

## Effects of a Whole-grain Paddy Rice Diet on the pH Distribution in the Gizzard and Retention Time of Digesta in the Crop of Broiler Chicks

Mari Nishii<sup>1</sup>, Masaharu Yasutomi<sup>1</sup> and Yoshiaki Sone<sup>2</sup>

<sup>1</sup>Kyoto Prefecture Agriculture Experiment Station, Ayabe-Shi 623-0221, Japan

<sup>2</sup>Mimasaka University, Tuyama-Shi 708-8511, Japan

We previously reported that a diet containing 65% paddy rice suppressed the colonization of *Campylobacter jejuni* in the cecum of broiler chicks, suggesting that this type of diet has positive effects on upper gastrointestinal tract function in broilers. To reveal the possible mechanisms involved in this antibacterial effect of the whole-grain paddy rice diet, we performed experiments comparing the digesta passage rate in the crop and gizzard, the development of gizzard, and the pH distribution in the gizzard between groups of chicks fed two different diets, such as ground corn and whole-grain paddy rice. During these experiments, we made the following observations: the chicks in the group fed the whole-grain paddy rice diet had more developed gizzards and significantly larger crop content than the chicks in the group fed the ground corn diet. The chicks fed the whole-grain paddy rice diet retained the digesta in the crop for much longer and had less variation in the pH values in the gizzard than those fed ground corn. On the basis of these observations, we concluded that the hardness of the rice hull in whole-grain paddy rice leads to a larger amount and longer retention of content in the crop, as well as the uniformity of the internal pH of the gizzard, by promoting gizzard activity. We speculate that the hardness of the rice hulls promoted the grinding activity of the gizzard, resulting in the long retention time of the digesta in the crop and uniformity of the internal pH of the gizzard, which may sterilize or suppress *Campylobacter* growth in the gastrointestinal tract of broiler chicks.

**Key words:** broiler chick, crop content, gizzard activity, mean retention time, whole-grain paddy rice

*J. Poult. Sci.*, 53: 181–191, 2016

### Introduction

Chickens have several natural barriers in their upper gastrointestinal tract that help prevent pathogenic bacterial infection (Moen *et al.*, 2012). For example, in the crop and gizzard, the most dominant bacteria is *Lactobacillus* (Rehman *et al.*, 2007), which exerts a bactericidal or bacteriostatic effect on invading pathogenic bacteria by producing organic acids and bacteriocins (Fuller, 1973; Jin *et al.*, 1996). In addition, the gizzard maintains a low pH, which produces a bactericidal environment for many bacteria, including *Campylobacter*, via the secretion of hydrochloric acid from the glandular stomach. As the gizzard plays a role in promoting digestive activity in the lower part of the digestive tract by triturating feed particles, its activity is influenced by factors such as hardness and the type and proportion of dietary fiber in feed (Nir *et al.*, 1994b); as a result, the gizzard pH decreases, and it takes longer for

gizzard contents to pass to the lower part (González-Alvarado *et al.*, 2007; Jiménez-Moreno *et al.*, 2009a). For example, by adding insoluble dietary fibers such as oat hulls in the diet of chickens, the gizzard pH decrease and this reduces the frequency of *Campylobacter* and *Salmonella* infection or a small number of bacteria in the intestinal tract in young broilers (Bjerrum *et al.*, 2005; Santos *et al.*, 2008).

In our previous study (Nishii *et al.*, 2015), we examined the inhibitory effect of a diet containing whole-grain paddy rice on the colonization of *C. jejuni* in the cecum of broiler chicks and reported that feeding a diet containing 65% Whole-grain paddy rice to broiler chicks from 2 weeks of age significantly inhibited *C. jejuni* colonization in the cecum of chicks inoculated with the bacteria at 4 weeks of age. We also observed that the mean ratio of gizzard weight to body weight of the chicks fed the whole-grain paddy rice diet was significantly higher than that of control chicks fed a ground corn diet. Therefore, we speculated that feeding of whole-grain paddy rice to chicks caused the inhibition of *C. jejuni* colonization in the cecum of broiler chicks, to the same extent as that caused by the feeding of oat hulls, by the improvement in gizzard activity, the decrease in gizzard pH, and the longer retention of contents in the gizzard.

Received: September 26, 2015, Accepted: December 26, 2015

Released Online Advance Publication: January 25, 2016

Correspondence: M. Nishii, Kyoto Prefecture Agriculture Experiment Station, Ayabe-Shi 623-0221, Japan.

(E-mail: m-nishii34@pref.kyoto.lg.jp)

In the present study, we focused on comparing the pH distribution and internal pH in the gizzard, bacteriological trend of the crop contents, and digesta passage rate in the crop and gizzard. Finally, we discussed the possible mechanism of the inhibitory effect of the whole-grain paddy rice diet on *C. jejuni* colonization in the upper gastrointestinal tract of broiler chicks.

## Materials and Methods

### Animal Care

All procedures involving animals and their care conformed to the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, 2006).

### Experiment 1

Chick rearing and feeding procedures were similar to those conducted in our previous study (Nishii *et al.*, 2015). Eighty 1-day-old female broiler chicks (Chunky) were raised in a flock-rearing area that was approximately 2 m<sup>2</sup> in size and covered with sawdust in a windowless broiler house. The rearing area temperature was controlled at 34°C on the first

day of rearing and was gradually lowered to 25°C until the chicks reached 14 days of age. After 15 days of age, the room temperature was kept between 20 and 25°C. The duration of light exposure was continuously controlled until 7 days of age, and after 8 days, it was maintained at 20 h per day. During the experiment, the feed intake and body weight of the birds were measured weekly. The chicks were given a starter feed until 14 days of age (Table 1) containing ground corn (68.5% on a weight basis) crushed into particles of less than 1.4 mm in size.

On day 14, the chicks were allocated into three groups: ground corn diet (GC), whole-grain paddy rice diet (WPR), and brown rice diet (BR). The diets for each group are shown in Table 1. The ground corn was supplied by Yao Feed Co., Ltd. (Osaka, Japan), and the whole-grain paddy rice (Momiroman) and brown rice were obtained from Kyoto Prefecture Agriculture Experiment Station (Kameoka-Shi, Kyoto, Japan). The particle size distributions of the three diets were determined by the dry sieving method and are summarized in Table 2. The feedstuff did not contain any

Table 1. Comparison of the experimental diets

Ingredients (%)	Starter diet <sup>1</sup> (1 to 14 days)	Grower diet (15 to 42 days)		
		Ground corn diet	Whole-grain paddy rice diet	Brown rice diet
Ground corn	65.0	70.0	10.0	10.0
Paddy rice	—	—	60.0	—
Dehulled rice	—	—	—	60.0
Soybean meal	20.0	20.0	20.0	20.0
Fish meal (65% crude protein)	7.0	5.0	5.0	5.0
Corn gluten meal	2.0	—	—	—
Soybean oil	3.0	2.0	2.0	2.0
Calcium carbonate	0.5	0.5	0.5	0.5
Tricalcium phosphate	0.6	0.6	0.6	0.6
Dicalcium phosphate	0.5	0.5	0.5	0.5
Manganese sulfate	0.015	0.015	0.015	0.015
Sodium chloride	0.25	0.15	0.19	0.19
DL-methionine	0.3	0.25	0.25	0.25
L-lysine HCl	0.5	0.4	0.4	0.4
Riboflavin	0.0004	0.00025	0.00025	0.00025
Copper sulfate	0.0005	0.001	0.001	0.001
Zinc sulfate	0.0005	0.004	0.004	0.004
Folacin	0.00004	0.00003	0.00003	0.00003
L-threonine	0.25	0.25	0.25	0.25
Choline chloride	0.05	—	—	—
Calcium pantothenate	0.0004	—	—	—
Nicotinamide	0.003	—	—	—
Vitamin/mineral premix <sup>2</sup>	0.3	0.3	0.3	0.3
Calculated				
Crude protein (%)	21.1	18.2	17.5	18.1
Metabolizable energy (kcal/kg)	3,056	3,120	2,748	3,120

<sup>1</sup> The starter diet was ground and then passed through a sieve with a mesh size of 1.4 mm.

<sup>2</sup> Vitamin and mineral premix including the following (per kg of the diet): retinol (retinyl acetate), 3,500,000 IU; cholecalciferol, 700,000 IU; vitamin E (DL- $\alpha$ -tocopheryl acetate), 600 mg; menadione, 250 mg; thiamine, 500 mg; riboflavin, 450 mg; pyridoxine, 350 mg; cyanocobalamin, 0.8 mg; nicotinamide, 1,700 mg; D-pantothenic acid, 750 mg; choline chloride, 35,000 mg; ZnCO<sub>3</sub>, 5,700 mg; MnSO<sub>4</sub>, 8,250 mg; FeSO<sub>4</sub>, 3,890 mg; CuSO<sub>4</sub>, 1,160 mg; CoSO<sub>4</sub>, 17 mg.

Table 2. Particle size distribution of the grower diet (supplied from 15 to 42 days of age)

Particle size class	Ground corn diet <sup>1</sup>	Whole-grain paddy rice diet <sup>2</sup>	Brown rice diet <sup>3</sup>
>2.8 mm	0.54	5.58	0.54
2.0-2.8 mm	14.80	60.64	5.68
1.4-2.0 mm	36.61	11.35	69.97
1.0-1.4 mm	17.08	6.28	7.66
0.5-1.0 mm	11.90	5.74	5.74
<0.5 mm	19.08	10.42	10.42

<sup>1</sup> The ground corn diet contained 70% corn.

<sup>2</sup> The whole-grain paddy rice diet contained 10% corn and 60% paddy rice.

<sup>3</sup> The brown rice diet contained 10% corn and 60% dehulled rice.

antimicrobials or coccidiostats. All chicks had free access to feed and water throughout all the experiments.

During the experiment, the birds' feed intake and body weight were measured weekly. At 28 and 42 days of age, eight chicks from each flock were randomly selected and weighed, killed by anesthesia overdose by intravenous injection (sodium pentobarbital, Somnopentyl; Kyoritsu Pharmacy, Tokyo, Japan), and then dissected. The contents of each segment in the digestive tract (crop, proventriculus, gizzard, duodenum, jejunum, ileum, and cecum) were removed onto a plastic plate under aseptic conditions and weighed. A portion of the content (0.1 g) was collected for bacterial culture, and the remaining portion was subjected to the following analyses. The content was added to two volumes of deionized water in a 50-ml centrifuge tube, and the suspension was mixed using a vortex mixer for 1 min. The pH of the diluted mixture was measured using a glass electrode pH meter (9615-10D; Horiba, Kyoto, Japan) twice for each mixture. The empty gizzard was then washed with water, dried with desiccant paper, and weighed.

The total viable bacterial counts of *Lactobacilli*, coliform bacteria, and lactose-negative enterobacteria in the contents of the crop, gizzard, ileum, and cecum were quantitatively detected. The contents of each segment were diluted 1:10 (w/v) in dilution anaerobic buffer solution [composition in 1000 ml of purified water:  $\text{KH}_2\text{PO}_4$ , 4.5 g;  $\text{Na}_2\text{HPO}_4$ , 6.0 g; L-cysteine hydrochloride, 0.5 g; Tween 80, 0.5 g; agar, 1 g (Mituoka, 1994)], and the first diluted suspension was serially diluted in 10-fold steps using the dilution anaerobic buffer solution. Subsequently, the suspensions were spread onto the following agar culture media: *Lactobacillus* selective LBS agar (BBL, Becton Dickinson and Company, Sparks, MD, USA) supplemented with 0.8% Lab-Lemco Powder (Oxoid Ltd, Basingstoke, Hampshire, England), 0.1 % sodium acetate trihydrate, and 0.37% acetate (modified-LBS) for *Lactobacilli* and MacConkey agar (Oxoid Ltd, Basingstoke, Hampshire, England) for coliform bacteria and lactose-negative enterobacteria. Modified-LBS agar plates were anaerobically incubated at 37°C for 48 h, and all growing colonies were counted. MacConkey agar plates were aerobically incubated at 37°C for 24 h, and coliform bacteria and lactose-negative enterobacteria were counted as red and colorless colonies, respectively.

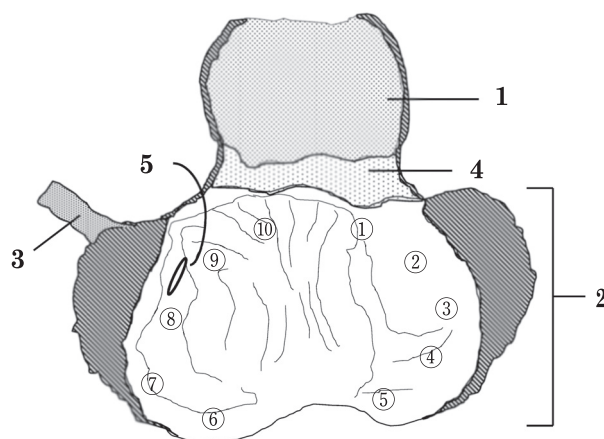


Fig. 1. Distribution of points and position for the measurement of pH in gizzard contents. The slanted line shows the ventral incision of the gizzard and proventriculus. 1, proventriculus; 2, gizzard; 3, proximal duodenum; 4, isthmus; 5, pylorus.

### Experiment 2

One hundred 1-day-old female broiler chicks (Chunky) were raised in the same manner as that in Experiment 1. On day 14, the chicks were allocated into two groups: GC and WPR. At 28 days of age, 17 chicks from each group were randomly selected and weighed and were killed by the same method as that in Experiment 1 (anesthesia overdose by injection). The gizzard and proventriculus were removed from the chicks, and the removed gizzard was incised from the ventral side of the proventriculus along the median line using surgical scissors to expose the gizzard content. Immediately after the incision was made, 10 portions of the content, approximately 0.5 g each, were gently removed from the exposed content using a small pair of tongs at the 10 positions shown in Fig. 1. These portions were put on the measurement section of a pH meter (B712; Horiba, Kyoto, Japan), added to 0.5 ml of deionized water, gently mixed, and the pH was then measured within 10 min after removal. The sites of content sample collection depicted in Fig. 1 are anatomically labeled as follows: ① and ⑩: the vicinity of the proventriculus (proximal site), ⑧ and ⑨: near the pylorus

leading to the duodenum (distal site), and ②-⑦: the others (middle site).

### Experiment 3

At 29 days of age, 32 birds from each group (GC and WPR) were randomly selected and orally administered 1 ml of titanium dioxide-water solution (0.0426 g/ml) (Wako Pure Chemical Industries, Osaka, Japan) using a 1-ml syringe attached to a flexible tube. Four birds from each group were killed by the same method as that in Experiment 1 (anesthesia overdose by intravenous injection) at 20, 40, 60, 80, 100, 120, 180, and 240 min after the administration of the titanium dioxide solution. Immediately after killing, the crop was removed from the birds killed at 20, 40, 60, 80, 100, and 120 min. The gizzard was also removed from the birds killed at 60, 120, 180, and 240 min. The contents of the crop and gizzard were transferred into 100-ml beakers and dried at 105°C overnight using a ventilation dryer. The dried content was crushed using a food mill and was then subjected to quantitative determination of titanium dioxide by the method by Short *et al.* (1996).

The mean retention time (MRT) of the digesta was estimated using the following calculation, where  $t$  (min) is the elapsed time after the administration of titanium dioxide to the chicks and  $R_{crop}(t)$  and  $R_{gizzard}(t)$  are the residual amounts of titanium dioxide in the crop and gizzard, respectively, assuming that the residual amount of titanium dioxide in the crop at the time of administration of titanium dioxide ( $t=0$ ),  $R_{crop}(0)$ , is one.

$$dR_{crop}(t)/dt = -a R_{crop}(t)$$

$$dR_{gizzard}(t)/dt = a R_{crop}(t) - b R_{gizzard}(t)$$

where the small italic letters,  $a$  and  $b$ , are constants.

By solving these differential equations, the following equations are obtained.

$$R_{crop}(t) = e^{-at} \quad (1) \text{ for the crop}$$

$$R_{gizzard}(t) = [a/(a-b)](e^{-bt} - e^{-at}) \quad (2) \text{ for the gizzard}$$

Finally, MRTs are calculated as follows (Dhanoa *et al.*, 1985):

$$\text{MRT}_{crop} = 1/a \quad (3) \text{ for the crop}$$

$$\text{MRT}_{gizzard} = 1/b \quad (4) \text{ for the gizzard}$$

The residual amounts of titanium dioxide in the crop and gizzard measured at each time point after its administration in the present experiment were substituted into equations (1) and (2), respectively, and the constants,  $a$  and  $b$ , were determined by the least squares method. These calculations were conducted using the solver function of Microsoft Excel 2010 (Redmond, WA, USA). MRTs in the crop and gizzard were calculated by equations (3) and (4), respectively.

### Statistical Analysis

In Experiment 1, the differences in the mean values of the chicks' body weight, relative weight of the gizzard, pH and weight of the content of the gizzard, and the bacterial counts in the contents of the crop, gizzard, ileum, and cecum between the chick groups were statistically analyzed by multiple comparisons using the Tukey-Kramer test. In Experiment 2, the differences in the pH values among the three sites were analyzed using two-way analysis of variance (ANOVA) (the factors were the chicks' groups and mea-

sured sites). When a significant difference was observed in the dispersion component, the mean value of the component was subjected to a significant difference test using the Tukey-Kramer method. The coefficient of variations of the individual chicks was calculated, along with the mean and the standard deviation of the pH values at the 10 measurement points of the gizzard for each chick. Significant differences of the mean of the coefficient of variation were analyzed using ANOVA.  $P$ -values less than 0.05 were considered statistically significant. Both tests were conducted using Microsoft Excel 2010 add-in software.

## Results

### Experiment 1

Table 3 shows the comparison of the change in the mean body weight of the chicks in the three experimental groups in addition to their feed intake during Experiment 1. Although the feed intake was not significantly different between the three groups during the observation period, the mean body weight of the chicks in the BR group was significantly higher than that of the GC and WPR groups at 28 ( $p=0.021$ ) and 42 ( $p=0.041$ ) days of age. Table 4 shows the comparisons of the relative weights of the gizzard and pH values in the crop and gizzard among the three groups. Statistical analysis (Tukey-Kramer test) showed significant differences in the relative weight of the gizzard (gizzard weight/100 g of body weight) of the chicks among the three groups: at 28 ( $p<0.001$ ) and 42 ( $p<0.001$ ) days of age, the value of this parameter was significantly higher in the WPR group than in the other two groups. The order of the relative weights of the gizzard was WPR>GC>BR. There was no significant difference between any pair of the mean pH values of the contents of the crop and gizzard at any age in the chicks.

Tables 5 and 6 show the weight of the content of the crop, proventriculus, gizzard, ileum, and cecum at 28 and 42 days of age, respectively. At 28 days of age, there was significantly more content in the crop in the WPR group than in the GC group ( $p=0.004$ ); in the lower gastrointestinal tract, there was significantly more content in the duodenum and ileum in the WPR group than in the BR and GC groups ( $p=0.018$ ). At 42 days of age, there was significantly more content in the crop in the WPR group than in the GC group ( $p=0.001$ ), and in the lower gastrointestinal tract, there was significantly more content in the ileum in the WPR group than in the GC group ( $p=0.005$ ).

Table 7 shows the populations of *Lactobacilli*, coliform bacteria, and lactose-negative enterobacteria in each segment at 28 and 42 days of age. At 28 days of age, the GC group had the lowest numbers of *Lactobacilli* in the gizzard among the three groups, and the number of *Lactobacilli* in the cecum of the GC group was significantly lower than that of the BR group ( $p=0.006$ ); further, there were no significant differences in the number of *Lactobacilli* in the crop or ileum among the three groups at either 28 or 42 days of age. The number of coliform bacteria did not significantly differ among the three groups, and neither was there any significant difference among the various segments or at any age, except

**Table 3. Mean body weights and feed intake in the three broiler chick groups from 14 to 42 days of age**

	n	Group			P-value
		GC <sup>1</sup>	WPR <sup>2</sup>	BR <sup>3</sup>	
Body weight (g)					
14 days	19	343±8	339±10	356±5	0.323
28 days	19	1388±21 <sup>b</sup>	1389±31 <sup>b</sup>	1484±28 <sup>a</sup>	0.021
42 days	11	2542±38 <sup>ab</sup>	2514±55 <sup>b</sup>	2732±89 <sup>a</sup>	0.046
Feed (g/bird)					
14 to 21 days		662	648	687	
21 to 28 days		628	677	657	
28 to 35 days		621	646	655	
35 to 42 days		990	974	977	

The body weight values are presented as the mean±standard error of the mean.

<sup>a-b</sup> Means within the same row with different superscripts are significantly different ( $p < 0.05$ ).

<sup>1</sup> The ground corn diet.

<sup>2</sup> The whole-grain paddy rice diet.

<sup>3</sup> The brown rice diet.

**Table 4. The mean pH values in the gizzard and crop and the relative weight of the gizzard contents in the three chicks' groups**

Group	pH <sup>1</sup>				Relative weight of gizzard (g/100 g body weight)	
	Crop		Gizzard		28 days	42 days
	28 days	42 days	28 days	42 days		
GC <sup>2</sup>	5.08±0.14	4.71±0.19	3.51±0.13	3.84±0.19	1.76±0.09 <sup>b</sup>	1.43±0.05 <sup>b</sup>
WPR <sup>3</sup>	5.00±0.12	5.04±0.01	3.88±0.11	4.01±0.17	2.13±0.03 <sup>a</sup>	1.74±0.06 <sup>a</sup>
BR <sup>4</sup>	4.83±0.04	4.75±0.10	3.83±0.14	3.92±0.13	1.47±0.04 <sup>c</sup>	1.17±0.03 <sup>c</sup>
	P-value					
Group	0.285	0.188	0.098	0.751	<0.001	<0.001

The data are presented as the mean±standard error of the mean,  $n=8$ .

<sup>a-c</sup> Means within the same column with different superscripts are significantly different ( $p < 0.05$ ).

<sup>1</sup> pH of gizzard contents diluted (1 : 2) with deionized water.

<sup>2</sup> The ground corn diet.

<sup>3</sup> The whole-grain paddy rice diet.

<sup>4</sup> The brown rice diet.

**Table 5. Weight of the contents in different parts of the intestine at 28 days of age**

Items (g)	Group			P-value
	GC <sup>1</sup>	WPR <sup>2</sup>	BR <sup>3</sup>	
Crop	6.6±1.4 <sup>b</sup>	16.0±2.9 <sup>a</sup>	13.1±1.6 <sup>ab</sup>	0.014
Proventriculus	1.6±0.1	1.5±0.1	1.2±0.2	0.235
Gizzard	12.5±0.8	14.5±0.6	13.8±0.8	0.190
Duodenum	5.7±0.2 <sup>ab</sup>	6.7±0.5 <sup>a</sup>	5.0±0.4 <sup>b</sup>	0.018
Jejunum	20.3±1.5	24.8±1.4	20.6±1.5	0.071
Ileum	16.5±0.8 <sup>b</sup>	19.5±0.7 <sup>a</sup>	14.9±0.6 <sup>ab</sup>	<0.001
Cecum	9.2±1.2	6.8±1.0	6.6±0.7	0.137

The data are presented as the mean±standard error of the mean,  $n=8$ .

<sup>a-b</sup> Means within the same row with different superscripts are significantly different ( $p < 0.05$ ).

<sup>1</sup> The ground corn diet.

<sup>2</sup> The whole-grain paddy rice diet.

<sup>3</sup> The brown rice diet.

Table 6. Weight of the contents in different parts of the intestine at 42 days of age

Items (g)	Group			P-value
	GC <sup>1</sup>	WPR <sup>2</sup>	BR <sup>3</sup>	
Crop	4.2±1.1 <sup>b</sup>	30.2±5.4 <sup>a</sup>	16.3±5.0 <sup>ab</sup>	0.001
Proventriculus	1.6±0.2 <sup>a</sup>	0.7±0.1 <sup>b</sup>	1.9±0.3 <sup>a</sup>	0.001
Gizzard	16.3±0.9	20.9±1.1	18.2±1.8	0.077
Duodenum	6.5±0.5	7.4±0.7	7.1±0.9	0.631
Jejunum	33.1±1.5	33.3±1.5	29.3±1.1	0.085
Ileum	18.2±0.4 <sup>b</sup>	24.5±1.7 <sup>a</sup>	20.9±1.1 <sup>ab</sup>	0.005
Cecum	9.8±1.4	6.3±1.1	9.5±1.2	0.123

The data are presented as the mean±standard error of the mean,  $n=8$ .

<sup>a-b</sup> Means within the same row with different superscripts are significantly different ( $p<0.05$ ).

<sup>1</sup> The ground corn diet.

<sup>2</sup> The whole-grain paddy rice diet.

<sup>3</sup> The brown rice diet.

Table 7. Bacterial counts (log<sub>10</sub> cfu/g) in the contents of different parts of the intestine ( $n=8$ )

Group	GC <sup>1</sup>		WPR <sup>2</sup>		BR <sup>3</sup>		P-value	
	Time (day)		Time (day)		Time (day)		group	
	28	42	28	42	28	42	28	42
<i>Lactobacilli</i>								
Crop	8.36±0.12 (8/8) <sup>4</sup>	9.36±0.19 (8/8)	8.41±0.24 (8/8)	9.20±0.10 (8/8)	8.69±0.16 (8/8)	9.05±0.20 (8/8)	0.459	0.437
Gizzard	5.62±0.30 <sup>b</sup> (8/8)	5.03±0.65 <sup>ab</sup> (8/8)	6.49±0.10 <sup>a</sup> (8/8)	6.34±0.30 <sup>ab</sup> (8/8)	6.63±0.08 <sup>a</sup> (8/8)	6.37±0.27 <sup>ab</sup> (8/8)	0.002	0.069
Ileum	8.52±0.15 (8/8)	7.89±0.42 (8/8)	8.49±0.16 (8/8)	7.86±0.20 (8/8)	8.64±0.11 (8/8)	8.06±0.17 (8/8)	0.739	0.692
Cecum	8.27±0.21 <sup>b</sup> (8/8)	8.83±0.40 <sup>ab</sup> (8/8)	8.66±0.08 <sup>ab</sup> (8/8)	9.22±0.21 <sup>ab</sup> (8/8)	8.99±0.07 <sup>a</sup> (8/8)	9.35±0.33 <sup>ab</sup> (8/8)	0.006	0.500
Coliform bacteria								
Crop	4.87±0.38 (8/8)	3.26±0.55 (8/8)	4.77±0.25 (8/8)	3.43±0.39 (8/8)	4.11±0.21 (8/8)	3.36±0.52 (8/8)	0.160	0.965
Gizzard	2.35±0.27 (2/8)	2.90±0.52 (5/8)	3.67±0.71 (6/8)	2.81±0.53 (5/8)	2.95 (1/8)	1.78 (1/8)		
Ileum	6.15±0.27 <sup>ab</sup> (8/8)	5.88±0.28 <sup>ab</sup> (8/8)	6.49±0.23 <sup>a</sup> (8/8)	5.14±0.35 <sup>ab</sup> (8/8)	5.20±0.32 <sup>b</sup> (8/8)	5.40±0.37 <sup>ab</sup> (8/8)	0.009	0.298
Cecum	7.36±0.29 (8/8)	7.28±0.22 (8/8)	7.59±0.27 (8/8)	7.16±0.32 (8/8)	6.94±0.29 (8/8)	6.99±0.30 (8/8)	0.285	0.764
Lactose-negative enterobacteria								
Crop	4.12±0.51 (5/8)	ND <sup>5</sup> (0/8)	4.02±0.24 (2/8)	2.15±0.15 (4/8)	3.17±0.37 (5/8)	2.21±0.43 (3/8)		
Gizzard	ND (0/8)	ND (0/8)	ND (0/8)	ND (0/8)	ND (0/8)	ND (0/8)		
Ileum	3.48 (1/8)	ND (0/8)	ND (0/8)	ND (0/8)	2.78 (1/8)	ND (0/8)		
Cecum	5.95±0.18 (4/8)	ND (0/8)	6.48±0.13 (5/8)	ND (0/8)	6.14±0.17 (3/8)	6.27±0.20 (4/8)	0.099	

Values are colony-forming units per gram of the content (Mean±SEM)

<sup>a-b</sup> Means within a line with different superscripts are significantly different ( $p<0.05$ ).

<sup>1</sup> The ground corn diet.

<sup>2</sup> The whole-grain paddy rice diet.

<sup>3</sup> The brown rice diet.

<sup>4</sup> Colonies detected on the plate/total number of colonies on the plate.

<sup>5</sup> ND=not detectable.

**Table 8. Comparison of the mean pH values at three different sites in the gizzard between the two groups of chicks**

Group	pH values at three different sites			P-value	Coefficient of variance <sup>6</sup>
	Proximal <sup>3</sup>	Middle <sup>4</sup>	Distal <sup>5</sup>		
GC <sup>1</sup>	3.06±0.52 <sup>b</sup> n=34	3.33±0.53 <sup>a</sup> n=102	3.58±0.55 <sup>a</sup> n=34	<0.001	0.1079±0.0402 <sup>x</sup> n=17
WPR <sup>2</sup>	3.23±0.43 n=34	3.41±0.56 n=102	3.47±0.49 n=34	0.136	0.0785±0.0293 <sup>y</sup> n=17

The data are presented as the mean ± standard error of the mean.

<sup>1</sup> The ground corn diet.

<sup>2</sup> The whole-grain paddy rice diet.

<sup>3</sup> Contents at the collected site ① and ⑩ in Figure 1.

<sup>4</sup> Contents at the collected site ②, ③, ④, ⑤, ⑥, and ⑦ in Figure 1.

<sup>5</sup> Contents at the collected site ⑧ and ⑨ in Figure 1.

<sup>6</sup> The coefficient of variance was calculated on the basis of 10 sites in the gizzard of individual chicks.

<sup>a,b</sup> Means within the same row with different superscripts are significantly different ( $p < 0.05$ ).

<sup>x,y</sup> Means within the same column with different superscripts are significantly different ( $p < 0.05$ ).

for between the WPR and BR groups in the ileum at 28 days of age. Lactose-negative enterobacteria were not detected in the gizzard of chicks in the three groups at any age.

### Experiment 2

Table 8 shows the comparison of the mean pH values in the three sites (proximal, distal, and middle site) between the GC and WPR groups. In the WPR group, there were no significant differences in the pH values among the three sites; conversely, in the GC group, the pH value at the proximal site was significantly lower than that of the other two sites. To clarify the uniformity of the pH distribution in the gizzard, we calculated and compared the coefficient of variations of the pH values at the 10 measurement sites for each group. As shown in Table 8, the coefficient of variation for the WPR group was significantly smaller than that of the GC group ( $p = 0.026$ ), which suggests that the variation of pH values in the gizzard of the chicks in the WPR group was less than that of the chicks in the GC group. In order to examine survival pH ranges of *Campylobacter*, we summarized the three pH range frequency in the gizzard in the two chick groups as Table 9. This table shows that the sampling sites with pH values lower than pH 3 and higher than pH 4 accounted for 29.1% and 15.0% (on average), respectively, of all the sampling points in each group ( $n = 170$ ).

### Experiment 3

Figures 2 and 3 show the amount of titanium dioxide detected in the crop and gizzard, respectively, after various lengths of time following the administration of titanium dioxide as a percentage of the initial amount of titanium dioxide. These figures also show the residual curves drawn using Equations (1) and (2). MRT was calculated based on these regression curves (Table 10). In the GC group, MRTs through the crop and gizzard were 41 and 34 min, respectively. In the WPR group, MRTs through the crop and gizzard were 115 and 21 min, respectively. Titanium dioxide was retained in the crop for longer in the WPR group than in the GC group, whereas there was no difference in MRT in the

**Table 9. Relative frequency of three classes of pH values at different sites in the gizzard**

Group	Relative frequency (%)		
	pH < 3	pH 3–4	pH > 4
GC <sup>1</sup>	31.8	54.7	13.5
WPR <sup>2</sup>	26.5	57.1	16.5
Mean	29.1	55.9	15.0

<sup>1</sup> The ground corn diet.

<sup>2</sup> The whole-grain paddy rice diet.

gizzard between the two groups.

## Discussion

The gizzard plays a role in grinding coarse feed particles until a certain minimal critical size by performing its muscular activity, before its contents are passed into the lower digestive tract. Therefore, when chicks are fed feed materials of certain grinding resistance, they develop stronger gizzard function (muscular activity) than when fed regular chicken feed. These phenomena have been reported for diets containing oat hull (Jiménez-Moreno *et al.*, 2009b; González-Alvarado *et al.*, 2010; Mateos *et al.*, 2012), coarsely crushed corn (Nir *et al.*, 1994a, b; Singh *et al.*, 2014), and whole wheat (Engberg *et al.*, 2004; Bjerrum *et al.*, 2005; Gabriel *et al.*, 2008). In the present experiment, the level of gizzard development was in the order of WPR > GC > BR (estimated by the relative weight of the gizzard; see Table 4). This observed order of gizzard development seems to be consistent with the frequency of resistance of grinding activity in the gizzard, when each feed material was soaked with water in the crop. This soft property of brown rice is confirmed by the fact that brown rice has a constant stiffness in the dry state, but absorbs water easily and softens when it is soaked for 15–30 min in water (Jagtap *et al.*, 2008; Kong *et al.*,

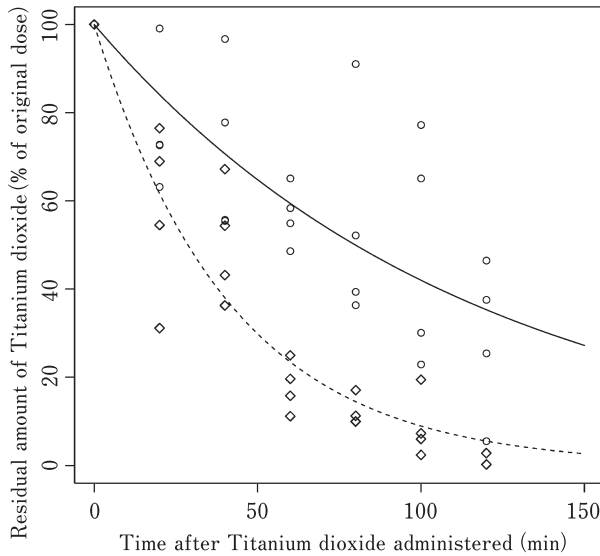


Fig. 2. The amount of titanium dioxide detected in the crop at various time points after its administration (percentage of the admitted dose) ( $n=4$ ).  $\circ$ : WPR,  $\diamond$ : GC. The residual curve calculated from Equation (1). Solid line: WPR;  $R_{crop}(t)=100e^{-0.0087t}$  ( $r^2=0.49$ ), dashed line: GC;  $R_{crop}(t)=100e^{-0.0242t}$  ( $r^2=0.77$ ).

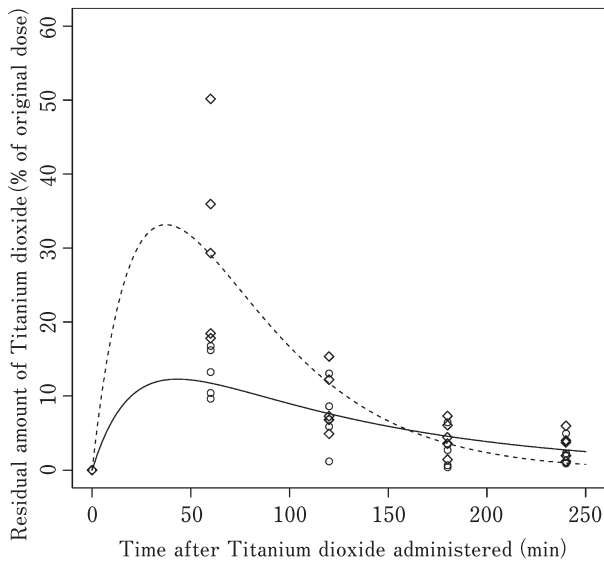


Fig. 3. The amount of titanium dioxide detected in the gizzard at various time points after its administration (percentage of the admitted dose) ( $n=4$ ).  $\circ$ : WPR,  $\diamond$ : GC. The residual curve calculated from Equation (2). Solid line: WPR;  $R_{gizzard}(t)=-21.75(e^{-0.0486t}-e^{-0.0087t})$  ( $r^2=0.48$ ), dashed line: GC;  $R_{gizzard}(t)=-450.47(e^{-0.0296t}-e^{-0.0242t})$  ( $r^2=0.33$ ).

Table 10. Estimates of the mean retention time of titanium dioxide in the crop and gizzard

Group	Mean retention time (min)	
	Crop	Gizzard
GC <sup>1</sup>	41	34
WPR <sup>2</sup>	115	21

<sup>1</sup>The ground corn diet.

<sup>2</sup>The whole-grain paddy rice diet.

2011). In contrast to brown rice, paddy rice is composed of brown rice and hulls; the proportion of rice hulls is equivalent to approximately 20% by weight of paddy rice. Rice hulls are highly resistant to grinding in the upper digestive tract because they have a silica layer inside the backbone of the epidermis, which forms a hard, rough surface that imparts a specific texture. A previous study reported that gizzard development is superior in chicks fed a diet containing 2.5% or 5% rice hull than in those fed a diet without rice hulls (Mateos *et al.*, 2012). In the present study, we used a whole-grain paddy rice diet containing 60% paddy rice (Table 1), which corresponds to a diet containing 12% rice hulls. Therefore, we presume that the texture of the hulls in paddy rice enhanced the grinding activity of the gizzard, thus promoting gizzard development.

In Experiment 1, there was no difference in the pH of the gizzard contents among the three groups. However, in Experiment 2, we found variations in pH in the gizzard contents in the GC and WPR groups; the mean coefficients of variation of the 10 pH measurement sites in the individual chicks were 0.1079 and 0.0785 for the GC and WPR groups, respectively (Table 8). We interpreted the mechanism of these pH variations observed in the gizzard as follows. The pH at the proximal site (⑩ in Fig. 1) was generally low because gastric juice from the proventriculus flows into this site. The higher pH at the distal site (Fig. 1 ⑧⑨) reflects the inflow of bicarbonate and bile from the duodenum associated with reverse peristalsis (Duke, 1992). In addition, calcium salts in the diet work as a buffer to raise the pH in the gizzard (Guinotte *et al.*, 1995; Lawlor *et al.*, 2005; Walk *et al.*, 2012). The inflow and outflow of feed particles simultaneously occur in the gizzard, producing dynamic changes in the pH; as a result, the gizzard contents have various pH values.

Additionally, we found that the coefficient of variance of pH in the gizzard contents in the WPR group was significantly smaller than that in the GC group (Table 8). There was no significant difference in the pH values at the three sites in the WPR group, whereas in the GC group, the pH value at the proximal site was significantly lower than that at the other two sites (Table 8). These results may explain the fact that the orally ingested *Campylobacter* survived in the gizzard of the WPR group in our previous study (Nishii *et al.*, 2015). The optimum pH of *Campylobacter* has been reported as pH 6.5–7.5 (Jackson *et al.*, 2009). In *in vitro* ex-



periments, Chaveerach *et al.* (2002) showed that the numbers of *Campylobacter* decreased at values lower than pH 4 and that *Campylobacter* is sterilized at pH values below 3. In the present study, the average pH range of the gizzard was pH 3.3 to 3.4, and the number of *Campylobacter* was reduced under these conditions. While the average pH in the gizzard was low enough to prevent bacterial growth, 15% of the points for the measurement of pH in the gizzard had values higher than pH 4, i.e., the viable pH range for bacterial growth, which would enable *Campylobacter* to survive (Table 9). Under such conditions (the gizzard has points where *Campylobacter* may be able to survive), the promoted grinding activity of the gizzard could contribute to the elimination of these high pH areas in the gizzard through uniform pH distribution if the gizzard content was sufficiently mixed. The results of our study show that when broiler chicks are fed whole-grain paddy rice, the coefficient of variation of the gizzard pH is significantly smaller than that in chicks fed ground corn. Thus, we surmised that the hardness (grinding resistance of the food material) of the whole-grain paddy rice promoted the development of the activity of the gizzard and the uniformity of the internal pH of the gizzard, thus eliminating the areas of higher pH in the gizzard where *Campylobacter* can survive.

MRT of the gizzard contents was similar between the GC and WPR groups (Table 10). The grinding activity of the gizzard crushes coarse feed particles until a certain critical size is selectively retained ingested feed in the gizzard, before passage of the food to the lower gastrointestinal tract (Svihus, 2011). Therefore, in this experiment, MRT in the gizzard may have been influenced by the rheological properties due to their particle sizes of the marker used. A previous study used Cr-mordanted sunflower hulls and titanium dioxide as markers and showed that MRT of titanium oxide in the gizzard was significantly shorter than that of Cr-mordanted sunflower hulls (Rougière and Carré, 2010). We speculate that the insoluble fraction such as hulls of paddy rice in WPR retained in the gizzard for long time than the marker of titanium dioxide, because whole-grain paddy rice was resistant to milling as Rougière and Carré (2010) found that sunflower hulls were retained in the gizzard.

In the present study, there was significantly more content in the crop in the WPR group than in the GC group (Tables 5 and 6). Thus, we discuss the possible causes for variations in the crop content and its inhibitory effect on *Campylobacter* below.

The crop is a storage organ that holds the ingested feed to be sent to the gizzard (Jackson and Duke, 1995). The storage of feed in the crop, therefore, is closely related to feed intake regulation, such as the frequency of feeding and the time spent feeding. The accumulation of feed in the crop (large crop content) has been reported in chickens subjected to time limitations for feeding, such as intermittent feeding, lighting schedule, and some feed types (Barash *et al.*, 1993; Duve *et al.*, 2011). In contrast, with *ad libitum* feeding, chickens eat small amounts approximately every half hour

and do not tend to store ingested food in the crop (Svihus, 2014). In the present study, the chicks were raised under *ad libitum* feeding conditions, and the amount of crop content in the GC group (Tables 5 and 6) was small ( $6.6 \pm 1.4$  g and  $4.2 \pm 1.1$  g at 28 and 42 days of age, respectively), which is in agreement with the findings reported by Svihus (2014). However, Tables 5 and 6 show that although the chicks in the WPR group were raised under *ad libitum* feeding conditions, they had significantly larger crop contents ( $16.0 \pm 2.9$  g and  $30.2 \pm 5.4$  g at 28 and 42 days of age, respectively) than those of the GC group. This phenomenon could be explained by Savory's (1985) findings that chickens had the largest crop and gizzard contents when they were fed diets nutritionally "diluted" by adding 40% cellulose. In our experiments, the whole-grain paddy rice diet contained 15% less metabolizable energy than the ground corn diet (Table 1) because the hulls of paddy rice are not digestible. In other words, the whole-grain paddy rice diet supplied in this experiment could be considered as a diluted feed similar to that described by Savory (1985).

Also, it is likely that the decrease in the passage of digesta to the lower gastrointestinal tract results from the hulls in the whole-grain paddy rice diet, which are resistant to grinding, and they are thus retained in the gizzard for a long time (Vergara *et al.*, 1989). It seems to become a limiting factor (regulator) of feed digestion of the entire gastrointestinal tract like as a time limit for feeding or food deprivation. We speculate that whole-grain paddy rice acts as a nutritionally "diluted" cereal and adds resistance to the grinding activity in the gizzard. Feeding whole-grain paddy rice to broiler chicks may result in the accumulation of large amounts of feed in the crop in order to avoid hunger, or in other words, to satisfy energy requirements by increasing the feed intake (Denbow, 1994; Ferket and Gernat, 2006; González-Alvarado *et al.*, 2010).

This larger crop content likely led to the effect of "the larger the crop content, the longer the retention in the crop" observed in this study (in Experiment 3; Fig. 2, Table 10). Table 10 shows that MRTs of the digesta in the crop were 41 and 115 min in the GC and WPR groups, respectively. We speculate that the longer retention time in the crop of the chicks in the WPR group played an important role in the suppression of *Campylobacter* in our previous report (Nishii *et al.*, 2015). The reason for this is that *Lactobacilli*, which are the dominant flora in the crop, suppress the survival of pathogenic bacteria that have entered via the oral route, depending on the contact time with *Lactobacilli*. Chaveerach *et al.* (2002) reported that when  $10^3$  CFU/g of *Campylobacter* were exposed to pH 4.5 or pH 5.0 water-diluted acetic acid, the number of bacteria decreased over time and were not detected after 2 or 8 h, respectively. In the present study, there was no significant difference in the number of *Lactobacilli* or in the pH of the crop content between the GC and WPR groups; however, the pH range in the crop was low enough to inhibit bacterial growth. Therefore, when the crop is invaded by *Campylobacter*, it is possible that the longer retention of the content in the crop in chicks fed whole-grain

paddy rice may have a sterilizing or bacteriostatic effect on the gizzard, bacterial growth was inhibited as the gizzard maintains a lower pH environment than that in the crop, which is supported by our observation that the populations of *Lactobacilli*, coliforms, and lactose-negative enterobacteria in the gizzard were significantly smaller than those in the crop (Table 7). The amount of *Lactobacilli* in the gizzard of the chicks in the WPR group, BR was significantly higher than that of GC, it is not obvious why the populations of *Lactobacilli* in the gizzard were different between three diets.

Although the different diets had no effect on the pH range in the gizzard, bacterial growth was inhibited as the gizzard maintains a lower pH environment than that in the crop, which is supported by our observation that the populations of *Lactobacilli*, coliforms, and lactose-negative enterobacteria in the gizzard were significantly smaller than those in the crop (Table 7). The amount of *Lactobacilli* in the gizzard of the chicks in the WPR group, BR was significantly higher than that of GC, it is not obvious why the populations of *Lactobacilli* in the gizzard were different between three diets.

In conclusion, we speculate that there are two reasons why a whole-grain paddy rice diet inhibits *C. jejuni* colonization in the cecum of broiler chicks. First, the hardness (grinding resistance of the food material) of the whole-grain paddy rice promoted the development of gizzard activity; thus, this stronger grinding activity of the gizzard eliminated the areas of higher pH in the gizzard where *Campylobacter* could survive. Second, the larger amount and longer retention of the content in the crop in the WPR group had a bacteriostatic effect due to the longer co-existence of the pathogenic bacteria with *Lactobacilli*, which exhibit bactericidal or bacteriostatic action, due to the low pH in the crop.

## References

- Barash I, Nitsan Z and Nir I. Adaptation of light - bodied chicks to meal feeding: Gastrointestinal tract and pancreatic enzymes. *British Poultry Science*, 34: 35-42. 1993.
- Bjerrum L, Pedersen K and Engberg M. The influence of whole wheat feeding on Salmonella infection and gut flora composition in broilers. *Avian Diseases*, 49: 9-15. 2005.
- Chaveerach P, Keuzenkamp DA, Urlings HAP, Lipman LJA and van Knapen F. In vitro study on the effect of organic acids on *Campylobacter jejuni/coli* populations in mixtures of water and feed. *Poultry Science*, 81: 621-628. 2002.
- Denbow DM. Peripheral regulation of food intake in poultry. *Journal of Nutrition*, 124: 1349S-1354S. 1994.
- Dhanoa MS, Siddons RC, France J and Gale DL. A multicompartmental model to describe marker excretion patterns in ruminant faeces. *British Journal of Nutrition*, 53: 663-671. 1985.
- Duke GE. Recent studies on regulation of gastric motility in turkeys. *Poultry Science*, 71: 1-8. 1992.
- Duve LR, Steinfeldt S, Thodberg K and Nielsen BL. Splitting the scotoperiod: effects on feeding behaviour, intestinal fill and digestive transit time in broiler chickens. *British Poultry Science*, 52: 1-10. 2011.
- Engberg RM, Hedemann MS, Steinfeldt S and Jensen BB. Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. *Poultry Science*, 83: 925-938. 2004.
- Ferket PR and Gernat AG. Factors that affect feed intake of meat birds: A review. *International Journal of Poultry Science*, 5: 905-911. 2006.
- Fuller R. Ecological studies on the lactobacillus flora associated with the crop epithelium of the fowl. *Journal of Applied Bacteriology*, 36: 131-139. 1973.
- Gabriel I, Mallet S, Leconte M, Travel A and Lalles JP. Effects of whole wheat feeding on the development of the digestive tract of broiler chickens. *Animal Feed Science and Technology*, 142: 144-162. 2008.
- González-Alvarado JM, Jiménez-Moreno E, Lázaro R and Mateos GG. Effect of type of cereal, heat processing of the cereal, and inclusion of fiber in the diet on productive performance and digestive traits of broilers. *Poultry Science*, 86: 1705-1715. 2007.
- González-Alvarado JM, Jiménez-Moreno E, González- Sánchez, Lázaro R and Mateos GG. Effect of inclusion of oat hulls and sugar beet pulp in the diet on productive performance and digestive traits of broilers from 1 to 42 days of age. *Animal Feed Science and Technology*, 162: 37-46. 2010.
- Guinotte F, Gautron J, Nys Y and Soumarmon A. Calcium solubilization and retention in the gastrointestinal tract in chicks (*Gallus domesticus*) as a function of gastric acid secretion inhibition and of calcium carbonate particle size. *British Journal of Nutrition*, 73: 125-139. 1995.
- Jackson DN, Davis B, Tirado SM, Duggal M, van Frankenhuyzen JK, Deaville D, Wijesinghe MAK, Tessaro M and Trevors JT. Survival mechanisms and culturability of *Campylobacter jejuni* under stress conditions. *Antonie Van Leeuwenhoek*, 96: 377-394. 2009.
- Jackson S and Duke GE. Intestine fullness influences feeding behaviour and crop filling in the domestic turkey. *Physiology & Behavior*, 58: 1027-1034. 1995.
- Jagtap PS, Subramanian R and Singh V. Influence of soaking on crushing strength of raw and parboiled rice. *International Journal of Food Properties*, 11: 127-136. 2008.
- Jiménez-Moreno E, González-Alvarado JM, de Coca-Sinova A, Lázaro R and Mateos GG. Effects of source of fibre on the development and pH of the gastrointestinal tract of broilers. *Animal Feed Science and Technology*, 154: 93-101. 2009a.
- Jiménez-Moreno E, González-Alvarado JM, González-Serrano A, Lázaro R and Mateos GG. Effect of dietary fiber and fat on performance and digestive traits of broilers from one to twenty-one days of age. *Poultry Science*, 88: 2562-2574. 2009b.
- Jin LZ, Ho YW, Abdullah N, Ali MA and Jalaludin S. Antagonistic effects of intestinal *Lactobacillus* isolates on pathogens of chicken. *Letters in Applied Microbiology*, 23: 67-71. 1996.
- Kong F, Oztop MH, Singh RP and McCarthy MJ. Physical changes in white and brown rice during simulated gastric digestion. *Journal of Food Science*, 76: E450-E457. 2011.
- Lawlor PG, Lynch PB, Caffrey PJ, O'Reilly JJ and O'Connell MK. Measurements of the acid-binding capacity of ingredients used in pig diets. *Irish Veterinary Journal*, 58: 447. 2005.
- Mateos, GG, and Jiménez-Moreno E, Serrano MP and Lázaro RP. Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. *Journal of Applied Poultry Research*, 21: 156-174. 2012.
- Mitsuoka T. In *Studies of Bifidobacteria*. Japan Bifidus Foundation. Tokyo. 1994.
- Moen B, Rudi K, Svihus B and Skånseng B. Reduced spread of *Campylobacter jejuni* in broiler chickens by stimulating the bird's natural barriers. *Journal of Applied Microbiology*, 113: 1176-1183. 2012.
- Nir I, Shefet G and Aaroni Y. Effect of particle size on performance. 1. Corn. *Poultry Science*, 73: 45-49. 1994a.

- Nir I, Hillel R, Shefet G and Nitsan Z. Effect of grain particle size on performance. 2. Grain texture interactions. *Poultry Science*, 73: 781-791. 1994b.
- Nishii M, Yasutomi M and Sone Y. Inhibitory effect of whole grain paddy rice feeding on the colonization of *Campylobacter jejuni* in the cecum of broiler chicks. *Journal of Poultry Science*, 52: 312-317. 2015.
- Rehman HU, Vahjen W, Awad WA and Zentek J. Indigenous bacteria and bacterial metabolic products in the gastrointestinal tract of broiler chickens. *Archives of Animal Nutrition*, 61: 319-335. 2007.
- Rougière N and Carré B. Comparison of gastrointestinal transit times between chickens from D+ and D- genetic lines selected for divergent digestion efficiency. *Animal*, 4: 1861-1872, 2010.
- Santos FBO, Sheldon BW, Santos Jr AA and Ferket PR. Influence of housing system, grain type, and particle size on *Salmonella* colonization and shedding of broilers fed triticale or corn-soybean meal diets. *Poultry Science*, 87: 405-420. 2008.
- Savory CJ. An investigation into the role of the crop in control of feeding in Japanese quail and domestic fowls. *Physiology & Behavior*, 35: 917-928. 1985.
- Short FJ, Gorton P, Wiseman J and Boorman KN. Determination of titanium dioxide added as inert marker in chicken digestibility studies. *Animal Feed Science and Technology*, 59: 215-221. 1996.
- Singh Y, Ravindran V, Wester TJ, Molan AL and Ravindran G. Influence of feeding coarse corn on performance, nutrient utilization, digestive tract measurements, carcass characteristics, and cecal microflora counts of broilers. *Poultry Science*, 93: 607-616. 2014.
- Svihus B. The gizzard: function, influence of diet structure and effects on nutrient. *World's Poultry Science Journal*, 67: 207-224. 2011.
- Svihus B. Function of the digestive system. *The Journal of Applied Poultry Research*, 23: 306-314. 2014.
- Vergara P, Ferrando C, Jiménez M, Fernández E and Goñalons E. Factors determining gastrointestinal transit time of several markers in the domestic fowl. *Quarterly Journal of Experimental Physiology*, 74: 867-874. 1989.
- Walk CL, Bedford MR and McElroy AP. Influence of limestone and phytase on broiler performance, gastrointestinal pH, and apparent ileal nutrient digestibility. *Poultry Science*, 91: 1371-1378. 2012.