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

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~~A~~ gizzard is the only gastrointestinal organ in birds for ~~a~~ mechanical digestion ~~by having some degree of muscular development~~. Many birds ingest and utilize grit~~s~~ in ~~a~~ gizzard to enhance mechanical digestion efficiency. ~~This study~~ conducted an experiment on the regulatory factors of 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 ~~by~~ two different types of diet (herbivory and non-herbivory) to test whether diet and gizzard muscularity of chicks ~~regulate~~ grit characteristics such as size, amount, and shape (circularity, roundness, and solidity) of different stages (ingested grits, gizzard grits, and excreted grits).

~~This experiment demonstrated that~~ ingested grit~~s~~ we~~re~~ larger and ~~that they have~~ lower circularity and solidity than ~~initial grits~~, regardless of ~~types of~~ diet and gizzard muscularity. ~~On the other hand,~~ the amount of ingested grit~~s~~ ~~differed by diet (~~greater in herbivorous groups~~)~~. The size and amount of gizzard ~~grits also differed by diet (~~larger and greater in herbivorous groups~~)~~, and the size of gizzard ~~grits further differed by gizzard muscularity (~~larger in the less-muscular gizzard group~~)~~. The shape indexes (circularity, roundness, and solidity) of gizzard ~~grits~~ were higher than those of ~~initial grits~~. The circularity and solidity of gizzard ~~grits differed by diet and gizzard muscularity (~~higher in herbivorous and muscular gizzard groups~~)~~. The size and solidity of excreted ~~grits~~ were smaller and higher than those of gizzard ~~grits.~~ The size and amount of excreted ~~grits differed by diet (~~larger in non-herbivorous groups~~)~~, and the size of excreted ~~grits further differed by gizzard muscularity (~~larger in less-muscular gizzard groups~~)~~.

These results show that diet ~~regulates~~ the characteristics of ingested and excreted grits, whereas gizzard muscularity regulates the characteristics of excreted grits. The use of large size and amount of gizzard grits in herbivorous groups as well as a high ability to retain small gizzard grits in less-muscular gizzard groups may be regulations upon needs of digesting hard, coarse materials. Flexible regulations on gizzard grit use might reflect the omnivorous nature of *Gallus gallus domesticus* and may aid their smooth diet shifts. The results also show that gizzard grit shapes do not reflect the shapes of ingested grit~~s unlike~~ previously ~~considered,~~ but ~~instead, gizzard grit~~ shape~~s~~ reflect diet and gizzard muscularity of chick~~s~~.

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ABSTRACT

A gizzard is the only gastrointestinal organ in birds for a mechanical digestion by having some degree of muscular development. Many birds ingest and utilize grits in a gizzard to enhance mechanical digestion efficiency. This study conducted an experiment on the regulatory factors of 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 by two different types of diet (herbivory and non-herbivory) to test whether diet and gizzard muscularity of chicks regulate grit characteristics such as size, amount, and shape (circularity, roundness, and solidity) of different stages (ingested grits, gizzard grits, and excreted grits).

This experiment demonstrated that ingested grits were larger and that they have lower circularity and solidity than initial grits, regardless of types of diet and gizzard muscularity. On the other hand, the amount of ingested grits differed by diet (greater in herbivorous groups). The size and amount of gizzard grits also differed by diet (larger and greater in herbivorous groups), and the size of gizzard grits further differed by gizzard muscularity (larger in the less-muscular gizzard group). The shape indexes (circularity, roundness, and solidity) of gizzard grits were higher than those of initial grits. The circularity and solidity of gizzard grits differed by diet and gizzard muscularity (higher in herbivorous and muscular gizzard groups). The size and solidity of excreted grits were smaller and higher than those of gizzard grits. The size and amount of excreted grits differed by diet (larger in non-herbivorous groups), and the size of excreted grits further differed by gizzard muscularity (larger in less-muscular gizzard groups).

These results show that diet regulates the characteristics of ingested and excreted grits, whereas gizzard muscularity regulates the characteristics of excreted grits. The use of large size and amount of gizzard grits in herbivorous groups as well as a high ability to retain small gizzard

grits in less-muscular gizzard groups may be regulations upon needs of digesting hard, coarse materials. Flexible regulations on gizzard grit use might reflect the omnivorous nature of *Gallus gallus domesticus* and may aid their smooth diet shifts. The results also show that gizzard grit shapes do not reflect the shapes of ingested grits unlike previously considered, but instead, gizzard grit shapes reflect 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 experienced morphological and physiological adaptations during their evolutionary history. Birds have acquired specialized gastrointestinal organs, including a crop for temporal storage of food (Proctor & Lynch, 1993), caeca for fermentation of plant fiber (Potter et al., 2006), and a gizzard for mechanical digestion of ingesta (Moore, 1999). In a gizzard, ingesta experience strong compressing and translational stress to be mechanically processed (Moore, 1998a). Large food particles are selectively retained in a gizzard until these are grinded to small size (Hetland et al., 2003; Moore, 1999)

For better digestion efficiency, birds ingest and retain grits 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 grits in cases where there is insufficient sands or gravels in their surrounding environment (McIlhenny, 1932). Grit use is especially common in herbivorous and granivorous birds for mechanical breakdown of rough, coarse food (Gionfriddo & Best, 1999). Previous works generally agree that grits improve digestion efficiency in birds, especially of the ones that feed on coarse, less-nutritional food (Fritz, 1937; Hetland et al., 2003; Jin et al., 2014; Smith & MacIntyre, 1959). Despite of the importance of grits for a mechanical digestion, the regulatory factors of grit use is not well-understood because previous studies are based primarily on grits collected from gizzards (e.g., Best & Gionfriddo, 1991; Gionfriddo & Best, 1996; Norris et al., 1975) although grit characteristics can be strongly modified in a gizzard (Buckner et al., 1926; Wings & Sander, 2007).

Here we report an experiment which tests changes in the amount, size, and shape of grits by different diet and gizzard muscularity, in order to understand the regulatory factors of grit use in domestic chickens. It is commonly assumed that diet affects grit use in birds (e.g., Fritz, 1937; Gionfriddo & Best, 1999; Hoskin et al., 1970; Norris et al., 1975; Soler et al., 1993), but our experiment also test effects of gizzard muscularity difference because gizzard with a higher muscularity is likely to have higher digestion efficiency by itself and may not require help of grits. The regulatory factors upon grit ingestion, retention in a gizzard, and excretion were tested 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) 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 gizzard muscularity (evaluated as relative weight of a gizzard and the body mass of the chick). Following Sacranie et al. (2012), the development of a gizzard muscularity was enhanced on half of the chicks (34 individuals) by feeding larger amount of insoluble fiber through providing the mixture of 70 wt% of commercial starter pellet and 30 wt% of rice hull, whereas only commercial starter pellet was fed on the other half. The chicks had *ad libitum* access to feed and water.

This experiment was conducted for one week, on four groups (17 individuals each) under four combinations of two factors: diets (herbivorous and non-herbivorous) and gizzard muscularity (muscular and less muscular) at the time of starting the experiment. These four groups are as followed: 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 grass (*Medicag sativa*) and Fabaceae grass (*Phleum pratense*), whereas non-herbivorous groups (nH-M and nH-lM) fed on dried fish (*Engraulis japonicas*). During the experiment, all chicks were raised in separated 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 grits per chicks, which were provided separate from feed. Feces on the last day of the experiment were collected to evaluate characteristics of excreted grits. 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.

Grit characteristics

The amount, size, and shape of grits (initial, remained, gizzard, and excreted grits) were evaluated. The amount of grits was evaluated in weights (grams). Size and shape were evaluated quantitatively using image processing program ImageJ (Schneider et al., 2012). A minor axis was employed as a grit size index. Circularity, roundness, and solidity were employed as the indexes of grit shape. 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. Solidity was calculated as an area of a grit divided by an area of convex hull. To take the images for the analyses, grits were positioned segregated from each other and were lighted from the background to obtain clear outlines.

Terminology

Initial grits were the stones, which were given *ad libitum* access to each chick (Fig. 1). Ingested grits were the stones, which were swallowed by the chicks out of the initial grits during the experiment. Remained grits were the stones, which were not ingested by the chicks out of the initial grits by the end of the experiment. Gizzard grits refer to the stones remained in the gizzards of the chicks after euthanization. Excreted grits were 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. Muscular gizzard groups were set by feeding on a mixture of rice hull and starter pellets for three weeks prior to the experiment and had relatively high gizzard muscularity, whereas less-muscular gizzard groups were set by feeding on starter pellets three weeks prior to the experiment and had relatively low gizzard muscularity. “Sharp” is used to describe grits with relatively low circularity, roundness, and/or solidity and “dull” is used to describe grits with relatively high shape indexes.

Ingested grits

The amount, size, and shape of ingested grits were evaluated by comparing the characteristics of initial and remained grits. To test size preference by the chicks, the size distribution of initial grits was controlled in advance. Grits were classified into six different size classes by sieving (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) and four grams of grits from each size classes were supplied in mixture as initial grits. Prior to the experiment, 500 grits were randomly chosen from each size classes of initial grits and circularity, roundness, and solidity of the grits were evaluated. At the end of the experiment, remained grits were collected and sieved into the six size classes. The amount of the ingested grits in each size classes was evaluated by subtracting the weights of the remained grits from weights of the initial grits by each size classes (4 grams each) to test size preferences. Average values of the shape indexes of the remained grits were compared with those of the initial grits to test if there was any shape preference on the ingested grits. The amount, size, and shape of the remained grits were then compared among different diet and gizzard muscularity groups.

Gizzard grits

Gizzard grits were separated from other stomach contents by a floatation method (decantation). Stomach contents were soaked with water in a beaker over one night, and low density food particles were gently disposed. This procedure was repeated until only grits ~~were~~ remained in the beaker. The amount, size, and shape of the gizzard grits were compared among different diet and different gizzard muscularity groups.

Excreted grits

Excreted grits were separated from fecal particles using the same method as separating gizzard grits. The amount, size, and shape of the excreted grits were evaluated and compared with those of the gizzard grits to test on selection of excretion. Grit characteristics were also compared among different diet and gizzard muscularity groups.

Statistical analyses

All statistical analyses were conducted using basic functions of software R (R Core Team, 2019). Tukey-Kramer HSD tests were performed for mean comparisons. Chicks, euthanized following the Hokkaido University rules prior to the end of the experiment, were excluded from the analyses. The data analyzed are provided as Supplemental Information (Data S1-S5).

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 following the Hokkaido University regulations before the end of the experiment. Therefore, all of the analyses were performed on total of 56 chicks.

Ingested grits

Large grits (>2.8 mm) were ingested significantly more than smaller grits (<2.8mm) in all groups (Table S1). The average amount of ingested grits was significantly higher in the herbivorous and less-muscular gizzard group than in the non-herbivorous and less-muscular gizzard group (H-lM > nH-lM) for grits larger than 1.7 mm (Fig. 2, Table S2). Remained grits generally had higher average circularity and solidity than initial grits in all groups (Fig. 3A, Table S3). This trend for circularity was statistically supported in nearly all of the girt sizes other than 1.0–1.4 mm. The trend for solidity was statistically supported for grits larger than 1.7 mm. There was generally no difference in average roundness between initial and remained grits. No general difference in size and shape of ingested grits among different diet and gizzard muscularity groups was confirmed (Table S1, Table S2).

Gizzard grit

Herbivorous groups retained significantly more gizzard grits than non-herbivorous groups in average weights relative to the body mass (H-M > nH-M, H-lM > nH-lM; Table S4). The average sizes of gizzard grits, normalized by cubic root of a body mass, were significantly larger in herbivorous groups than in non-herbivorous groups (H-M > nH-M, H-lM > nH-lM; Table S5). Within non-herbivorous groups, the less-muscular gizzard group contained larger gizzard grits in respect to their body mass than the muscular gizzard group (nH-lM > nH-M). The average circularity and solidity of gizzard grits were significantly larger than those of initial grits in all groups (Table S6). Average roundness of gizzard grits were also significantly higher than those of initial grits in herbivorous groups (H-M and H-lM). The average circularity and solidity of gizzard grits of herbivorous groups were significantly higher than those of non-herbivorous groups (H-M > nH-M, H-lM > nH-lM; Fig. 3B; Table S7). Within herbivorous groups, the gizzard grits of the muscular gizzard group ~~have~~ significantly higher average circularity (with exception of gizzard grits sized 1.4-1.7 mm and 2.8-3.35 mm) and solidity (with exception of gizzard grits sized 1.4-1.7 mm and <2.0 mm) than those of the less-muscular gizzard group (H-M > H-lM).

Excreted grits

The average sizes of excreted grits were significantly smaller than those of gizzard grits of corresponding groups (Table S8). The average solidity of excreted grits was also significantly smaller than the gizzard grits of the corresponding groups except in the non-herbivorous and muscular gizzard group (nH-M). At the same time, the average circularity and solidity of excreted grits were higher than those of initial grits. Amount of excreted grits are 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). The average sizes of excreted grits were significantly larger in non-herbivorous groups than in herbivorous groups (nH-M > H-M, nH-lM > H-lM; Table S9). The average size of the excreted grits of the non-herbivorous and less-muscular gizzard group was also significantly larger than that of the non-herbivorous and muscular gizzard group (nH-lM > nH-M). The average circularity, roundness, and solidity of excreted grits did not differ significantly among different diet or muscularity groups (Table S9).

DISCUSSION

Amount regulations

The larger amounts of ingested and gizzard grits in herbivorous groups than in non-herbivorous groups (H-lM > nH-lM; Fig. 2, Tables S2, S4) are concordant with previous studies (comprehensive review done in Gionfriddo and Best, 1999). The larger amounts of excreted grits in non-herbivorous groups than in herbivorous groups (nH-M > H-M, nH-lM > H-lM) suggests that the large amounts of gizzard grits in herbivorous groups are regulated by both large amount of grit ingestions and limited grit excretions. These regulations to retain larger amount of gizzard grits are likely to benefit herbivorous groups to supply higher digestion ability for breaking down tough plant fibers, since larger amount of gizzard grit improves digestive performance in domestic chickens (Bale-Therik et al., 2012) as long as the amount is not excessive (Moore, 1998b).

Size regulations

Since the size of gizzard grits is unlikely to affect digestion efficiency in domestic chickens (Smith, 1960), or larger gizzard grits may even have lower digestion efficiency than smaller gizzard grits (Moore, 1998c), dominances of grits larger than 2.8 mm among the ingested grits in all groups (Table S1) may simply be due to ease to pick large grits. The smaller size of excreted grits than gizzard grits (Table S8) in all groups suggest that size is one of the primary factors that determine which grits to be excreted in domestic chickens. While the excretion of small grits is concordant with a trend in domestic chicken (Smith, 1960), it is controversial with this trend in house sparrow (Gionfriddo & Best, 1995). Therefore, the size regulation of excreted grits may vary taxonomically.

The larger sizes of ingested and gizzard grits in herbivorous groups than in non-herbivorous groups (H-M > nH-M, H-lM > nH-lM) are 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 larger size of excreted grits in non-herbivorous groups than in herbivorous groups (nH-M > H-M, nH-lM > H-lM) suggests that the herbivorous groups have higher ability to retain small gizzard grits than the non-herbivorous groups do. The high ability to retain small gizzard grits in the herbivorous groups is likely to result in retaining larger amount of gizzard grits, which provide better digestion efficiency (see above). The larger sizes of gizzard and excreted grits in the non-herbivorous and less-muscular gizzard group than in the non-herbivorous and muscular gizzard group (nH-lM > nH-M) indicate that gizzard muscularity also contributes to the size regulation of gizzard grits.

Shape regulations and abrasions

The greater sharpness of ingested grits than that of initial grits in all groups (Fig. 3A, Table S3) is consistent with previous knowledge in domestic chickens (Smith, 1960) as well as in House Sparrows and Northern Bobwhite (Best & Gionfriddo, 1994). Since sharp gizzard grits function as “blades” in a gizzard, this selection would increase digestion efficiency (Moore, 1998c). The active ingestions of sharp grits in all groups are likely to be a congenital behavior unlike the amount of ingested/excreted grits which are likely to be regulated upon demands (see above). The gizzard grits are duller than the initial grits in all groups (Table S6), which contradicts with the sharper ingested grits than the initial grits. Since the excreted grits were also duller than the initial grits (Table S9), the best explanation is that the gizzard grits are severely abraded inside of the gizzards upon mechanical digestion of ingesta.

The dominance of dull gizzard grits in herbivorous groups more than in non-herbivorous groups (H-M > nH-M, H-lM > nH-lM; Fig. 3B, Table S7), as well as in muscular gizzard groups than in less-muscular gizzard groups (H-M > H-lM, nH-M > nH-lM), strongly suggests that diet and gizzard muscularity affect the degree of abrasions on gizzard grit. 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 the primary factor which determines the degree of gizzard grit abrasion. Correlations between gizzard grit shape indexes (average values of circularity, roundness, and solidity per individual) and gizzard muscularity ($p < 0.05$) further support this assumption. Therefore, the shapes of gizzard grits are unlikely to reflect grit selection patterns in domestic chickens unlike previously considered in birds (Best & Gionfriddo, 1991; Gionfriddo & Best, 1996). Instead, our experiment suggests that the differences in gizzard grit shapes reflect differences in diets and gizzard muscularities, although investigations in broader taxonomic sets are essential to test the assumption.

Regulations of chick grit use behaviors

This study is the first attempt to examine the regulatory factors of chicken grit uses throughout ingestion, retention, and excretion among different diets and gizzard muscularities. This experiment strongly suggests that chick grit use behaviors are primarily regulated by a diet and secondarily by a gizzard muscularity (Fig. 4, Table 1). The flexible regulations 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 gizzard grit use behaviors 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 grit use behaviors demonstrated that chicks had a selection on size, amount, and the shape of ingested and excreted grits. It also revealed that gizzard grit shapes were greatly modified through abrasion therefore did not reflect the original shapes upon ingestion. Instead, gizzard grit shapes reflected diets and gizzard muscularities of chicks. Ingestion of sharp grits regardless of diet and gizzard muscularity suggested that the behavior which facilitates better digestion efficiency was congenial to chicks. On the other hand, the ingestion and retention of larger amount of grits by herbivorous groups and non-herbivorous, muscular gizzard groups may be behavioral adaptation to supple digestion ability upon need of digesting coarse ingesta. The flexibility might be reflecting the omnivorous nature of chickens.

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Table 1(on next page)

Summarized results of the present experiment.

	Stone characteristics
Ingested grits	
Size	Larger than the initial grits
Amount	H-lM > nH-lM
Shape	Sharper than the initial grits
Gizzard grits	
Size	H-M > nH-M, H-lM > nH-lM nH-lM > nH-M
Amount	H-M > nH-M, H-lM > nH-lM
Shape	More dull than the initial grits H-M > nH-M, H-lM > nH-lM H-M > H-lM
Excreted grits	
Size	Smaller than the gizzard grits nH-M > H-M, nH-lM > H-lM nH-lM > nH-M
Amount	nH-M > H-M, nH-lM > H-lM
Shape	Sharper than the gizzard grits, duller than the initial grits

1

Figure 1

Visualized terminology regarding the grits treated in this study.

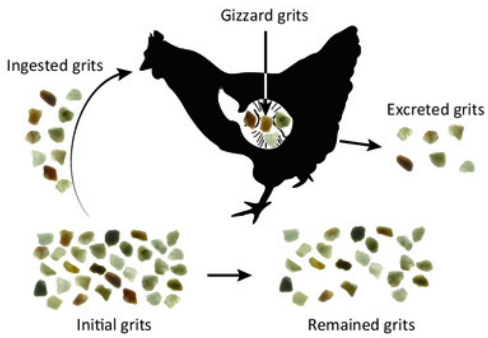

Gizzard grits
Ingested grits
Excreted grits
Initial grits
Remained grits

Figure 2

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

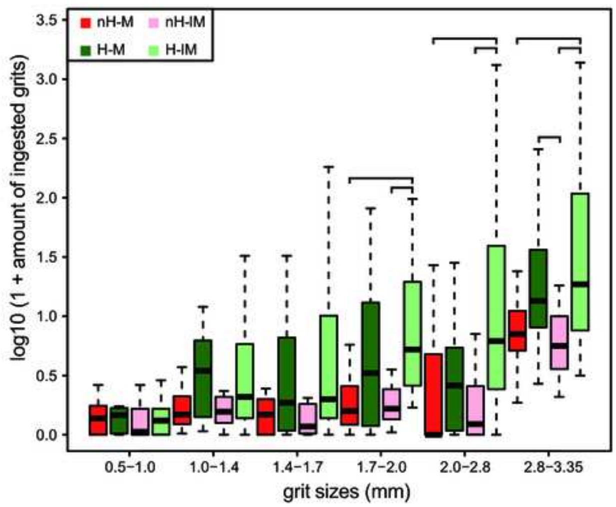

nH-M
nH-IM
H-M
H-IM
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
log10 (1 + amount of ingested grits)
0.5−1.0
1.0−1.4
1.4−1.7
1.7−2.0
2.0−2.8
2.8−3.35
grit sizes (mm)

Figure 3

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.

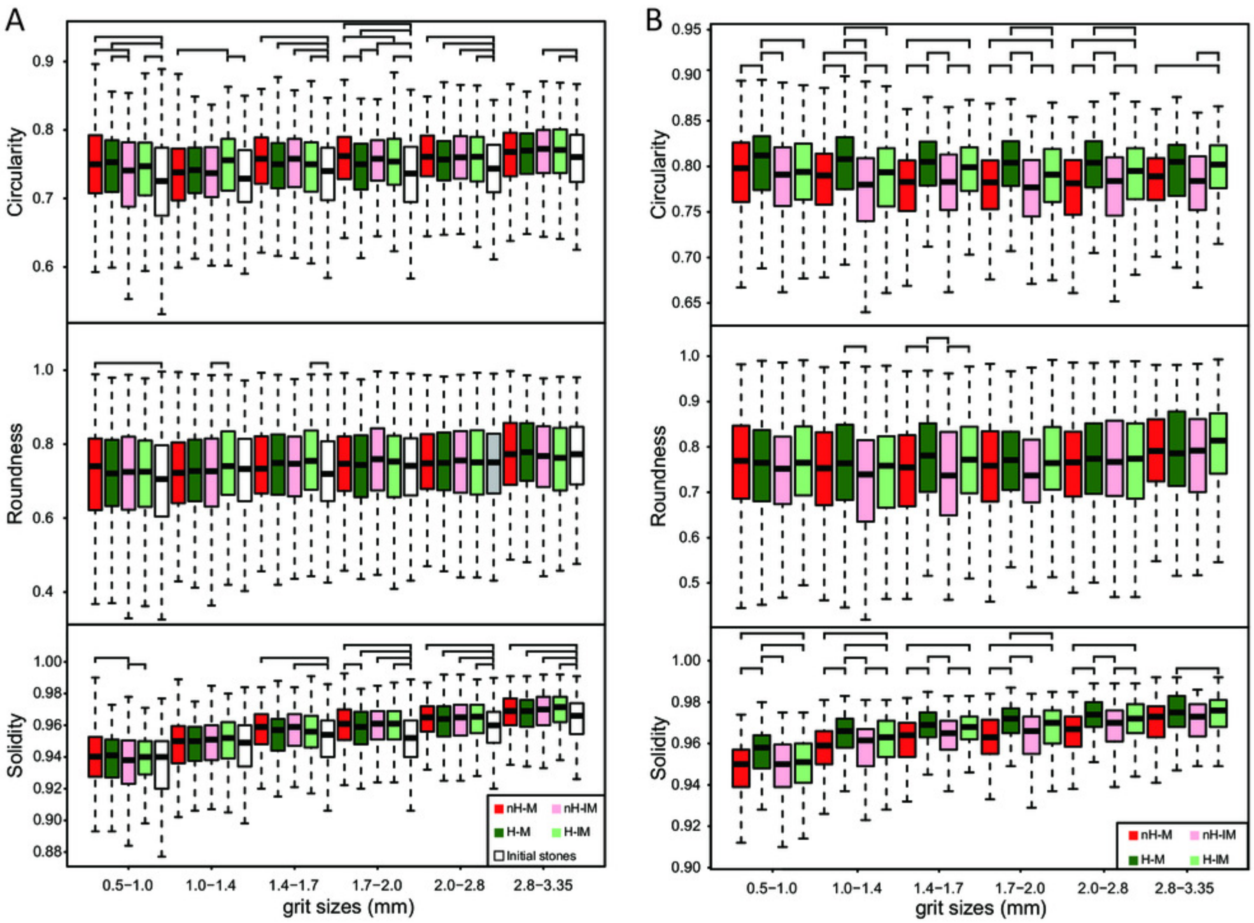
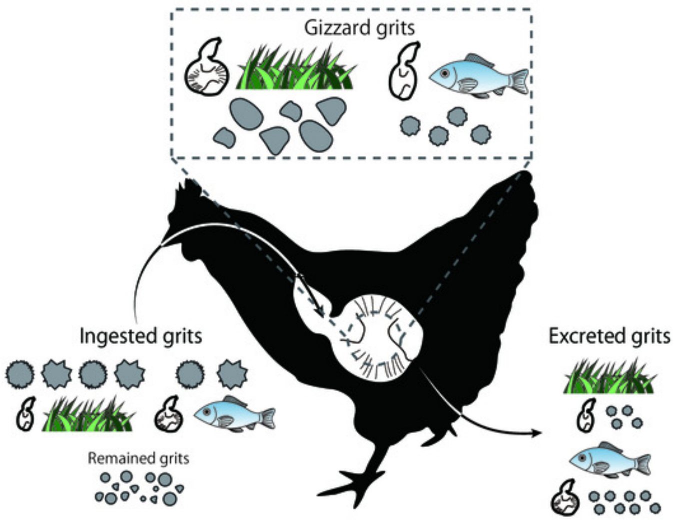

A
Circularity
Roundness
Solidity
0.5–1.0
1.0–1.4
1.4–1.7
1.7–2.0
2.0–2.8
2.8–3.35
grit sizes (mm)
nH-M
nH-IM
H-M
H-IM
Initial stones
B
grit sizes (mm)

Figure 4

Schematic summary of the results this experiment


Gizzard grits
Ingested grits
Excreted grits
Remained grits