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Effects of Different Feeding Frequencies on Broiler Chickens' Growth Performance and Intestinal Villus Development

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Abstracts

This experiment was conducted to determine the effects of feeding frequency on growth performance and development of small intestine in broiler chickens from 1 to 14 days of age. Forty Aber acres broiler chicks at 1 day old were randomly assigned to 4 treatments as follows - T1: feeding once daily (100 % diet at 6:30), T2: feeding twice daily (50 % diet at 6:30 and 18:30), T3: feeding 4 times per day (25 % diet at 6:30, 11:30, 16:30 and 21:30) and T4: feeding 6 times per day (16.67 % diet at 6:30, 9:30, 12:30, 15:30, 18:30 and 21:30). Chickens were fed with commercial broiler rations for 14 days. Growth performance parameters including BW, BW gain, FI and FCR were determined. Changes in intestinal villus morphology were examined by hematoxylin and eosin (H&E) dye staining. Results showed that chickens fed 4 and 6 times per day had lower ($p < 0.05$) BW, BW gain and FI when compared with chickens fed once daily, from 1 to 7 days of age. However, at 14 days of age, there were no significant differences in all parameters of growth performance. There were no changes in gross anatomy of the small intestine. Fixed feeding 4 and 6 times per day resulted in the villus height of the jejunum and ileum being higher ($p < 0.05$) than when feeding once a day. Feeding 6 times per day resulted in the highest villus density in jejunum among feed regimes. The results suggested that both once and multiple daily feedings were suitable approaches to achieve optimum growth performance for broiler chickens from 1 to 14 days of age. Fixed feeding 6 times per day being the most beneficial for improving intestinal villus development.

Keywords: Broiler, Feed restriction, Compensatory growth, Villus, Small intestine

Introduction

Poultry meat is the most favored protein source. It is an available and economical source of animal protein. Consumption of poultry meat is estimated to increase globally to 154 million tons by 2031 with poultry meat projected to account for 47 % of the protein consumed from meat sources [1]. In response to consumer demand, over last 30 years the broiler industry has developed genetics, nutrition and controlled environments, aiming to produce chicken with high growth rates [2,3]. Rapid growth and high feed efficiency are 2 key factors for effective broiler production nowadays. High growth rate of broilers could be accomplished where chickens consumed feed *ad libitum*, along with effective disease control and continuous lighting [4,5]. Unfortunately, it has been reported that the chickens fed *ad libitum* had an accompanying increased rate of mortality and metabolic diseases. Moreover, feeding more than chicken maintenance and production requirements resulted in increased feed wastage and feed costs as well as low carcass quality [5-7]. Therefore, novel strategies to overcome these problems are essential. Feed restriction is a well-known technique to manipulate the growth curve of broiler chickens. Previous reports revealed that feed restriction alleviated health problems, improved carcass quality and increased feed efficiency [2, 8-10]. The compensatory growth effect is a key mechanism of effectiveness in feed restriction. This

phenomenon was observed in feed restricted chickens as producing a market weight similar to that of chickens fed *ad libitum* [2,5,11,12].

Although, feeding frequency is one method of feed restriction, the effects of frequent feeding on broiler growth performance remains unclear. Aziz *et al.* [13] reported that increased frequency of feeding could increase overall BW and feed efficiency in broiler chickens. The study showed feeding for 3 times a day gave the best results on final BW, BW gain and feed efficiency. This is in agreement with study of Farghly and Hassanien [14] which reported that increased frequency of feeding caused a decrease in abdominal fat and improved growth performance of broilers. In contrast, Adikari *et al.* [4] observed that there were not significant differences in weight gain, daily weight gain and feed conversion ratio in chickens fed once, twice, thrice and 4 times per day. Similarly, Aziz and Masoud [15] reported no significant differences in overall weight, average daily gain and feed conversion ratio among chickens with one feed, 2 equal feeds and 3 equal feeds per day. Furthermore, Liu *et al.* [16] demonstrated that geese fed *ad libitum*, thrice, 4 and 5 times per day had no significant difference on final BW, average daily gain and feed efficiency. They suggested all feeding regimes were suitable for geese to achieve optimal production [16]. Whereas, Kareem-Ibrahim *et al.* [17] reported that increased frequency of feeding could decrease growth parameters. The study revealed that broiler chickens fed twice per day had a better impact on final weight and average daily gain than those of chickens fed thrice per day.

Almost all nutrients are digested and absorbed at small intestine. Thus, changes in gross anatomy and histology of the small intestine may alter digestive and absorptive abilities affecting growth performance. In broiler chicken, previous reports have demonstrated that the height of villus and cell area in feed restricted chickens were decreased and recovered after refeeding *ad libitum*. The final BW of feed restricted chickens was also decreased when compared with those of chickens fed *ad libitum* [18]. This is in agreement with the study of Dastar *et al.* [19] who observed that decrease in overall BW of feed restricted chickens was associated with decreases in width, height and surface area of villus of duodenum, jejunum and ileum. These results imply that feed restriction had negative effect on intestinal villus development. However, these studies were performed using the method of feed withdrawal [18,19], but the effects of other feed restriction methods including feeding frequency remains unknown. Liu *et al.* [16] reported that increasing feeding frequency in geese resulted in no significant difference in length of duodenum, jejunum, ileum and small intestine but unfortunately, changes in histology were not examined [16]. The present study aimed to investigate the effects of feeding frequency on growth performance in broiler chickens from 1 to 14 days. Changes in anatomy and histology of the small intestine by difference in frequency of feeding were also examined. Here, we provide the first report that the compensatory growth effects by frequently feeding was at least mediated through adaptation of villus morphology in the small intestine along with improved feed consumption.

Materials and methods

Animal and experimental design

This experiment was approved by the Institutional Animal Care Use Committee, Mahasarakham University (AICUC-MSU), Thailand. Approval number: AICUC-MSU-35/2022. Sample size was calculated by using G* Power version 3.1 for ANOVA [20]. To achieve 95 % power of study to detect an effect size from means ($f = 0.705$) with 5 % margin of error, the required sample size was 10 chickens for each group. All chickens were obtained from a commercial hatchery in Thailand. A total of 40 Arber Acres, 1-day old broiler chickens were randomly divided into 4 feeding frequency treatments; once, twice, 4 and 6 times per day in a completely randomized design as is shown in **Table 1**. Each feeding frequency treatment was replicated ten times.

Table 1 Experiment design.

Treatments	Feed amount (%)	Time					
		First meal	Second meal	Third meal	Fourth meal	Fifth meal	Sixth meal
T1	100 %	06:30	-	-	-	-	-
T2	50 %	06:30	18:30	-	-	-	-
T3	25 %	06:30	11:30	16:30	21:30	-	-
T4	16.67 %	06:30	09:30	12:30	15:30	18:30	21:30

Animal diet and housing

The experiment was performed at the Faculty of Veterinary Sciences, Mahasarakham University, Thailand. The chickens were reared in separated pens which were placed on a concrete floor. Rice husk was used as the litter material and the pens were equipped with drinkers and feeders. Drinking water was provided *ad libitum* under a continuous lighting system. 100 W electric bulbs were used for heating during the brooding period from 1 to 7 days of age. The chickens were vaccinated by eye-drop against Newcastle's disease and infectious bronchitis disease at 7 days of age. All chickens were fed on commercial broiler starter with 21.35 % crude protein with differences in frequency of feeding from 1 to 14 days. Adjustment of daily feed amount accorded with the recommendation made by Aviagen brand 2022. The feeders and drinkers were cleaned daily, and the drinking water was replaced by fresh water every day.

Parameters measured

Body weight (BW) was measured at 1, 7 and 14 days of age. The body weight gain for the designated period of study was calculated as the difference between final BW and initial BW. Feed intake (FI) was daily determined by measuring given feed and leftover feed per pen. In addition, feed conversion ratio (FCR) was calculated (feed intake (g)/ weight gain (g)) as described by Aziz and Masoud [15]. At the end of experiment (day 14), the chickens were ethically killed by cervical dislocation and then dissected for determining changes in gross anatomy and histology of the small intestine. The lengths of duodenum, jejunum, ileum and overall small intestine were measured as described by Liu *et al.* [16]. The jejunum was located between pancreatic loop and Meckel's diverticulum, while the ileum was located between Meckel's diverticulum and ileocecal junction.

Changes in histology were observed by microscopic examination. In brief, sample tissue was fixed with 10 % buffered formalin and then subjected to sectioning and staining with hematoxylin and eosin (H&E) dye as described by Aengwanich and Suchint [21]. Villus length, crypt depth and villus density of duodenum, jejunum and ileum were measured as described by Khempaka *et al.* [22] using a Nikon Eclipse E200 microscope and analyzed with NIS-Elements program.

Statistical analysis

Analysis of variance was determined using one-way ANOVA. Differences among treatments were determined using Duncan's multiple-range test. The level of significance was set at $p < 0.05$.

Results

Performance: Body weight, Body weight gain, Feed intake and Feed conversion ratio

The effects of feeding frequency on growth performance in broiler chickens is shown in **Table 2**. At 7 days of age, the BW difference was significant ($p < 0.05$) among treatment groups. The BW of broiler chickens fed 4 and 6 times daily was less ($p < 0.05$) than that of chickens fed once daily. However, it was observed that the BW was not significant different ($p > 0.05$) among feeding regimes at 14 days of age. The BW gain of chickens during experimental period from 1 to 7 days of age had significant differences ($p < 0.05$) among groups of treatments, but not for the period of 1 to 14 days of age. BW gain was examined from 1 to 7 days of age, the BW gain of chickens fed 4 and 6 times per day was less ($p < 0.05$) than that of chickens fed once daily. Additionally, FI of chicken for a period of 1 to 7 days was affected by feeding regimes. Accumulative FI from 1 to 7 days for chickens fed once daily was greater ($p < 0.05$) than that of chickens fed 4 and 6 times per day. However, there were no differences in feeding frequencies ($p > 0.05$) in FI of broiler chicken for the period of 1 to 14 days of age. There were no significant differences ($p > 0.05$) of FCR among chickens fed once, twice, 4 and 6 times per day for both 1 to 7 and 1 to 14-day periods.

Table 2 Effects of feeding frequency on growth performance in broiler chickens.

Parameter	Treatment				SEM	*p*- value
	T1	T2	T3	T4		
BW (g)						
1 day	42.00	42.00	41.00	41.00	0.57	0.868
7 days	169.00[a]	153.50[a,b]	142.00[b]	134.00[b]	27.56	0.021
14 days	299.00	277.00	271.00	277.00	6.10	0.393
BW gain (g/bird)						
1 to 7 days	127.00[a]	111.50[a,b]	101.00[b]	93.00[b]	4.32	0.027

Parameter	Treatment				SEM	*p*- value
	T1	T2	T3	T4		
8 to 14 days	130.00	123.00	129.00	143.00	7.31	0.824
1 to 14 days	257.00	235.00	230.00	236.00	6.09	0.421
Feed intake, FI (g/bird)						
1 to 7 days	165.50[a]	156.00[a,b]	138.50[b,c]	126.00[c]	4.53	0.006
8 to 14 days	422.00	420.50	389.50	381.50	79.38	0.701
1 to 14 days	587.50	558.50	528.00	507.50	11.09	0.051
Feed conversion ratio (FCR)						
1 to 7 days	1.33	1.42	1.39	1.41	0.03	0.660
1 to 14 days	2.30	2.40	2.31	2.18	0.03	0.063

Note: [a, b, c] Means with different superscripts in a row are significantly different ($p < 0.05$).

Digestive development: Intestinal length, length of villus, crypt depth and number of villus

The effects of feeding frequency on small intestine development were examined. Changes in gross anatomy and histology of the small intestine were investigated at 14 days old. Results are shown in **Table 3**. The chickens fed once daily had length of jejunum and ileum longer ($p < 0.05$) than of chickens fed 6 times per day. However, differences in frequency of feeding had no effects on total length of small intestine ($p > 0.05$). Changes in intestinal villus morphology were determined by using H&E staining. The results revealed that the villus height of jejunum and ileum of those chickens fed once daily was less ($p < 0.05$) than that of chickens fed 4 and 6 times per day. There were no significant differences ($p > 0.05$) in crypt depth of duodenum, jejunum and ileum among the feeding regimes. Difference in feeding regimes did effect the villus density of the small intestine. The results indicated that the number of villi per unit at jejunum was the highest ($p < 0.05$) with fixed feeding 6 times per day and lower in other feeding regimes.

Table 3 Effects of feeding frequency on small intestine development in broiler chickens.

Parameter	Treatment				SEM	*p*-value
	T1	T2	T3	T4		
Intestinal length (cm)						
Small intestine	112.20	111.68	102.95	100.55	1.94	0.062
Duodenum	18.65	19.85	18.60	19.50	0.43	0.673
Jejunum	47.05[a]	45.55[a,b]	41.40[a,b]	40.20[b]	1.01	0.043
Ileum	46.50[a]	46.28[a,b]	42.95[a,b]	38.85[b]	0.97	0.011
Height of villus (µm)						
Duodenum	1044.02	1149.94	1230.40	1151.53	39.08	0.425
Jejunum	752.45[b]	880.34[a,b]	980.23[a]	941.45[a]	30.42	0.038
Ileum	531.53[b]	516.70[b]	744.02[a]	692.87[a]	31.08	0.012
Crypt depth (µm)						
Duodenum	160.94	173.78	160.01	174.04	6.23	0.775
Jejunum	135.13	140.18	157.69	144.23	4.72	0.382
Ileum	106.39	96.52	115.44	117.44	3.97	0.227
Number of villus (per 100 µm, unit)						
Duodenum	0.54	0.54	0.44	0.48	0.03	0.466
Jejunum	0.50[b]	0.44[b]	0.49[b]	0.65[a]	0.02	0.003
Ileum	0.76	0.76	0.62	0.75	0.03	0.145

Note: [a-b] Means with different superscripts in a row are significantly different ($p < 0.05$).

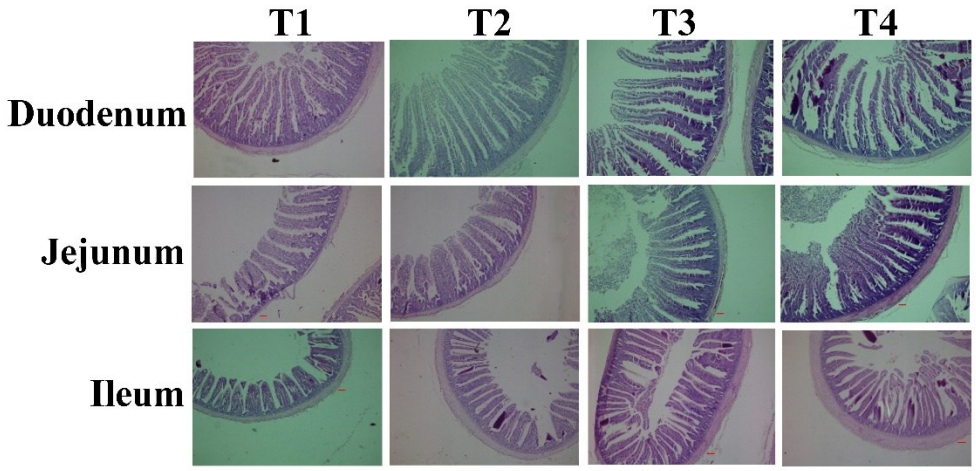

Figure 1 Effects of feeding frequency on histology of small intestine in broiler chickens. Tissues of duodenum, jejunum and ileum were prepared and stained by H&E dye. Feeding frequency treatments were once (T1), twice (T2), 4 (T3) and 6 (T4) times per day. Scale bar = 100 μm, magnification: ×40.

Discussion

Chicken growth performance depends on several factors including disease status, FI, feed digestibility, feed absorption and feeding pattern. The present study revealed that, at first week of age, growth parameters; BW, BW gain and FI were decreased in feeding groups 4 and 6 times per day. This implied that increasing feeding frequency could induce growth suppression at an early phase of treatment and this may be involved with a decrease in daily FI. Feed restriction could induce feed limitation or absence in broiler chickens. Thus, feed restriction by feeding frequency method may contribute to a lack of nutrients for cell growth [23,24] and changes in patterns of growth associated with hormone secretion, growth hormone, thyroid and insulin-like growth factor-1 (IGF-1) [25,26] resulting in growth suppression. The present findings were in agreement with a previous report that, in the early phase of treatment, chickens fed twice and thrice daily had lower BW and FI than those of chickens fed *ad libitum* although these were comparable at the end of the study [14]. Also, Liu *et al.* [16] reported that the BW and FI in geese fed *ad libitum* were significantly higher than those of geese fed 3, 4 and 5 times per day in early treatment, but at the end of experiment the final BW, average daily gain, average daily FI and feed conversion were equal.

In comparison with early phase of treatment, in the final phase, the present study showed final BW of those chickens fed 4 and 6 times per day were comparable to chickens fed once daily ($p > 0.05$). Also, FI from 8 to 14 days of age were equal among group of treatments. This implied that groups of feeding 4 and 6 times daily adapted by increased FI per day for weight compensation. In the present study, the compensatory growth effects of broilers fed 4 and 6 times daily may occur from 8 to 14 days. Compensatory growth is a phenomenon in which the chicken experiences abnormally rapid growth relative to age. This allowed feed restricted chicken to attain a market weight similar to that of chickens fed *ad libitum* [2,5,12]. The present results indicated that increased FI was at least a mechanisms of compensatory growth. This was agreed with previous reports, studies have revealed that the compensatory growth by increased feeding frequency occurred in association with increase in FI per unit time [14,16]. The key mechanisms of compensatory growth is not only depends on increased FI, but also increased digesta load, decreased maintenance requirement and digestive organs adaptation [2].

Feed digestibility and absorptive capacity also play important roles in high broiler growth performance, not only increased feed consumption. The small intestine is an important organ of the digestive system as almost all nutrients are digested and absorbed there. Thus, changes in gross anatomy and histology could have an effect on the area available for digestion and absorption thereby affecting growth performance. Previous studies have shown that feed restriction had negative effects on intestinal villus development [18,19]. Yamauchi *et al.* [18] reported that the villus height and cell area in the duodenum of feed restricted chickens were decreased after 3 days feed withdrawal. Similarly, Dastar *et al.* [19] demonstrated that feed restriction could decrease the height, width and surface area of villi of the duodenum, jejunum and ileum in broiler chickens. At the end of experiments, overall BW in restrictively fed chickens was decreased [18,19]. In the present study, frequent feeding at 4 and 6 times per day resulted

in decreased length of the jejunum and ileum when compared with fixed feeding for once daily. Whereas, villus height at jejunum and ileum and villus density at jejunum of chickens fed 4 and 6 times per day were increased, the total length of small intestine was not changed. It was noted that fixed feeding 6 times per day resulted in the highest villus density at jejunum among group of treatments, this feeding regimen appears to offer the most effective solution for enhancing intestinal villus development. These results imply that the loss of area for digestion and absorption may be compensated by marked increase in villus height and villus density and these resulted in no difference in overall BW among feeding regimes, at the end of study. Differences in results reported in this and previous studies [18,19] may be due to difference in method of feed restriction. Feed restriction by the feed withdrawal method was reported in previous studies [18,19]. In the present study, it was noted that improvement of intestinal villus morphology was associated with compensatory growth effects of chickens fed 4 and 6 times per day. The digestive adaptation was one important factor contributing to growth compensation [2]. Our study demonstrated firstly that the compensatory growth could be achieved by promoting improvement of intestinal villus development and feed consumption when broiler chickens were fed frequently. However, our study did not investigate beyond 2 weeks of age. The effects of frequency of feeding on broiler performance and intestinal development at the market age should be further study.

Conclusions

The present study suggests that both a single daily feed and feeding more than once daily at 2, 4 and 6 times per day may be a suitable approach for achieving optimum production while feeding at least 6 times per day proved to be the best approach for enhancing intestinal villus development in broilers aged 1 to 14 days. Here, we provide the first report that the compensatory growth effects by frequently feeding was at least mediated through adaptation of villus morphology in the small intestine along with improved feed consumption.

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