

Research Note: Integrated gut microbiome and short-chain fatty acids responds to dominance hierarchy in roosters

Siyu Chen,^{*,1} Chao Yan,^{†,1} Wen Liu,[†] Kecheng Chen,^{*} Limin Xing,^{*} Hua Li,^{*} and Xingbo Zhao^{*,†,2}

^{*}Guangdong Provincial Key Laboratory of Animal Molecular Design and Precise Breeding, School of Life Science and Engineering, Foshan University, Foshan, China; and [†]College of Animal Science and Technology, China Agricultural University, Beijing, 100193, China

ABSTRACT The dominant chickens have priority over the use of resources, such as resting places and the announcement of dawn. While cooperation from the subdominant animal is of great help to reduce conflict and maintain the sustainability of a group. However, whether the dominance hierarchy is associated with individuals' health is not yet known. In this study, we first determined the dominance hierarchy within a group of roosters, to figure out its effects on individuals' health status by the determination of microbial composition and short-chain fatty acids (SCFAs). Sixteen Weining roosters were kept in a group in order to fix and determine the ranking of dominance hierarchy, as R1 (the highest-ranking rooster), R2, R3, and R4. Results show that the R1 roosters had the highest aggression behavior

followed by R2, R3 and R4 ($P < 0.05$). The alpha diversity of R1, R2, and R4 was higher than R3 roosters ($P < 0.05$). There were several top 10 phylum and genus microbes among the different ranking roosters ($P < 0.05$). The acetic acid, propionic acid, butyric acid, and valerate acid concentrations were higher, while isobutyric acid concentration was lower in the higher rank roosters (R1 and R2) than the lower rank roosters, respectively (R3 and R4) ($P < 0.05$). Our results show that the variation of dominance hierarchy contributes to changes of microbial composition, diversity and metabolites. Dominant roosters seem to benefit from SCFAs activities while subdominant roosters profit from microbial functions.

Key words: rooster, social rank, gut microbiome, metabolome, short-chain fatty acids

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INTRODUCTION

Dominance hierarchy, described as the repeated win of one individual over another, commonly exists in all social species. As known, animals with different social status show various behavioral responses such as boldness and explorative tendencies, which are usually consistent across time and/or context. The highest ranking rooster has the priority to announce the break of dawn, implying a prominent status of high rank individuals (Shimmura et al., 2015). A positive correlation between social rank and the outcome of competition for food resources in layers (Shimmura et al., 2010), suggests a priority of higher social rank in layers. Higher social dominance can contribute to benefits on reproductive

success and immune response and thereby fitness. While subdominant animals are confronted with a lack of resources or threats from higher ranking individuals, resulting in a stressful status (Shimmura et al., 2010). From the above literatures, we consider that the higher dominance animal may be of significance for the selection for breeders. Notably, a cooperation of dominant and subdominant individuals, such as the least conflict, aggressive behavior, and reasonable resource allocation, is of great help for the sustainability of a group. It is known that low-status monkeys are also more considerate to others' behavior and only express their learning ability when segregated from higher ranking animals, indicating a balance of social rank with priority to resources (Drea and Wallen, 1999). In mice, lower social rank rodents can learn to make and observe rules to resolve conflict, which in turn enhances long-term benefit and payoff equity within a stable group (Choe et al., 2017). That is to say, the role of the lower social rank animal is also essential. Thus, it would be interesting to know whether resource priority or personality for dominance hierarchy is associated with the health of individuals.

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¹These authors contributed equally to this work.

²Corresponding author: zhxbcau@126.com

The gut microbial community due to its association with the host health and behaviors has been paid increased attention in a broad range of animal species (Chen et al., 2019). Numerous studies have shown that the role of intestinal flora includes nutrient absorption, mucosal barrier fortification, angiogenesis, immunity, and response to infection (Morais et al., 2021). The decreased microbial diversity is associated with diseases and brain function related to anxiety-like and stress-related behaviors in mice (Morais et al., 2021). Short-chain fatty acids (SCFAs) are mainly produced by gut microbiota fermentation of dietary fiber and play a critical role in microbiota-gut-brain crosstalk (Dalile et al., 2019). Collectively, SCFAs have been implicated in numerous physiological functions including gastrointestinal functionality, host metabolism, circadian rhythm and immune functions (Wouw et al., 2018). Emerging evidence indicates that SCFAs can regulate appetite, energy homeostasis and physiological processes. Therefore, an integrated study combining gut microbiome and SCFAs is essential and would help to better understand the benefits of social dominance.

The study was aimed to explore the relationship between the social dominance and gut microbiota, as well as its metabolites, so as to provide a more detailed profile of social dominance on health of Weining chickens. This would also give a new insight into the selection strategies of breeder roosters.

MATERIALS AND METHODS

Animals and Treatments

This study was approved by the Animal Care Committee of Foshan University (Approval ID: FOSU#080). A total of 16 Weining roosters, a local breed in Southwest China, were randomly selected from a 100-rooster group, reared in a free-range farm. The roosters were housed in four groups of four roosters. They were 180 days old when they entered the experiment with an average body weight of 1,850 g (standard deviation = 40.06). To avoid the inner interruption of crowing and environmental noise from other groups, these birds were moved to a single barn 15 d before the test and each group was kept in an individual cage (1.5 m × 0.5 m × 0.8 m) with a single light- and sound-tight room. Animals had ad libitum access to water and commercial breeder feed (corn-soybean meal diets from the New Hope Group, Sichuan, China) renewed at 8:00 am daily and were marked using colored leg rings combined with painting different colors on the back and head feathers.

Behavioral Traits

Maintenance Behaviors After 14-d of familiarity with the environment and mates, behaviors including aggressive (attacking another bird with the beak or claws by prolonged episodes of pecking and scratching or continuous performance of this behavior), being aggressive

(attacking object) and crowing were recorded for 2 h by 2 video cameras (Sony, Japan) after feeding for 3 consecutive days. Continuous behavioral samplings were analyzed by one observer (the same as below).

Confrontation Test Then, each group was moved to a test arena (2.0 m × 2.5 m × 1.5 m) enclosed by solid panels. Aggressive behaviors, being aggressive behaviors, and crowing behaviors were counted for 10 min.

Food Competition Test Birds were deprived of food from 18:00 the day before the food competition test. Briefly, in each group, 2 randomly combined birds in a pair (6 pairs: rooster 1&2, 1&3, 1&4, 2&3, 2&4, 3&4 paired) were placed in a test arena (2.0 m × 2.5 m × 1.5 m) for 5 min. One leg of the pair of birds was tied by a 1 m length rope to limit their free activities. The arena contained a feeder, which only allowed one bird at a time to feed. The aggressive behaviors, and the time that birds occupied the feeder were recorded.

Dominance Hierarchy From the record of the aggressive interactions from the above three events, the dominance value of individual roosters was calculated by using the Index of Clutton-Brock (ICB) was calculated (Shimmura et al., 2015). The formula was as follow: dominant value = $(B + \sum b + 1) / (L + \sum l + 1)$. Where B = number of individuals that a rooster beat; $\sum b$ = total numbers that all roosters beat excluding the number of the subject itself; L = number of individuals that the rooster lost to; $\sum l$ = total number that all roosters lost to excluding the subject itself. Accordingly, rank 1 (R1), R2, R3, and R4 roosters with the descending order of the social rank were calculated.

Gut Microbiome The day after the food competition test, 16 birds were humanely euthanized, and the cecum contents of each bird were collected. They were stored in dry ice and then at -80 °C until further processing. Cecal DNA was extracted using the QIAamp DNA stool mini kit (Tiangen Biotech, Beijing, China) according to the manufacturer's protocol and then tested for quality to construct a library for sequencing, which was described in our previous study (Chen et al., 2019). Based on the OTU information, the relative abundance of taxonomic ranks and alpha diversity were analyzed through bioinformatics. Alpha diversity concerning the Shannon diversity index was used to explore within-group sample diversity. Higher index numbers represent higher alpha diversity.

Gut-Microbial Metabolisms of SCFAs Cecum contents from each bird were also detected for gut-microbial metabolisms of SCFAs. The 100 mg of cecum contents were added with 100 μL 15% phosphoric acid, 50 μg/mL internal standard (isohexanoic acid) solution, and 400 μL ether for homogenization for 1 min. The mixtures were vortexed for 30 s and shaken for 15 min at 4° C. The homogenates were centrifuged at 12,000 rpm for 10 min at 4°C. All Chenomx Nuclear Magnetic Resonance (NMR) data were obtained using a gas mass spectrometry (Agilent) at 600 MHz with a TXI z-gradient probe at 25°C. Serum samples were added to the phosphate buffer solution at the same final concentration as the intestine samples. Metabolites were assigned

using the Chenomx NMR Suite 8.2 software (Chenomx Inc., Edmonton, Alberta, Canada).

Statistical Analyses

All the data were analyzed by SPSS version 25. The dominant value was calculated using the frequency of aggressive and being aggressive behaviors in maintenance behaviors, confrontation test and food competition test. The frequency of aggressive behavior, crowing, being aggressive behavior data did not meet the assumptions for parametric analysis, and could not be successfully transformed to meet the assumption, which was thereby analyzed by Kruskal-Wallis H and Post-Hoc tests. All values with $P < 0.05$ were regarded as statistically significant.

RESULTS AND DISCUSSION

The dominant position of an individual is determined by various factors including body size, kinship, prior dominance experience and residency. In order to fix and determine the dominance hierarchy, behavioral tests were conducted. Our results (mean \pm SE) show that the R1 (63.25 \pm 10.56) roosters performed more aggressive behavior followed by R2 (40.00 \pm 6.33), R3 (25.25 \pm 3.25), and R4 (4.75 \pm 2.02) ($P = 0.005$), while the received aggressive behavior was seen highest in R4 (91.25 \pm 8.03) roosters than R3 (21.75 \pm 5.66), R2 (12.5 \pm 1.44), and R1

(4.75 \pm 1.65) roosters ($P = 0.004$). These suggest a linear position of dominance hierarchy of individuals within a group. However, the frequency of crowing behavior was not different among the four ranking roosters, which is inconsistent with Shimmura et al. (2015) who reported that the highest-ranking roosters have a priority to announce the break of dawn. We assume that this may be due to the timing to observe crowing, and crowing for dawn is probably associated with the dominance hierarchy, but not for crowing for other motivations.

We next examined the effect of dominance hierarchy on gut microbiome and metabolites to figure out the implication of health-related aspects. The alpha diversity of R1, R2, and R4 was higher than R3 roosters in terms of Shannon diversity analyses ($P = 0.005$, Figure 1A), but did not differ among R1, R2, and R4. Previous works showed caged rearing reduced stress in chicken (Chen et al., 2019) and maternal separation stress in mice could result in decreased gut microbial diversity (Bailey et al., 2010). Besides, social rank greatly influences animals' access to resources and is therefore a stressor to lower ranking animals. However, our results did not show a descending order of the alpha diversity from higher ranking to lower ranking roosters. This may imply that a stable group under the cooperation of dominant and subdominant individuals is of great significance and has a profound role on maintaining an individual's benefits. The relative abundance of the top three microbes at the phylum level was *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, which is in agreement with our previous study

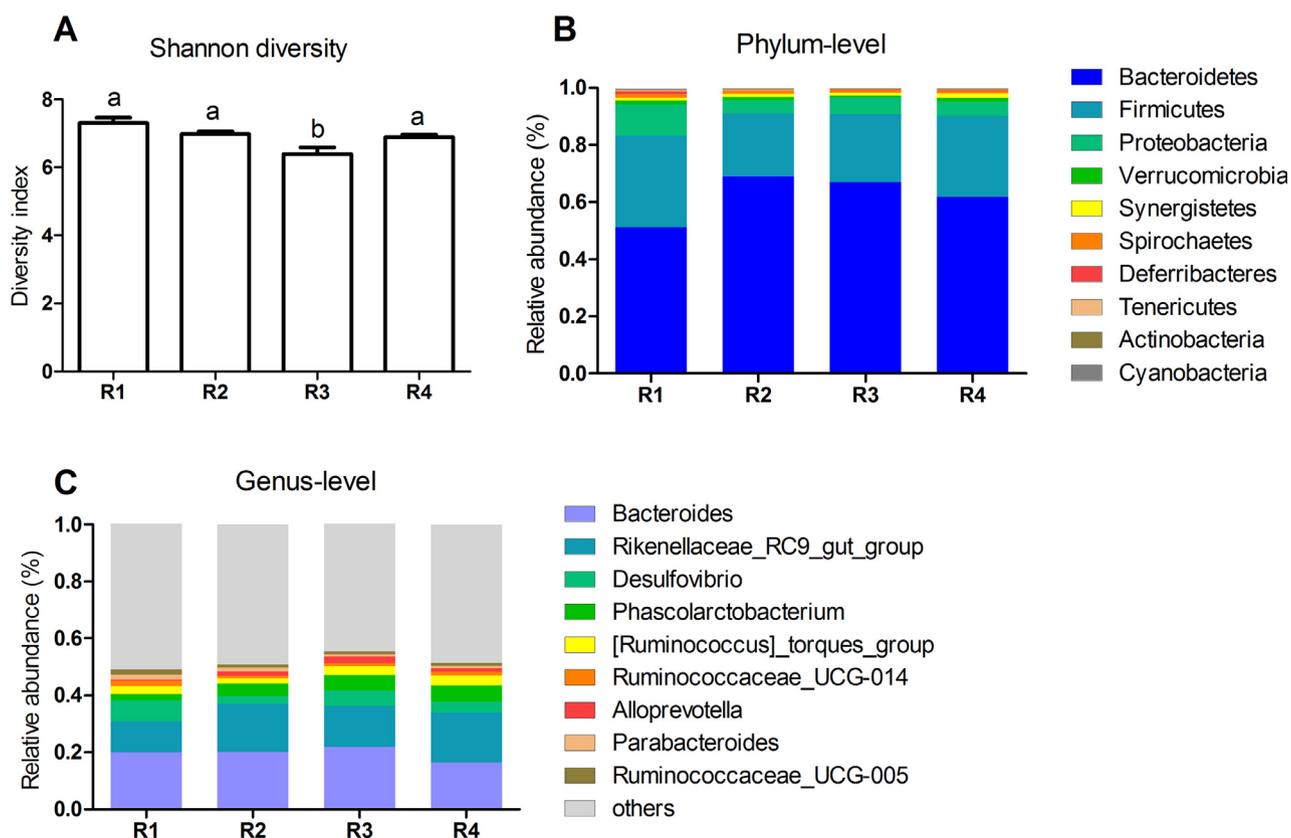


Figure 1. Gut microbiota: alpha diversity (A) and relative abundance (%) at the phylum level (B) and at the genus level (C). R1: highest rank; R2: second rank; R3: third rank; R4: lowest rank.

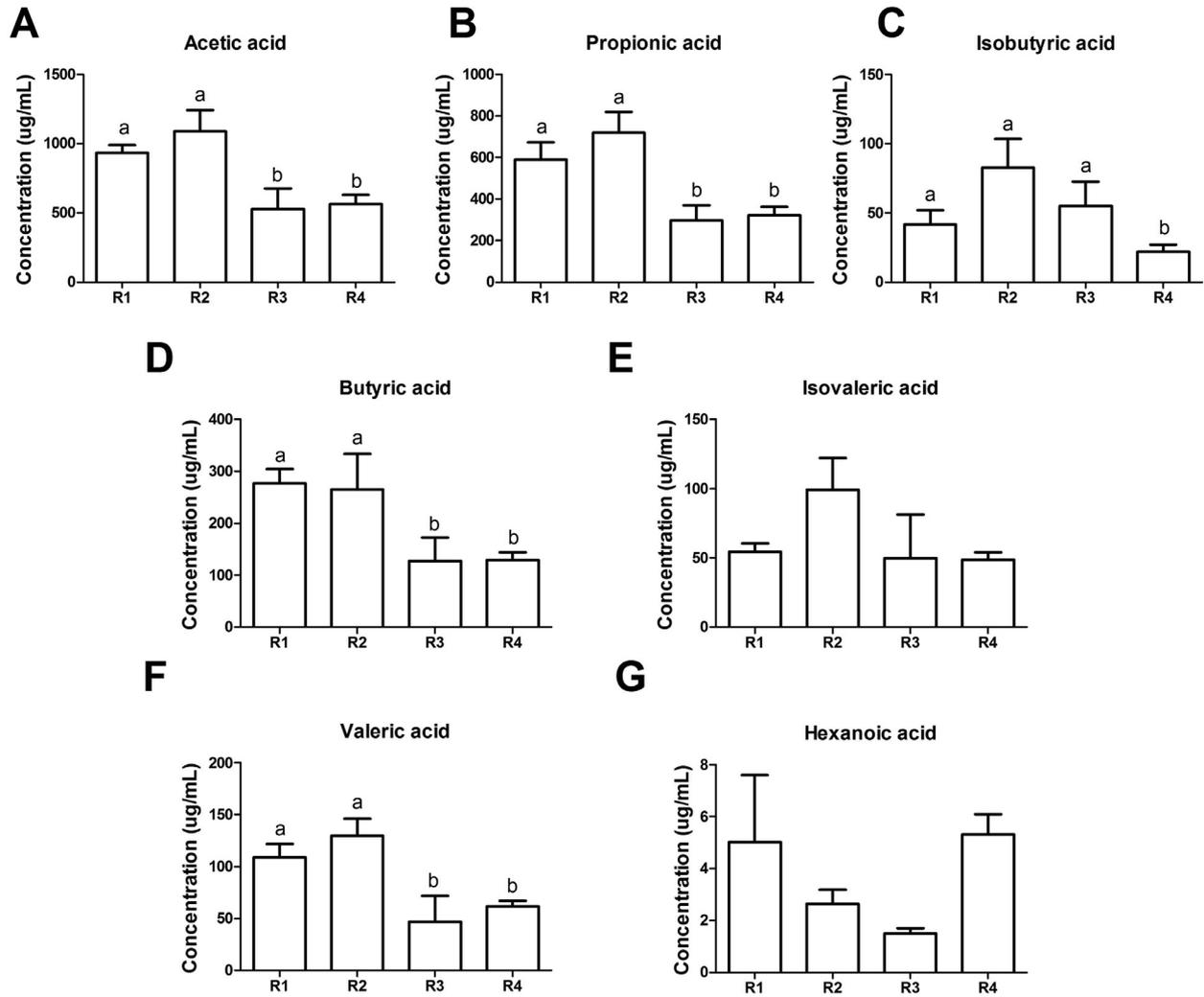


Figure 2. Concentrations (ug/mL) of the different short-chain fatty acids: (A) acetic acid, (B) propionic acid, (C) isobutyric acid, (D) butyric acid, (E) isovaleric acid, (F) valeric acid, and (G) hexanoic acid. R1: highest rank; R2: second rank; R3: third rank; R4: lowest rank.

(Chen et al., 2019). *Bacteroidetes* is the lowest in the R1 ($P = 0.001$), while *Proteobacteria* ($P = 0.004$) had the highest abundance as compared to other ranking roosters, and *Firmicutes* ($P = 0.020$) in the R1 was higher than the R2 (Figure 1B). The higher abundance of *Firmicutes* than *Bacteroidetes* in the gut is helpful to absorb energy and thus is leading to obese and unhealthy status in humans (Morais et al., 2021). At the genus level, the relative abundance of *Desulfovibrio* in the R1 was higher than in the R2 and R4 roosters ($P = 0.002$, Figure 1C). *Desulfuricus* produces sulfide, which is toxic to colonic epithelial cells and has previously been linked to ulcerative colitis. The relative abundance of *Phascolarctobacterium* in the R1 was lower than that in the R4 roosters ($P = 0.039$, Figure 1C). *Phascolarctobacterium* belongs to *Firmicutes* and is associated with SCFAs production, which plays an important role in promoting animal intestinal development and reducing the level of inflammation (Wu et al., 2017). From the balance of microbes, it seems that changes in the abundance of these microorganisms in lower ranking roosters may indicate better health conditions as compared to higher ranking roosters.

Butyric acid can be used as an inhibitor of histone deacetylase (HDAC) to change the expression of

various functional genes, regulate cell activity, and inhibit inflammation and cancer (Dalile et al., 2019). SCFAs receptor (G-protein-coupled receptors 43, GPR43) belongs to G protein-coupled receptor family, which plays an important role in glucose metabolism and fat metabolism. Acetic acid and propionic acid are the most effective agonists of GPR43, which participate in appetite regulation and gastrointestinal peptide secretion (Dalile et al., 2019). In this study, there are differences in SCFAs between high- and low-social status roosters, which seems to indicate that SCFAs can regulate the behavior of the host, similarly to previous studies which found that SCFAs regulate the expression of stress-related behaviors (Wouw et al., 2018). Further, with respect to affective symptomatology, lower fecal SCFAs concentrations are associated with depression in humans (Skonieczna-Zydecka et al., 2018). Roosters with high social status have higher SCFAs concentration, which may indicate that individuals with high social status have better health status. According to the energy allocation theory, high selection pressure on production traits will reduce the high energy-consuming behavior of chickens, and then more energy will be allocated to maintain and improve production performance.

The higher ranking roosters may consume more energy on behaviors and activities to maintain their social rank, which in turn compromise the gut microbiome balance.

Dominant roosters seem to benefit from SCFAs activities while subdominant roosters seem to benefit from microbial functions. However, this implication has the limitation that the low number and small-scale individuals and relatively inadequate duplicate groups, as well as lack of health-related parameters, such as immune- or neural-related index. A comprehensive study should further be carried out to figure out the mechanism of interaction of dominance hierarchy with the health parameters, so as to contribute to the selection and rearing strategies of breeder roosters.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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