the ingestion and retention of larger amount of grit by herbivorous groups may be behavioral adaptation to supple digestion ability upon need of digesting coarse ingesta. The grit use flexibility might be reflecting the omnivorous nature of chickens. Grit in gizzard being less-sharp than ingested grit suggests strong abrasion of grit inside gizzard. Herbivorous and muscular gizzard groups having sharp grit in gizzard than non-herbivorous and less-muscular gizzard further suggest that grit abrasion is enhanced by herbivory and large gizzard muscularity.

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Table 1 (on next page)

Average values of chick status, grit amount, and grit size.

Note that average sizes of offered and remained grit cannot be calculated, but assumed from amount of stones ingested per size class. See Fig. 3 and Table S3 for detail.

	Chick		Grit amount			Grit size			
	Body mass	Gizzard mass	Ingested	Gizzard	Feces	Offered	Remained	Gizzard	Feces
Raw values									
H-lM	182.736	7.531	4.853	1.305	0.09*	NA	NA	1.884	0.960
H-M	153.403	7.863	3.657	1.118	0.26*	NA	NA	1.743	0.975
nH-lM	225.163	6.636	1.879	0.781	1.34*	NA	NA	2.004	1.273
nH-M	250.977	8.205	2.318	0.919	0.83*	NA	NA	1.883	1.138
Relative to body mass									
H-lM	NA	0.041	0.026	0.007	NA	NA	NA	0.332	0.169
H-M	NA	0.051	0.022	0.007	NA	NA	NA	0.326	0.182
nH-lM	NA	0.030	0.009	0.003	NA	NA	NA	0.329	0.209
nH-M	NA	0.033	0.009	0.003	NA	NA	NA	0.298	0.180

*Total amount excreted on the last day of the experiment

1

Table 2(on next page)

Table 2. Average values of shape indexes.

	Circularity				Roundness				Solidity			
	Offered	Uningested	Gizzard	Feces	Offered	Uningested	Gizzard	Feces	Offered	Uningested	Gizzard	Feces
H-lM	0.734	0.749	0.789	0.773	0.732	0.742	0.762	0.760	0.950	0.954	0.961	0.947
H-M	0.734	0.746	0.798	0.783	0.732	0.740	0.764	0.763	0.950	0.953	0.964	0.953
nH-lM	0.734	0.747	0.776	0.774	0.732	0.738	0.745	0.762	0.950	0.954	0.959	0.953
nH-M	0.734	0.752	0.780	0.777	0.732	0.741	0.752	0.753	0.950	0.955	0.954	0.951

Figure 1

Visualized experimental design.

(A) Terminology regarding grit treated in this study. (B) Grit shape evaluation procedure. (C) Schematic explanation on grit shape indexes.

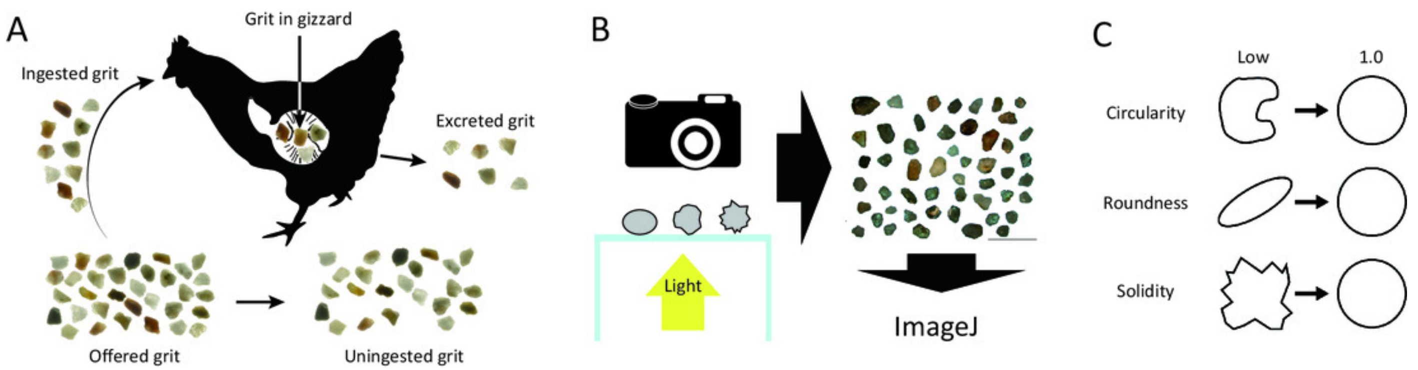

Figure 2

Boxplots showing chick information, grit amount, and size of grit in gizzard.

(A) Comparisons by raw values. (B) Comparisons by normalized values.

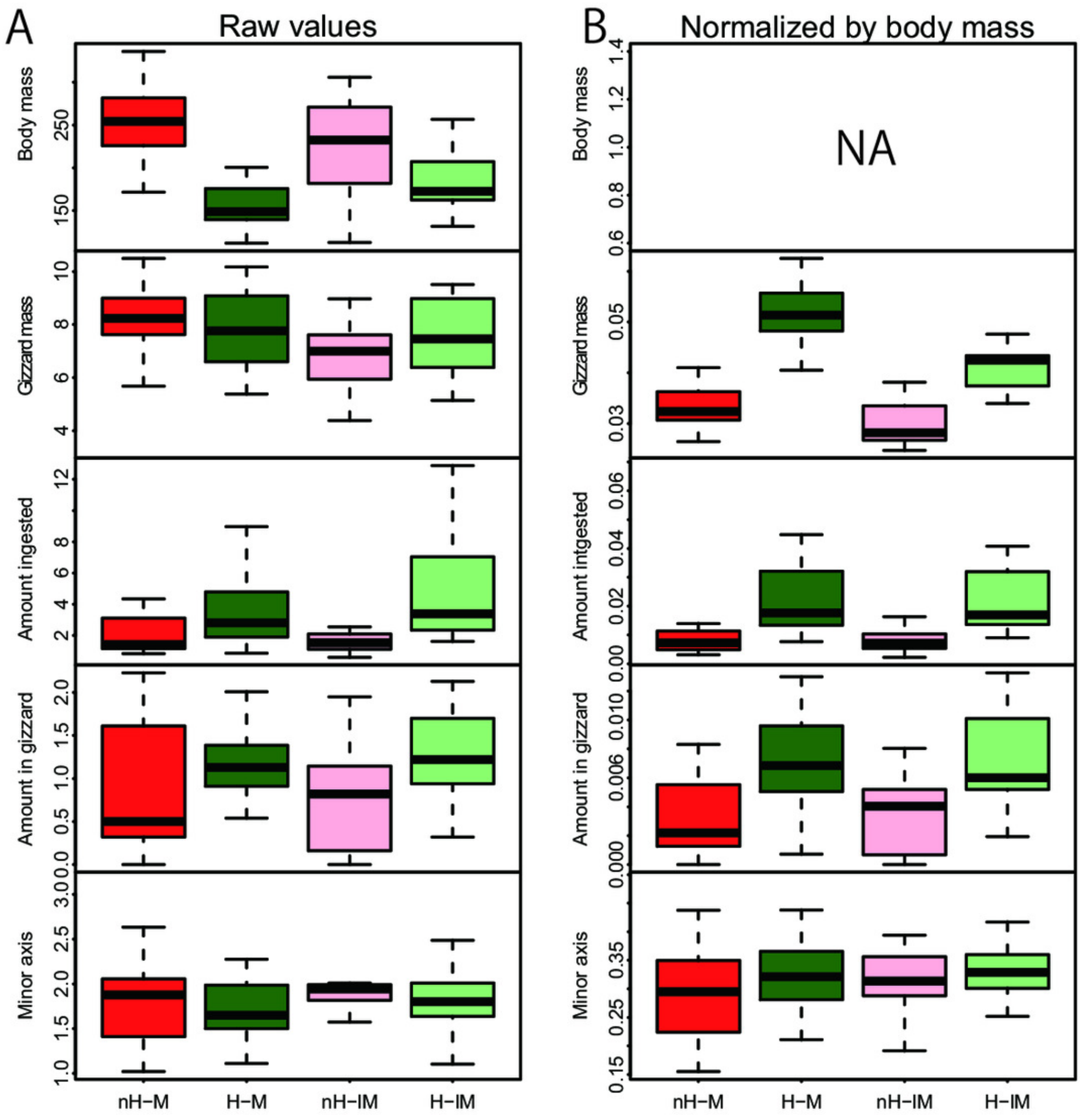

Figure 3

Boxplots showing amount of ingested grit by experimental groups, shown per grit size categories.

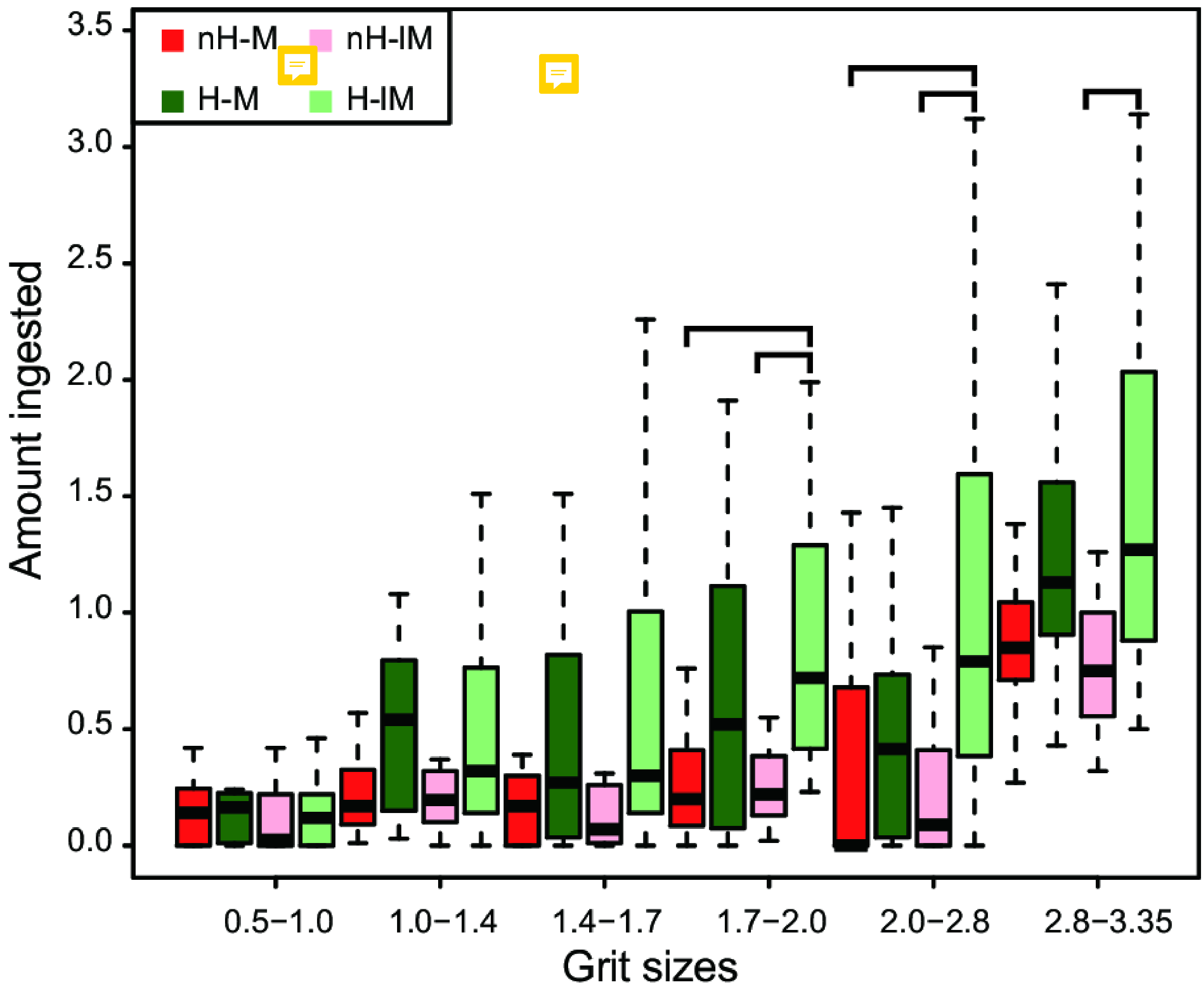

Figure 4

Boxplots comparing grit shapes.

(A) Boxplots comparing shapes of the initial grits and the remained grits by each experimental group, shown per grit size categories. (B) Boxplots comparing shapes of the stones by the experimental groups, shown per stone size categories. The brackets represent p-values < 0.05.

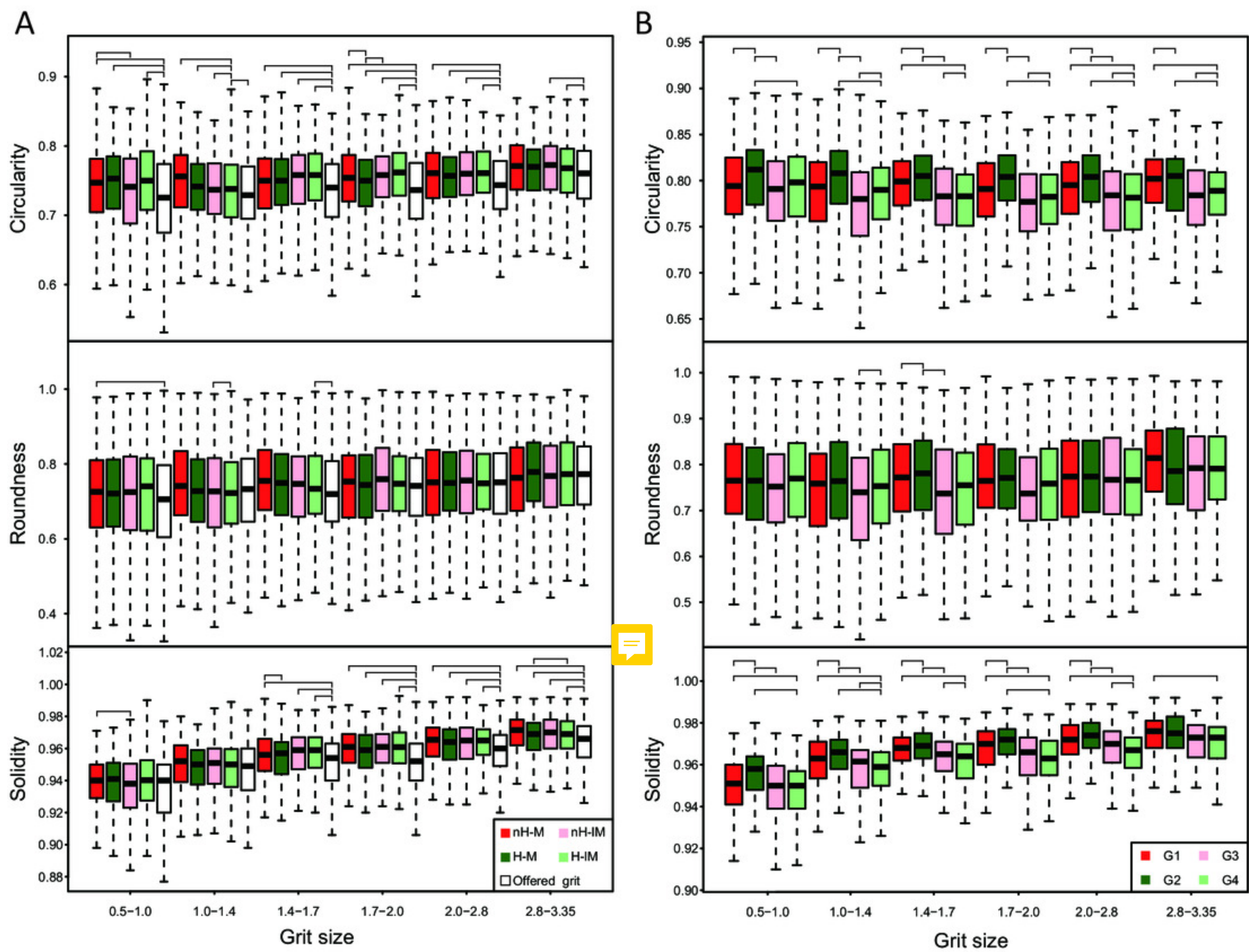

Figure 5

Schematic summary of the results this experiment