Research Article

The Effects of Feeding Time Restriction on Carcass Yield Characteristics, Gastrointestinal and Immune Organs of Broiler

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Abstract | A study was conducted to investigate the effect of the feeding time restriction with two cycles free access to feed 2 hours/day and 4 hours/day on the body weight of broiler, carcasses characteristics, gastrointestinal tract and immune organs at the end feed restricted and re-alimentation period. This research used two hundred of seven days-old unsexed commercial strain Lohmann of broiler chicken. The experimental design was completely randomized, with 5 treatments and 4 replications. As a control, chickens were fed *ad libitum* (FR-0); chickens were given free access to feed during two periods of 2 hours from age 7-14 days (FR-1) and age 7-21 days (FR-2); chickens were given free access to feed during two periods of 4 hours from age 7-14 days (FR-3) and age 7-21 days (FR-4). Body weight, carcass yield, breast, and tight muscle weight of broilers subjected to feed time restriction were significantly lower than broilers fed *ad libitum* at the end of the feed restriction. The empty of crop and gizzard weight were significantly higher than the broilers fed *ad libitum* at age 14 and 21 days. The weight of bursa Fabricious increased (P<0.05) with the feeding time restriction at age 14 days. However, there were not significant different in all of parameter at the end re-alimentation period (42 days). Based on our findings, feeding time restriction in broilers by 4 hours/day had no negative effect on body weight at slaughter age, carcass characteristics, or gastrointestinal and immune organs.

Keywords | Feed restriction, Carcass yield, Crop, Gizzard, Bursa fabrcious

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INTRODUCTION

The improvements in genetics, nutrition, and the growth performance of broiler chickens has greatly improved in the past few decades. This advantage can provide to reach the target body weight at slaughter age in a shorter time and have better production indexes. However, the rapid growth rate of broilers has caused health problems, while higher nutrient supplies have led to increased fat deposition (Tumova and Teimouri, 2010). Therefore, feed restriction is used as a management strategy to prevent these problems. Feed restriction has the potential to control body growth and some metabolic disorders related to the fast growth rates of broiler chicken genetic lines (de Jong et al., 2012; Butzen et al., 2013). Similarly, restricted feeding becomes a preference to improve the production efficiency of broilers (Sahraei, 2012) and increase resistance to heat stress (Xie et al., 2015).

The feed restriction program can be implemented through qualitative feed restriction such as provide feed diluted with rice hull and oats hull (Lesson et al., 1991, 1992), wood charcoal (Fanooci and Torki, 2010), lignocellulose (Oikeh et al., 2019), or quantitative feed restriction through limited feed supply or free access to the feed during certain times of the day (Al-Khair et al., 2017; Fondevila et al., 2020). There are many variations of feed restriction including quantitative feed restriction (Adeyemi et al., 2015; Butzen

et al., 2015; Shabani et al., 2015; Attia et al., 2017; Tumova and Chodova, 2018; Al-Khair, 2019; Khurshid et al., 2019; van der Klein et al., 2017), limiting by time or feed withdrawal (Aliakbarpour et al., 2013; Svihus et al. 2013; Butzen et al., 2015; Shafiei et al., 2018; Orso et al., 2019; Livingston et al., 2019; Fondevila et al., 2020), and skip a day feeding (Omolola and Olutoye, 2020; Shawkat et al., 2021; Akinsola et al., 2021) have been evaluated, however, it is not easy to find the appropriate method, because different restriction methods will give different results (Tumova et al., 2019).

According to previous investigations, feeding time restriction with a schedule on a specific day is easier to implement and is a less severe form of feed restriction. (Susbilla et al., 2003; Zhang et al., 2007; Azis et al., 2012), However, there are a number of differences in the results on final body weight and carcasses characteristics (Al-Khair et al., 2017; Shafieia et al., 2018; Farghly et al., 2019; Livingstone et al., 2019), gastrointestinal tract (Fondevila et al., 2020), immune organs (Farghly and Makled, 2015), and immune response (Mahmood et al., 2007). These differences are due to differences in the method of feed restriction and its application.

The implementation of feed restriction through restriction by feeding time, Fargly et al. (2019) found that intermittent feeding for 4 hours and 4 hours of fasting did not decrease the growth performance of the broiler in implementing feed restriction through restriction by feeding time. Similarly, Fondevila et al. (2020) demonstrated that restricting broilers from eating for less than 6 hours/day resulted in comparable body weight gain to broilers fed *ad libitum*. However, Butzen et al. (2013) concluded that chickens had time feeding 8 hours/day is considered most severe in broilers but chickens were able to reach a similar final weight at 42 d of age. According to these findings, broilers will likely adapt quickly to a prolonged restricted feeding regimen to achieve full compensatory growth performance. (Fondevila et al., 2020). Moreover, the immune organs, especially bursa Fabricius, can be affected by nutritional stress due to feed restrictions. In an early report, Griffiths et al. (1985) reported that nutritional stress causes thymus atrophy and a decrease in the weight of the Fabricius bursa. Jahanpour et al. (2015) found that quantitative feed restriction for 7 days did not affect the relative weight of immune organs but that 25 and 50% feed restriction for 14 days reduced the bursa of Fabricius weight. This fact suggests that the severity of feed restriction may be attributed to stress, which can stimulate corticosteroid secretion and inhibit immune cell proliferation. Therefore, feeding time restriction or intermittent feeding as a mild feed restriction system can be applied in a specific time interval to reduce the severity of feed restriction and stress. Based on these facts, it is unknown what intermittent of feed access during two periods of 4 hours/day may be reduce the severity of feed restriction. Studies in this respect are necessary, in order to know the response of feeding time restriction with different length of restriction periods on the carcasses characteristics, gastrointestinal tract and immune organs at the end feed restriction and re-alimentation period. It was hypothesized that the final body weight of broilers restricted during the starter period would catch up after restriction and show no effect on carcasses characteristcs, gastrointestinal tract and immune organs.

The present work is conducted to investigate the influence of the feeding time restriction with two cycles free access to feed 2 hours/day and 4 hours/day on the body weight, carcasses characteristics, gastrointestinal tract and immune organs at the end feed restriction and re-alimentation period. Besides that, the other goal of this study is to adapt broilers to the new feed-saving strategy and to develop specific broiler production practices to reduce costs while maintaining or increasing productivity.

MATERIALS AND METHODS

The chickens were raised under the standard procedures for rearing and treating farm animals outlined in the Law of the Republic of Indonesia No. 18, 2009, on animal husbandry and health.

Chickens, feed and housing

The experiment used 200 unsexed 7-days of age broiler chickens of strain Lohmann. Chickens were fed a commercial starter diet (22% crude protein) from age 1 to 21 days and a finisher diet (20% crude protein) from age 22 to 42 days. The chickens were raised in an open-sided house with natural cyclic temperatures (minimum, 20°C; maximum, 34°C) in 20 pens with perforated wooden floors.

Treatments and experimental design

At seven days of age, all chickens were weighed and randomly assigned to 20 cages with ten chicks/cage (l × w × h; 1 × 1.25 × 0.75 m). The study designed using a completely randomized design, with 5 treatments. The treatment included: chickens were fed *ad libitum* as a control (FR-0); chickens were given free access to feed during two periods of 2 hours (08:00-10:00 am and 04:00-06:00 pm) from age 7-14 days (FR-1) and age 7-21 days (FR-2); chickens were given free access to feed during two periods of 4 hours (08:00-12:00 am and 04:00-08:00 pm) age 7-14 days (FR-1) and age 7-21 days (FR-4). The chickens were fed *ad libitum* until 42 days of age at the end of the feeding time restriction. Each treatment was replicated 4 times, and the experimental unit for all measurements was placed in the pen with a perforated wood floor.

Variables measurement and statistical analyses

At 14, 21, and 42 d of age, 2 chickens close to the average of each group were selected for measurement of body weight, carcass, abdominal fat, and viscera organ weights. The chickens were fasted for 6 h, individually weighed, and then slaughtered according to the Islamic method by severing the jugular vein. Chickens slaughtered at 14, and 21 days of age were plucked using dry plucking. The chickens slaughtered at 42 days of age were scalded at 70°C for one minute, de-feathered using a machine picker, and then carcasses chilled with ice water for 6 hours. The carcass was drained for 5 minutes and eviscerated before determining the carcass weight. Manually, fat in the abdomen was removed from the carcass and weighed. The carcass and the abdominal fat weight were expressed as a percentage of body weight. The carcass fraction was dissected into breasts and legs. The breast included meat and skin without sternum. Leg comprises thigh and drumstick meat without femur and tibia. The viscera had been manually eliminated, and gastrointestinal organs such as crop, proventriculus, gizzard, small intestine, pancreas, and liver weights had been recorded. The total weight of carcass fractions was compared to the eviscerated carcass weight, while the weight of various gastrointestinal organs was compared to the body weight. The organs of the immune included the spleen, thymus, and bursa Fabricius, were collected and weighed, expressed as mg/g body weight.

The analyses of variance were done using the GLM procedure (SAS Institute, 2001). Duncan's test compared the differences among treatments. The significant differences were based on testing at P<0.05.

RESULTS AND DISCUSSION

Body weight

The effects of the treatments on body weight and carcass characteristics were shown in Table 1. The body weight of broilers was lower (P<0.05) by the feeding time restriction than those of control broilers from 7 to 14 or 21 days of age. During the feeding time restriction from 7 to 14 days of age, the body weight of the treatment FR-3 and FR-4 was higher (P<0.05) than that of FR-1 and FR-2. FR-4 was lower (P<0.05) than FR-0 during the period of restriction from 7 to 21 days of age but higher (<P0.05) than FR-1, FR-2, and FR-3. At 42 days of age, there were no significant differences in body weight between broilers subjected to feed restriction and control broilers.

Table 1: Effect of feeding time restriction during the starter period on body weight (BW), carcass weight (CW) and abdominal fat weight.

Age	Variables	Treatments					SE	p value
		FR - 0	FR - 1	FR - 2	FR - 3	FR - 4		
14 days	Body weight; BW (g/chick)	443.3^{a}	382.0^{c}	374.0^{c}	409.3^{b}	419.8^{b}	8.21	0.0002
	Carcass weight; CW (% BW)	61.9	64.8	63.3	62.2	62.1	1.89	0.7939
	Breast muscle (% CW)	20.2^{a}	17.5^{b}	18.1^{b}	20.1^{a}	20.8^{a}	0.43	0.0002
	Thigh muscle (% CW)	9.51	9.64	9.76	10.23	10.01	0.50	0.8498
	Drumstick muscle (% CW)	8.22	8.06	8.21	8.88	8.70	0.32	0.3391
	Abdominal fat (% BW)	1.31	1.43	1.20	1.10	1.28	0.12	0.4149
21 days	Body weight; BW (g/chick)	865.0^{a}	776.5^{c}	745.3^{d}	763.3^{cd}	805.3^{b}	7.79	<0.0001
	Carcass weight; CW (% BW)	73.8^{a}	72.8^{ab}	71.7^{bc}	73.8^{a}	71.1^{c}	0.54	0.0084
	Breast muscle (% CW)	22.8^{a}	19.7^{cd}	18.3^{d}	21.6^{ab}	20.3^{bc}	0.61	0.0012
	Thigh muscle (% CW)	11.2^{a}	11.1^{a}	11.6^{a}	10.2^{b}	10.3^{b}	0.25	0.0049
	Drumstick muscle (% CW)	8.67	8.75	8.68	8.52	8.69	0.26	0.9766
	Abdominal fat (% BW)	1.98	2.02	1.76	2.11	1.80	0.12	0.2411
42 days	Body weight; BW (g/chick)	2573.3	2470.3	2540.8	2481.5	2523.8	60.13	0.7370
	Carcass weight; CW (% BW)	77.8	78.3	75.8	77.6	77.0	0.59	0.0799
	Breast muscle (% CW)	24.6	24.5	24.1	24.0	26.3	1.01	0.5102
	Thigh muscle (% CW)	10.8	10.9	11.6	11.4	10.9	0.62	0.8667
	Drumstick muscle (% CW)	6.87	6.77	7.71	6.63	7.57	0.34	0.1262
	Abdominal fat (% BW)	3.28	3.18	2.19	3.12	2.82	0.29	0.1092

$^{a, b, c, d}$ Means within same row with no common superscrips differ at p<0.05. Chicks fed *ad libitum* (FR-0); chickens fed *ad libitum* for two cycles of 2 h periods during age 7-14 d (FR-1) and during age 7-21 d (FR-2); chickens fed *ad libitum* for two cycles of 4 h periods during age 7-14 d (FR-3) and during age 7-21 d (FR-4).

Table 2: Effect of feeding time restriction during the starter period on gastrointestinal organs.

Age	Variables	Treatments					SE	p-value
		FR - 0	FR - 1	FR - 2	FR - 3	FR - 4		
14 days	Crop (%BW)	0.54[b]	0.78[a]	0.88[a]	0.76[a]	0.80[a]	0.06	0.0149
	Proventriculus (%BW)	0.69	0.67	0.71	0.72	0.68	0.04	0.9174
	Gizzard (%BW)	1.97[c]	2.35[b]	2.81[a]	2.32[b]	2.06[c]	0.10	0.0002
	Pancreas (%BW)	0.48	0.44	0.42	0.41	0.42	0.02	0.2709
	Liver (%BW)	3.04	2.96	3.04	2.93	2.77	0.11	0.4644
	Small Intestine (%BW)	6.40	5.71	5.65	5.36	5.83	0.37	0.2146
21 days	Crop (%BW)	0.36[b]	0.50[ab]	0.64[a]	0.41[b]	0.58[a]	0.05	0.0050
	Proventriculus (%BW)	0.52	0.58	0.53	0.58	0.56	0.03	0.5380
	Gizzard (%BW)	1.53	1.58	1.55	1.50	1.57	0.07	0.9170
	Pancreas (%BW)	0.33	0.39	0.34	0.32	0.37	0.04	0.6242
	Liver (%BW)	2.31	2.50	2.37	2.40	2.45	0.12	0.8228
	Small Intestine (%BW)	3.52	3.88	4.13	3.78	4.08	0.27	0.5082
42 days	Crop (%BW)	0.29	0.31	0.35	0.27	0.33	0.03	0.4168
	Proventriculus (%BW)	0.32	0.33	0.38	0.31	0.32	0.03	0.5413
	Gizzard (%BW)	0.93	0.84	0.94	0.91	0.87	0.05	0.5855
	Pancreas (%BW)	0.25	0.22	0.24	0.22	0.21	0.01	0.3300
	Liver (%BW)	1.98	2.26	1.96	2.01	1.93	0.13	0.4373
	Small Intestine (%BW)	2.64	2.81	2.83	2.67	2.82	0.20	0.9321

[a, b, c] Means within same row with no common superscrips differ at $p<0.05$. Chicks fed *ad libitum* (FR-0); chickens fed *ad libitum* for two cycles of 2 h periods during age 7-14 d (FR-1) and during age 7-21 d (FR-2); chickens fed *ad libitum* for two cycles of 4 h periods during age 7-14 d (FR-3) and during age 7-21 d (FR-4).

Carcass characteristics

There were similar carcass yields at 14 days of age between the broiler subjected to the feed restriction and the control broiler. FR-1 and FR-2 were lower ($P<0.05$) breast muscle weights than FR-3, FR-4, and FR-0, while the breast muscle weight of FR-3 and FR-4 were similar to FR-0. However, the carcass weight of FR-0, FR-1 and FR-3 were higher ($P<0.05$) than FR-2 and FR-4 at 21 days of age. The breast muscle weight of FR-1, FR-2, and FR-4 were lower ($P<0.05$) than FR-3 and FR-0. The thigh muscle weight of FR-3 and FR-4 were lower ($P<0.05$) than FR-0, FR-1, and FR-2. There were no significant differences in the relative carcass weight among the restricted and control broilers at age 42 days. The weight of the breast, thigh, and drumstick muscles was not different between the broiler subjected to the feed restriction and the control broiler. Similarly, abdominal fat weight showed no significant differences among all treatments at 14, 21, and 42 days of age.

Gastrointestinal organs

The results of feeding time restriction on the gastrointestinal organs are presented in Table 2. At the end of the feeding restriction at age 14 days, the relative empty crop and gizzard weight of the broilers subjected to the feed restriction was higher ($P<0.05$) than the control. The relative gizzard weight of FR-4 was not different from FR-0. While the end feed restriction at age 21 days, the relative empty crop weights of FR-1 and FR-3 were no different from FR-0, while the relative empty crop weight of FR-2 and FR-4 were higher than FR-0 ($P<0.05$). No differences were found in the gastrointestinal organs between the broiler subjected to the feed restriction with the control broiler at the end of re-alimentation or recovery period (42 days of age).

Immune organs

Mean values regarding some immune organs weight of the broilers from the different treatments of feeding time restriction are shown in Table 3. Broilers who had to feed restriction for 7 days (7-14 days of age) had higher ($P<0.05$) bursa Fabricius weight than the control broiler. During this period, the spleen and thymus have similar weights ($P>0.05$) among all the treatments. At the ages 21 and 42 days, the bursa Fabricius, spleen, and thymus weight did not differ in the mean values among all the treatments.

Feed restriction has been widely used in the broilers, but it is not easy to find a suitable method, because different restriction methods will produce different results. In the present study show that the broilers had feed restriction by limiting feeding time during two periods of 2 hours (08:00-10:00 am and 16:00-18:00 pm) and 4 hours (08:00-12:00 am and 16:00-20:00 pm) resulted lower body weight than those of the broiler fed *ad libitum* at 14 and 21 days of age.

Table 3: Effect of feeding time restriction during the starter period on immune organs.

Age	Variables	Treatments					SE	p-value
		FR - 0	FR - 1	FR - 2	FR - 3	FR - 4		
14 days	Bursa Fabricius (mg/g BW)	1.72[b]	3.06[a]	2.64[a]	2.46[ab]	2.92[a]	0.25	0.0130
	Spleen (mg/g BW)	0.85	0.79	0.70	0.70	0.73	0.08	0.6665
	Thymus (mg/g BW)	3.01	4.01	2.79	4.50	3.09	0.53	0.1648
21 days	Bursa Fabricius (mg/g BW)	2.92	3.07	3.20	2.49	2.86	0.23	0.2815
	Spleen (mg/g BW)	0.82	0.94	0.91	0.85	0.89	0.14	0.9698
	Thymus (mg/g BW)	4.20	3.57	3.81	4.30	3.90	0.36	0.6199
42 days	Bursa Fabricius (mg/g BW)	1.28	1.29	1.44	0.93	1.65	0.23	0.2833
	Spleen (mg/g BW)	1.34	1.86	1.57	1.49	1.46	0.28	0.7517
	Thymus (mg/g BW)	2.27	2.23	2.19	2.01	1.93	0.16	0.5286

[a, b] Means within same row with no common superscrips differ at $p<0.05$. Chicks fed *ad libitum* (FR-0); chickens fed *ad libitum* for two cycles of 2 h periods during age 7-14 d (FR-1) and during age 7-21 d (FR-2); chickens fed *ad libitum* for two cycles of 4 h periods during age 7-14 d (FR-3) and during age 7-21 d (FR-4).

Body weight of broilers reduces by 13.82% (FR-1) and 7.67% (FR-3) during 1 wk feed restriction from age 7 to 14 days, while feeding condition during 2 wk from age 7 to 21 days, body weight was reduced by 13.84% (FR-2) and 6.91% (FR-4). Reduced feed intake is one factor that contributes to decreases in energy retention and weight gain. Besides that, the mechanism of decreased growth during the feed restriction can also be due to reduced metabolism; as a result, decreased circulation hormone triiodothyronine (T_3). The current study reduced feed intake by 20 to 30% of *ad libitum* during feed restriction. Susbilla et al. (2003) noted that protein retention in chickens was significantly reduced to 77% during feed restriction. Moreover, Győrffy et al. (2009) found that the broilers subjected to feed restriction of 70 to 85% *ad libitum* significant decrease the concentration of T_3. Therefore, because of the limited time available to access feed during the restriction, there is a limit to providing energy and nutrients to support growth (Azis et al., 2011, 2013). In this regard, Farghly et al. (2019) reported that 12 hours/day fasting of broilers was implemented immediately after hatching, and body weight was reduced by 7.9% at 21 days. In the other report, broilers subjected to the time restriction (8 hours/day) from age 8 to 16 days were reduced 32% compared with the control (Orso et al., 2019). Similar to our result, Livingston et al. (2019) also reported that the body weight of broilers had a time-limited feeding from 09:00 to 17:00 hours were lower than those the control at age 14 days (470 vs. 513 g) and age 21 days (958 vs. 1047 g). In our research, there was no significant difference in body weight between the broilers subjected to the feed restriction and those broilers fed *ad libitum* at the end of the re-alimentation period (42 days of age). Based on these results indicated that the broilers were able to show compensatory growth after feed restriction. In general, compensatory growth was expected after restricted feeding to achieve normal body weight at marketing or slaughter age. In line with Butzen et al. (2013, 2015), broilers with a time restriction of eight hours/day from age 8 to 16 days could recover their final body weight at 42 days of age. Furthermore, according to Shafiei et al. (2018), broilers subjected to feed withdrawal for 8 and 10 hours per day from 8 to 14 days of age were able to compensate for weight loss at 42 days of age. Additionally, Farghly et al. (2019) observed that the broilers subjected to intermittent feeding for 4 hours and 4 hours of fasting had similar body weight with control broilers until the marketing at 42 days (2177 vs. 2144 g). Moreover, Orso et al. (2019) reported that broilers subjected to restriction through by-time programs (8 hours/day) from age 8 to 16 days had a similar body weight with control at 42 days of age (2931 vs. 3001 g).

Feeding time restriction for 7 days had no negative impact on relative carcass weight at 14 days, but feeding time restriction for 14 days reduced relative carcass weight from 7 to 21 days of age. In the current experiment, the carcass weight loss was 16.3 and 10.3% under FR-2 and FR-4, respectively. In line with this, breast and thigh muscle weights were decreased during the feeding time restriction. The reduction in carcass, breast and thigh muscle weight is mainly attributed to a lack of nutrient intake, especially protein, to support muscle growth. In the present study, the breast muscle weight loss was 32.55% at 21 days under feeding time 4 hours/day from age 7 to 21 days. Butzen et al. (2013) found that breast weight loss was 34% when fed eight hours/day from age 8 to 16 days, which is consistent with our findings. The current study suggests that breast muscle weight loss due to feeding time restrictions during two weeks was higher than that reduction for body weight. The decline in breast yield of broilers with feed restriction may be due to a decreased amino acid intake (Melo et al., 2021). These results indicate that decreasing feed intake during feed restriction is a factor in reducing skeletal muscle growth. Trocino et al. (2015) reported that broilers

fed 80% *ad libitum* from age 13 to 21 days had significantly lower breast muscle yield than *ad libitum*-fed broilers. Similarly, Gratta et al. (2017) reported that lighter early-restricted chickens from 13 to 21 days had lower breast yield than the chicken fed *ad libitum*. Velleman et al. (2014) reported that feed restriction affects the organization of the muscle fibers and increases muscle mass. Furthermore, the condition of nutritional stress due to feed restriction can cause a decrease in breast muscle growth due to the decline in the number of satellite cells in the pectoralis major muscle (Ayansola et al., 2023).

At age 42 days, our findings show that the relative carcass weight of broilers subjected to feeding time restriction (FR-1, FR-3, and FR-4) was not significantly different with the broiler fed *ad libitum* (FR-0), except FR-2, was significantly lower the relative carcass weight with other one. It can be caused by young broiler chickens' inability to adapt to feed restrictions. This inability to adapt was evident in broilers with severe (4 hours/day) restricted levels for an extended period from 7 to 21 days of age. However, it was not seen in 8 hours/day restricted broilers. Similarly, Aliakbarpour et al. (2013) also found that the relative carcass weight significantly decreased due to the intermittent feeding program with 5 feeding times. However, another report that broilers subjected to feed restriction with 2 cycles/d had a similar effect with broilers fed *ad libitum* on relative carcass weight (77.7 vs. 77.9%) at 6 weeks of age (Farghly and Makled, 2015). Likewise, David and Subalini (2015) reported that broiler chickens' carcass characteristics at 41 days were not significantly affected by withdrawal for 7 hours (9:00 to 16:00) from age 8 to 30 days. A similar study by Al-Khair et al. (2017) found that the broilers subjected to the limiting of feeding time by 3 and 6 hours/day from age 8 to 28 days had similar carcass weight with control broilers. Furthermore, there were similarities in the relative breast, thigh, and drumstick muscle weight between feeding time restriction and control at 42 d of age. Farghly and Makled (2015) found that broilers subjected to intermittent feeding had a similar effect with broilers fed *ad libitum* on relative breast weight (24.3 vs. 24.0%) except on drumstick at 6 weeks of age. In another research, van der Klien et al. (2017) reported that none of the feed restrictions applied affected breast muscle weight at day 35. The abdominal fat weight was similar among all the treatments at ages 14, 21, and days. These results indicate that the feeding time restrictions were not effective in reducing abdominal fat weight. This study corroborates with several studies that there were no significant differences in abdominal fat due to feed restriction (Shabani et al., 2015; Farghly et al., 2019; Jahanpour et al., 2020). Several researchers reported that feed restriction of 70% of *ad libitum* in 2 weeks (van der Klein et al., 2017) and feed withdrawal (2 hours/day) from 8-35 days of age (Saleh et al., 2019) were able to reduce abdominal fat. The difference between studies may be related to broiler strain, climate, and the intensity of feed restriction.

The broilers subjected to feeding time restriction had higher relative empty crop and gizzard weight than the control broiler at age 14 days. These results suggest that feed restriction by time for 4 hours/day or 8 hours/day leads to increased crop and gizzard weight. Our result is in line with the study of Sacranie et al. (2012) that the weight of the empty gizzard increased with intermittent feeding. Similarly, Svihus et al. (2013) stated that feed restriction increases crop size and storage capacity. In the previous report, Zubair and Leeson (1994) observed that the weight of crop, proventriculus, and gizzard of restricted broiler chickens was significantly heavier than those of control broiler chickens at the end feed restriction. It is related to the change of mature tissues. Jones (1995) observed that late-mature organs such as gizzards develop earlier when feed restriction is applied.

Furthermore, Govaerts et al. (2000) concluded that during the period of feed restriction, the physical development of chickens takes precedence over the development of more important organs in early development, such as the stomach (proventriculus and gizzard). Likewise, Tumova and Chodova (2018) concluded that the growth of internal organs takes precedence over muscles during feed restriction. Another study by Fondevila et al. (2020) reported that restricting feeding for 4 to 8 hours/day from 8 to 19 day of age will stimulate the broiler's anticipatory feeding behaviour and crop development. In this regard, fasting broilers learn to modify their feeding behaviour by voluntarily increasing their feed consumption immediately before the beginning of the feed restriction (Fondevila et al., 2020). Concerning feeding time restriction, the other research, such as intermittent lighting, Shynkaruk et al. (2019) observed that lighting treatments with increasing levels of darkness increased the size of the crop due to the increased utilization of the crop as a storage organ. At the slaughter age (42 days of age), there were no significant differences among treatments on the weight of all gastrointestinal organs. It showed that these organs quickly responded to refeeding and returned to a normal weight at the slaughter age. Butzen et al. (2013) stated that the broilers had a quick adaptation during refeeding, and the internal organs recovered more quickly than other parts. Furthermore, Butzen et al. (2013) reported that broilers subjected to time restriction for 8 hours/days from 8 to 16 days of age did not affect the weight of viscera. Contrary to the previous report that broilers had free access to feed for 8 hours/day from age 1 to 21 days and 12 hours/days from age 22 to 35 days had higher internal organs such as

the liver and small intestine than the control broilers at age 35 days (Azis et al., 2019).

The feeding time restriction affected bursa Fabricius at 14 days of age; however, there were no negative effects of feed restriction on immune organs (bursa Fabricius, spleen, and thymus) at 21 and 42 days of ageIn comparison to the control broiler during 7 to 14 days of age, the feeding time restriction for 7 days from age 7 to 14 days had the significantly higher weight of bursa Fabricius; however, the weight of the spleen and thymus were similar to the broiler fed *ad libitum* during this period. These results indicate that the development of bursa Fabricius had better than those of control broilers. According to Jahanpour et al. (2015), quantitative feed restrictions of 25 and 50% for 14 days reduced the relative weight of the bursa Fabricius. On the other hand, Cazaban et al. (2015) recommended a minimum 0.11 bursa to body weight ratio standard from 7 to 42 days of age. These differences might be due to implemented method and feed restriction timing. In our study, the weight of the bursa Fabricius, spleen and thymus were unaffected by feeding time restriction at the end of the re-alimentation period. This finding supported to study of Davoodi-Omam et al. (2019), who found that the broilers subjected to quantitative feed restriction 20% for 14 days from 8 to 21 days of age had similar weight of immune organs (bursa Fabricius, spleen and thymus) at 42 days of age.

CONCLUSIONS AND RECOMMENDATIONS

It was concluded that the feeding time restriction in broilers with free access to feed during two periods of 2 hours (4 hours/day) advised daily intake during 7 to 21 days of age did not have a negative effect on body weight at slaughter age, carcass characteristics, gastrointestinal and immune organs at 42 days of age. During the feeding time restriction period, crop growth and gizzard were precedences over muscle growth.

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NOVELTY STATEMENT

The study reveals that the feeding time restriction by free access to feed during two periods of 2 hours (08:00-10:00 am and 04:00-06:00 pm) from 7 to 21 day of age can be recommended as feed restriction for broilers without effect on body weight at slaughter age, carcass characteristics, gastrointestinal and immune organs.

AUTHOR'S CONTRIBUTION

Abdul Azis: Conducted the study, investigation, data analysis and interpreted the data, writing draft manuscript. **Afriani Afriani:** searching literature, monitoring research implementation, collection and tabulation data, and assistant of preparing manuscript finishing.

Conflict of interest

The authors have declared no conflict of interests.

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