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The Effect of Limiting the Time of Feed Access during the Growing Phase on the Performance of Broilers

Research Article

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ABSTRACT

This study was carried out to investigate the effect of feed restriction during the first week of the grower period on the performance, intestinal morphology, and some blood parameters of broilers. Seventy five one-day-old Ross 308 male broilers were used in a completely randomized design with 3 treatments, the control group and groups with feed restriction program at the first week of the grower phase for 6 or 12 hours (6HR or 12HR). In the grower phase, the 12HR program reduced feed intake and daily weight gain. The feed conversion ratio during the grower phase decreased by feed restriction. In the finisher phase and whole the experimental period, no difference was observed in the performance traits. Serum cholesterol levels decreased in the 12HR group compared to the HR group. Serum malondialdehyde levels decreased in both groups with dietary restriction compared with the control group. The aspartate transaminase activity was higher in the 12HR group than in the 6HR group. In the duodenum, a significant decrease in the height of the villi was observed due to feed restriction. The lowest crypt depth at the duodenum was also observed in the 12HR group. The ratio of villi height to crypt depth in the duodenum was lower in the 12HR group than in the other two groups. In the jejunum, the highest villi height was recorded in the 12HR, control, and 6hr groups. In the ileum, like in the jejunum, the highest height of villi was observed in the 12HR group, and the difference with the control group was significant. The diameter of villi in the Ileum was also not affected by feed restrictions. The depth of the crypt increased due to feed restriction. The results of this study suggest that 6 hours of dietary restriction in the first week of the grower period did not affect the production traits of broilers and 12 hours of dietary restriction in the mentioned period, although reduced feed intake and growth in the grower period, at the end of rearing period sufficient compensatory growth occurred. On the other hand, it seems that the 12-hour diet restriction in the first week of growth has positive effects on the morphology of the small intestine by increasing the absorption surface and also has a positive effect on the peroxidative state of the body, which needs further research.

KEY WORDS broilers, feed Restriction, intestine morphology performance, serum parameters.

INTRODUCTION

Over the past decades, restricted feeding methods have been used to reduce the incidence of metabolic disorders such as sudden death syndrom and ascites (Yu and Robinson, 1992; Buys *et al.* 1998). On the other hand, with genetic advances in new broiler strains, higher growth rates have always increased the risk of such high growth rate-related abnormalities. Improving the growth rate can also affect carcass quality by increasing fat storage in the body

(Fouad and El-Senousey, 2014). Targeted reduction of nutrient intake and especially energy, in addition to reducing the incidence of metabolic diseases, can also control carcass fat deposits and also improve feed conversion ratio (Apeldoorn *et al.* 1999).

Feed restriction can be done qualitatively, *i.e* diluting the diet, and also quantitatively, *i.e* reducing the amount of available feed or reducing the access time to the feed (Lee and Leeson, 2001; Butzen *et al.* 2015). The main purpose of a proper diet is to achieve compensatory growth after returning to free feeding, which, in addition to compensating for weight loss, improves feed conversion ratio and reduces body fat reserves (Hornick *et al.* 2000) .

An overview of the results of previous research on dietary restriction programs in broilers has sometimes found conflicting reports. This may be due to the influence of factors such as environment, sex, severity, and duration of dietary restrictions, and genetic differences in the experimental birds (Lippens *et al.* 2000; Lee and Leeson, 2001; Khetani *et al.* 2009).

The results of some recent research suggest that feed restriction should be done in the second week of life rather than the first week and immediately after hatching (Noy *et al.* 2001). Applying a 20% early dietary restriction in the first week of life increases fat storage in the pectoral muscle (Velleman *et al.* 2014), but the same level of dietary restriction in the second week of life did not affect intramuscular and abdominal fat deposits (Jalal and Zakaria, 2012; Velleman *et al.* 2014). In another report, a 20 percent diet restriction at 8 to 16 days of age did not result in a difference in body weight, feed conversion ratio, chest muscle weight, or body fat at 35 days of age. This level of dietary restriction increased body fat only in females Butzen *et al.* (2013).

Novel *et al.* (2009) showed that a 50% fee restriction program in the second week of life reduced the live weight of broilers at 42 days, but a 25% feed restriction at the same period did not have such an effect, and feed restriction regardless of its level, did not affect feed conversion ratio. Kubikova *et al.* (2001) applied a dietary restriction in 13-week-old female broilers by inserting a 30% wood chip into the diet and reported comparable results. In the study of Dastar *et al.* (2017), a 6 hours daily feed restriction between 10to 38 days of age reduced the height and width of the villi, the ratio of villi height to crypt depth and villi surface in all segments of the small intestine.

Due to the improved growth rate and feed conversion ratios of modern broiler strains compared to the past few decades, the results of the previous research on dietary restriction programs may need to be updated (Zuidhof *et al.* 2014).

Ths study aimed to investigate the effect of feed access time restriction during the first week of the grower period on the performance, intestinal morphology, and some blood parameters of broilers.

MATERIALS AND METHODS

The experimental procedures were approved by the animal welfare committee of the department of animal science, University of Mohaghegh Ardabili, Iran. In this experiment, 75 one-day old Ross 308 male broilers with an average weight of 44 g ± SE were used. The birds were randomly distributed into 15 cages and the experiment was conducted in a completely randomized design with 3 treatments, 5 replications, and 15 chicks per replicate. Experimental treatments were: 1- control treatment with free access to feed throughout the experimental period (C), 2- group with 6 hours of daily feed restriction in the first week of the grower period (11 to 18 days of age; HR6) and 3- group with 12 hours of daily feed restriction in the first week of the grower period (HR12). The 6-hour feed restriction was performed between 9 am to 3 pm and the 12-hour restriction program was performed between 4 a.m. to 4 p.m. Table 1 shows the composition and the calculated chemical analysis of the basal diets based on the NRC (1994).

The lighting program of the poultry house was 1, 2, and 3 hours of darkness for the 1, 2, and 3 days of age, respectively, and then 4 hours of darkness until the end of the rearing period. The vaccination program was performed according to veterinary recommendations. The chickens were sprayed with LaSota vaccine in the hatchery and on the farm on days 10 and 17 the LaSota vaccine and on days 23 and 29 the clone 30 vaccine were used as a spray. The weight gain and feed consumption values were recorded at the end of the starter, grower, and finisher periods, and the feed conversion ratio was calculated as the consumed feed (kg) per kg weight gain. On day 42, two birds per cage (10 birds from each treatment) were randomly selected from each replicate and 2 ml of blood was collected through the wing vein. Blood samples were kept at room temperature for 4 to 6 hours to separate serum and then centrifuged at 3500 rpm for 10 minutes to ensure that no clots remained in the serum. The collected serum samples were transferred into micro tubes and stored at -20 ˚C. Then, the selected birds were slaughtered and carcass traits including internal organ weights were measured.

Evaluation of antibody titer against Newcastle disease virus

Antibody titers against Newcastle virus were measured in serum samples by hemagglutination inhibition test.

Table 1 Composition of the experimental diets

Ingredient (g/kg)	Starter	Grower	Finisher
Corn	501.17	569.93	599.19
Soybean meal	385.00	366.25	339.91
Corn gluten meal	36.18	-	-
Oil, vegetable	20.58	22.29	23.44
DL-methionine	3.24	2.67	2.99
L-lysine*HCl	3.11	2.10	1.95
L-threonine	1.18	0.57	0.83
Choline chloride	0.65	0.52	0.44
Di Ca-phosphate	20.74	15.16	12.82
$CaCO_3$	7.48	7.20	6.64
Na-bicarbonate	2.33	1.12	1.03
NaCl	1.44	1.30	1.37
Vitamin and mineral premix[1]	5.00	5.00	5.00
Chemical analysis (calculated) (g/kg)			
Dry matter %	891.42	888.56	887.96
Crude protein %	236.5	207.9	198.87
AMEn kcal/kg	2892.00	2950.00	3000.00
Lysine (SID) %	13.0	11.50	10.82
Methionine (SID) %	9.50	5.48	5.70
Met + Cys (SID) %	14.1	8.26	8.40
Threonine (SID) %	8.61	7.30	7.25
Calcium %	9.80	8.90	8.10
Avaailable phosphorus %	4.92	4.46	4.05
Sodium %	1.80	1.50	1.50

[1] Vitamin and mineral premix provided the following per kilogram of diet: vitamin A (retinyl acetate): 9000 IU; vitamin D (cholecalciferol): 5500 IU; vitamin E (DL-α-tocopheryl acetate): 68 IU; Menadione: 9.0 mg; Pyridoxine: 7.0 mg; Riboflavin: 26.0 mg; Ca-pantothenate: 26.3 mg; Biotin: 0.41 mg; Thiamine: 3.66 mg; Niacin: 75 mg; Cobalamin: 0.03 mg; Folic acid: 3.70 mg; Fe: 82 mg; Mn: 60 mg; Zn: 115 mg; Cu: 15 mg; I: 0.85 mg and Se: 0.4 mg.
AMEn: apparent metabolizable energy corrected for nitrogen.

Measurement of blood metabolites and antioxidant activity

A spectrophotometric autoanalyzer (Alcyon 300, USA) and commercial kits were used to measure serum cholesterol, triglyceride, uric acid, albumin, and total protein concentration (Pars Azmoon, Iran), activity of the enzymes aspartate aminotransferase (AST) and hepatic alanine aminotransferase (ALT) (Pars Azmoon, Iran), malondialdehyde (MDA) (Zellbio, Germany) and total antioxidant capacity (Randox, UK).

Investigation of intestinal morphology

To examine the morphology of the small intestine, 2 cm long sections were removed from the middle parts of the duodenum, jejunum, and ileum segments. The samples were washed twice with saline phosphate solution (PBS) to remove the contents and transferred to plastic containers containing 6-7 mL of 10% formalin. Paraffin wax method was used to prepare thin tissue slides.

Morphological experiments were performed according to the method of Iji *et al.* (2001). A paraffin mold from a microtome was used for cutting. The incisions were about 6 micrometers thick. Eosin was used for staining and a computer-connected light microscope was used for morphological examination. The parameters of villi height, crypt depth, and epithelium thickness in 4 fields of each slice tissue were measured in micrometers using a light microscope and Dino-Lite digital lens, and Dino Capture 2 software, and the ratio of villus height to crypt depth was obtained by calculation. The average of these 4 fields represents the data related to that slide.

Statistical analysis

The data were statistically analyzed using General Linear Models (GLM) procedures of SAS (2001) as a completely randomized design. To compare the means, Duncan's multiple range test was used at a significance level of 0.05.

RESULTS AND DISCUSSION

In this study, feed restriction reduced the mortality rate compared to the control group, but the observed difference was not significant. Table 2 shows the effect of dietary restriction on the production traits of broilers.

In the starter period, there was no difference in production traits among the experimental groups. During the grower period, the 12-hour diet restriction program (12HR) significantly ($P<0.05$) reduced feed intake and daily weight gain compared to the control and the 6-hour feed restriction group (6HR).

Table 2 Effect of dietary feed restriction on the performance of broiler chickens

Item	Starter (1-10 d)			Grower (11-24 d)			Finisher (25-42 d)			Total (1-42 d)		
	Gain (g/b/d)	Feed intake (g/b/d	Feed conversion ratio	Gain (g/b/d)	Feed intake (g/b/d)	Feed conversion ratio	Gain (g/b/d)	Feed intake (g/b/d)	Feed conversion ratio	Gain (g/b/d)	Feed intake (g/b/d	Feed conversion ratio
Control	22.72	25.69	1.13	66.21^a	92.83^a	1.40^a	116.23	192.84	1.66	75.84	116.93	1.54
6HR	23.14	25.79	1.11	67.62^a	92.66^a	1.37^b	116.87	196.48	1.68	75.80	117.59	1.55
12RH	23.04	25.52	1.10	60.76^b	83.23^b	1.36^b	116.41	194.34	1.67	74.07	114.36	1.54
SEM	0.46	0.41	0.009	1.37	1.38	0.010	1.99	2.29	0.015	0.89	1.01	0.009
P-value	0.80	0.89	0.14	0.003	0.0001	0.04	0.97	0.53	0.64	0.28	0.07	0.77

Control: free access to feed throughout the experimental; HR6: 6 hours of daily feed restriction in the first week of the grower period and HR12: 12 hours of daily feed restriction in the first week of the grower period.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Because chickens under food restriction had shorter access to feed, they consumed less energy and lost weight. Interestingly, the 6HR program did not affect feed intake and weight gain compared to the control group, indicating that the 6 hours of feed restriction was insufficient to affect feed intake and the birds were well able to compensate for growth, after access to feed. Feed restriction decreased the grower phase's feed conversion ratio (P<0.05). In the finisher period and whole the experimental period, no difference was observed in the performance traits of the experimental groups. This indicated compensatory growth in food-restricted birds.

Feeding programs that delay the early growth of broilers have been widely used to reduce mortality and improve feed conversion ratio. Restricting feed access time is a much simpler practical method than other conventional methods of feed restricting by diluting the diet. Abdel-Hafeez *et al.* (2017) also reported improved feed conversion ratio and reduced feed intake in feed-restricted birds. The expected improvement in production traits after the removal of feed restriction probably is due to decreased maintenance requirements or basal metabolic decline because of smaller body size during early growth (Urdaneta-Rincon and Leeson, 2002). The type and severity of feed restriction have a significant effect on the results of this type of feeding program. Santoso (2002) suggested that to achieve full compensatory growth and improve feed conversion ratio, feeding restrictions should be applied to at least 25% of free feeding for 6 days. The same researcher, by exposing broilers to the mentioned feed restriction during the second week of the rearing period, showed that the compensatory growth process is not complete until the age of 56 days.

Cristofori *et al.* (1997) restricted feed intake in broilers at one of three ages: 7 to 21 days, 7 to 28 days, or 21 to 35 days, and observed that birds were unable to compensate for weight at 42 days of age. Oyedeji *et al.* (2003) showed that in chickens exposed to skip a day feeding in one of the 1, 2, 3, 4, or 5 weeks of age, feed intake decreased but the feed conversion ratio was not affected.

Oyedeji *et al.* (2003), reported that a 50% feed restriction in the second week of age resulted in higher weight gain and a better feed conversion ratio at the end of the rearing period.

The use of this type of dietary restriction in the third week of life did not affect broilers and dietary restrictions after the third week of age reduced production traits. If a balanced feed restriction program is used at the appropriate time of the rearing period, an improvement in production traits can be observed (Oyedeji *et al.* 2003). Table 3 shows the effect of dietary restriction on the serum parameters of broilers.

Serum cholesterol levels significantly (P<0.05) decreased in the 12HR group compared to the 6HR group and the control group did not differ from the other two groups (Table 3). There was no difference in serum triglyceride levels of the experimental groups, although numerically, dietary restriction increased serum triglyceride (TG) levels. A decreasing trend in serum high-density lipoprotein cholesterol (HDL) levels was also observed due to dietary restriction, although the differences were not statistically significant. Obviously, the effect of dietary restriction on serum cholesterol cannot be easily attributed to the severity dietary restriction because the control group does not show a significant difference between the two groups under dietary restriction. Previous reports have sometimes presented very different results.

Anbasilar *et al.* (2009) restricted broiler chickens feeding for 4 hours from the age of 7 to 21 days and found that cholesterol levels were not affected by dietary restriction, but triglyceride levels were increased in restricted-fed birds. Demir *et al.* (2004) placed broilers under a dietary restriction of 25 and 50% of the previous day's feed intake of the control group as well as a dietary limit of 8 and 16 hours per day and observed that cholesterol levels in all of these groups were more than the birds with free access to feed. Abdel-Hafeez *et al.* (2017) also reported a reduction in serum cholesterol levels due to dietary restriction. Kubikova *et al.* (2001) found comparable results in 13-week-old female broilers fed a diet containing 30% wood chips.

Table 3 Effect of feed restriction on the serum lipoprotein fractions, nitrogen-containing metabolites, and antioxidant capacity

Item	Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	Uric Acid (mg/dL)	Albumin (g/dL)	Total Protein (g/dL)	AST (U/L)	ALT (U/L)	TAC (mmol/L)	MDA (nmol/Ml)	NDV antibody titer
Control	119.25[ab]	30.75	53.33	1.83	1.12	2.62	348.25[ab]	9.00	1.06	1.72	4.15
6HR	133.80[a]	33.80	51.60	2.59	1.18	2.74	334.20[b]	7.20	1.31	1.44	3.33
12RH	115.40[b]	33.40	44.66	2.85	1.12	2.58	366.40[a]	8.00	1.27	1.42	4.33
SEM	5.57	1.88	4.17	0.37	0.05	0.09	9.86	1.18	0.09	0.12	0.46
P-value	0.068	0.52	0.50	0.13	0.69	0.43	0.03	0.603	0.144	0.04	0.27

Control: free access to feed throughout the experimental; HR6: 6 hours of daily feed restriction in the first week of the grower period and HR12: 12 hours of daily feed restriction in the first week of the grower period.

HDL: high-density lipoproteins; AST: aspartate aminotransferase; ALT: alanine aminotransferase; TAC: total antioxidant capacity; MDA: malondialdehyde and NDV: newcastle disease virus.

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

Similar results have also been reported by Al-Rawashdeh *et al.* (1995); Santoso (2002). Santoso (2001) in 7-day-old broilers fed a dietary restriction of 25, 50, or 75% for 6 to 9 days and observed a significant reduction in triglyceride and no change in serum cholesterol. Santoso (2002) reported an increase in plasma triglyceride levels due to feeding at 75% of the control group over 4 to 6 days, which in the present study was a numerically similar result. On the other hand, Mohammadalipour *et al.* (2017) and Rajman *et al.* (2006) did not observe changes in serum cholesterol and TG levels of broiler chickens under dietary restriction.

It has been reported that various factors such as genetics, nutrition, climate, rearing procedure, age, and sex can affect blood lipid content (Li *et al.* 2002). But the most important reason for the discrepancy in the results seems to be differences in the sampling time. Zhan *et al.* (2007) restricted the broiler chickens feeding from 1 to 21 days of age for 4 hours and observed that blood triglyceride levels at the end of the dietary restriction were significantly decreased. However, at 63 days of age, the results were quite the opposite, and triglyceride levels were lower in the free-feeding group than in the diet-restricted group. Although in the present experiment, blood samples were taken at the end of the rearing period (42 days), it seems that due to the long refeeling period, the birds were able to adapt to the conditions.

Feed restriction did not affect ALT, total antioxidant capacity (TAC), uric acid, albumin, and total protein levels, but serum MDA levels significantly (P<0.05) decreased in both groups with dietary restriction compared with the control group. Serum uric acid level seems to be directly related to dietary protein intake (Szabo *et al.* 2005) and due to no significant difference in feed intake between experimental groups at the end of the experiment; the comparable serum uric acid concentrations can be justified. Uric acid is excreted due to protein metabolism in poultry and the unchanged serum's total protein and albumin level is consistent with the no significant difference in serum uric acid levels in the present study.

AST activity was higher in the 12 HR group than in the 6HR group (P<0.05), but both groups had no different from the control group. Contradictory results have been reported on the effect of dietary restriction on serum parameters.

Mohammadalipour *et al.* (2017) reported that dietary restriction reduced ALT and AST activity in cold stressed chickens but did not affect the total protein. ALT and AST aminotransferases are indicators of liver cell damage (Pratt and Kaplan, 2000) and their activity is increased by damaging hepatocyte membranes (Pratt and Kaplan, 2000; Rajman *et al.* 2006). In addition, an association between increased AST activity and heart damage had been reported (Daneshyar *et al.* 2009).

In the present study, the change in serum AST level was not completely consistent with the degree of dietary restriction and it seems that the more severe dietary restriction affected the liver. Although, ALT activity did not show a statistically significant change, but numerically reduced due to dietary restrictions, and this effect could be significant if the experiment was performed with a large number of repetitions. Arce *et al.* (1992) suggested that dietary restriction allows for better development of internal organs including the liver and cardiovascular system by restricting physical growth.

Increased serum malondialdehyde level is an indicator of lipid peroxidation in the body (Osawa *et al.* 2005). In the present study, a decrease in MDA levels and a numerical increase in total serum antioxidant capacity indicate the effect of dietary restriction on reducing lipid peroxidation in broiler chickens. However, Sherif and Mansour (2019) exposed broilers in the second week of rearing to a 20 to 40 percent dietary restriction and showed no effect on MDA and TAC.

The antibody titer against Newcastle virus in the serum of 12HR group was higher than the other two groups, but the difference was not statistically significant (Table 3). It has been reported that a slower growth rate due to dietary restriction or genetic potential improves humoral immunity of broilers (Orso *et al.* 2019).

The effect of dietary restriction on carcass traits of broilers is shown in Table 4. Dietary restriction did not affect the percentage of carcass, breasts, legs and relative weights of the liver, gizzard, spleen, heart, proventriculus, bursa of Fabricius, pancreas, as well as small intestine length.

The reported results of the effect of dietary restriction on carcass traits are also very diverse. While in the present study an insignificant increase was observed in the abdominal fat of restricted-fed chickens, Abdel-Hafeez *et al.* (2017) reported a reduction in carcass fat of broilers due to dietary restrictions. Petek (2000) from 5 to 37 days of age limited the feeding hours of broilers to 18 hours per day and observed a lower abdominal fat at 42 days of age. Abdel-Hafeez *et al.* (2017) reported a decrease in body fat of broilers that were exposed to 20 to 40 percent dietary restriction and suggested that there was a relationship with the severity of feed restriction. Zhong *et al.* (1995) attributed the reduction in the abdominal fat in restricted broilers to a reduction in fat cell volume due to a decrease in lipogenesis.

Liver weight was not affected by dietary restriction in this study. Petek (2000), as well as Fontana *et al.* (1992), also reported no effect of dietary restriction on liver percentage. Herzberg and Rogerson (1990) reported that in broilers fed a standard diet, about 28% of total body lipogenesis occurs in the liver. Zhong *et al.* (1995) also showed that dietary restriction at 7 to 12 days of age reduced lipogenesis in broilers at 14 days of age and affected liver weight.

However, in most previous studies, liver weight loss due to dietary restriction has been reported. Plavnik and Yahav (1998) observed a significant reduction in relative liver weight at 8 weeks of age with a dietary restriction of 60% from 6 to 12 days of age. Bartov (1996) reported that dietary restriction for 10 to 24 hours, regardless of diet, significantly reduced liver weight compared to controls, but there was no difference between the two periods of dietary restriction.

Ramlah *et al.* (1996) showed that early dietary restriction up to 75% of free feeding in the second week of rearing period, reduced liver weight at 42 days of age.

Palo *et al.* (1995) exposed broilers to dietary restriction and observed a decrease in liver percentage based on carcass weight. The experiment also showed a reduction in the number and size of liver cells, and it was also reported that organs such as the proventriculus, gizzard, small intestine, liver, and pancreas were less affected by dietary restriction and are renewed faster than the whole body during at re-feeding period.

Katanbaf *et al.* (1989) reported the effect of skipping a day of feeding on pancreas weight in 160-day-old pullets. In the present study, there was a small but non-significant increase in the pancreas weight of chickens under dietary restriction.

The effect of dietary restriction on the morphology of the small intestine of broilers is shown in Table 5. In the duodenum, a significant (P<0.05) decrease in the height of the villi was observed due to feeding restriction, but the diameter of the villi was not affected. The lowest crypt depth at the duodenum was also observed in the 12HR group (P<0.05), so the difference observed with the 6hr group was significant, and in contrast, the diameter of the crypt was higher in the 6hr group than in the 12HR group (P<0.05). The ratio of villi height to crypt depth (VH/CD) in the duodenum was lower in the 12HR group than in the other two groups. In the jejunum, the highest villi height was recorded in the 12HR, control, and 6HR groups, respectively (P<0.05), and again the diameter of the villi was not affected by feed restriction. Crypt depth and diameter were not significantly different in the experimental groups. There was no difference in VH/CD ratio in the jejunum.

In the ileum, like in the jejunum, the highest height of villi was observed in the 12HR group (P<0.05), and the difference with the control group was significant. The diameter of villi in Ileum was not affected by feed restrictions.

Table 4 Effect of fed restriction on carcass parameters of broiler chickens (as a ratio to live weight)

Item	Carcass	breast	Legs	Liver	Abdominal fat	Gizzard	Heart	Spleen	Pancreas	Proventriculus	Bursa of fabricius	Small intestine length
Control	67.71	27.71	19.45	2.28	1.57	0.96	0.34	0.08	0.18	0.37	0.12	6.88
6HR	68.21	27.20	19.55	2.74	1.73	0.90	0.37	0.09	0.21	0.36	0.10	7.14
RH12	69.30	27.50	20.65	2.34	1.47	0.93	0.36	0.09	0.19	0.33	0.11	6.39
SEM	0.59	0.67	0.34	0.23	0.16	0.06	0.018	0.016	0.012	0.032	0.016	0.25
P-value	0.20	0.86	0.055	0.36	0.51	0.80	0.80	0.93	0.23	0.70	0.79	0.15

Control: free access to feed throughout the experimental; HR6: 6 hours of daily feed restriction in the first week of the grower period and HR12: 12 hours of daily feed restriction in the first week of the grower period.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effect of fed restriction on small intestine morphology of broiler chickens

Item	Duodenum					Jejunum					Ileum				
	VH (μm)	VT (μm)	CD (μm)	CT (μm)	VH/CD	VH (μm)	VT (μm)	CD (μm)	CT (μm)	VH/CD	VH (μm)	VT (μm)	CD (μm)	CT (μm)	VH/CD
Control	1471.43^{a}	108.41	226.95^{ab}	10.30^{ab}	6.62^{a}	1083.46^{b}	98.39	174.12	11.87	6.32	358.67^{b}	68.53	107.56^{b}	13.83	3.40
6HR	1328.93^{b}	131.77	174.60^{b}	13.93^{a}	7.63^{a}	832.68^{c}	57.76	178.47	16.77	4.73	417.52^{ab}	80.18	168.02^{a}	9.23	2.48
RH12	1173.24^{c}	126.35	246.15^{a}	7.60^{b}	4.88^{b}	1253.54^{a}	94.73	187.40	9.44	6.71	498.15^{a}	74.81	156.64^{ab}	11.29	3.35
SEM	35.89	12.72	16.97	1.73	0.46	50.52	12.41	14.18	2.87	0.41	33.73	1072	15.88	1.85	0.36
P-value	0.0008	0.43	0.03	0.08	0.007	0.0008	0.08	0.80	0.23	0.19	0.04	0.75	0.054	0.26	0.19

Control: free access to feed throughout the experimental; HR6: 6 hours of daily feed restriction in the first week of the grower period and HR12: 12 hours of daily feed restriction in the first week of the grower period.
VH: villi height; VT: villi thickness; CD: crypt depth; CD: crypt thickness and VH/CD: villi height to crypt depth ratio.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The depth of the crypt increased due to feeding restriction, but only the difference between the 6HR group and the control group was statistically significant (P<0.05). The diameter of the crypt and VH/CD ratio were not affected by feed restrictions.

An increase in the height of the villi in the jejunum, which is the main site for nutrient uptake, and the ileum, which plays a key role in the re-absorption of water and electrolytes, may indicate an increase in absorption of nutrients at the re-feeding period and this is associated with gastrointestinal hypertrophy (Yu and Robinson, 1992). Azouz *et al.* (2019) reported an increase in villi height and crypt depth in broilers exposed to food restrictions. Furlan *et al.* (2001) also reported the effect of dietary restriction on weight loss and intestinal size, which affect the normal process of digestion and absorption and increase the feed conversion ratio as reported in some research on feed restriction in broilers. Jalilvand *et al.* (2017) reported that dietary restriction did not affect intestinal morphology. However, in the study of Dastar *et al.* (2017), dietary restriction reduced the height of the villi, the width of the villi, the ratio of the height of the villi to the depth of the crypt, and the area of the villi in all three parts of the small intestine. A decrease in the VH/CD ratio is a sign of increased intestinal turnover and metabolic activity because of the increased migration of enterocytes from the crypt depth to the villi top (Teimouri *et al.* 2005).

CONCLUSION

The results of this study suggest that 6 hours of dietary restriction in the first week of the grower period did not affect the production traits of broilers and 12 hours of dietary restriction in the mentioned period, although reduced feed intake and growth in the grower period, at the end of rearing period sufficient compensatory growth occurred. On the other hand, it seems that the 12-hour diet restriction in the first week of growth has positive effects on the morphology of the small intestine by increasing the absorption surface and also has a positive effect on the peroxidative state of the body, which needs further research.

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