Digestive Enzymatic Responses of Chickens Feed-restricted and Refed as Affected by Age

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The effect of feed restriction and refeeding on enzymatic activity and gastrointestinal organ weight was evaluated in chickens at two ages, 7 and 35 d of age. At each age, the birds were 70% feed-restricted for 7 d (30% of *ad libitum* intake) followed by *ad libitum* refeeding for 3 d. The control groups were fed *ad libitum* during equivalent periods (10 d). Pancreatic activity of chymotrypsin, trypsin, amylase and lipase, and intestinal activity of sucrase and maltase were analyzed, and the weights of the proventriculus, gizzard, pancreas, small and large intestine were obtained. Trypsin and amylase activity were lower in the feed-restricted group than in the control groups at d14, whereas sucrase activity was lower in the feed-restricted group than in the control group at d42, showing that the effect of feed restriction on enzyme activity was age-dependent. Feed restriction decreased the weight of all organs in the starter and finisher periods. Upon refeeding, the organ weight increased and reached that of the control group only in chickens that were feed-restricted from d35 to d42. In the starter period, proventriculus and gizzard were less affected by feed restriction. In the finisher period, the gizzard was also the least affected organ, while the small intestine was the most affected. All effects of feed restriction were reversed after 3 days of *ad libitum* refeeding, indicating that the alterations were fast and reversible. Moreover, response to feed restriction and refeeding is age dependent.

Key words: digestive enzymes, feed restriction, gastrointestinal organs, refeeding

J. Poult. Sci., 51: 289–296, 2014

Introduction

It is known that feed restriction affects chicken performance, leading to a decrease in the weight of the body and some organs, such as the pancreas and those of the digestive tract (Camacho *et al.*, 2004; Wijten *et al.*, 2010). It can be expected that a decrease in digestive organ mass leads to similar alterations in digestive activity. In fact, a 70% reduction was observed in pancreatic enzyme activity for trypsin in 80% feed-restricted chickens (Palo *et al.*, 1995), and 25% and 10% reductions were observed for carboxypeptidase A and general proteolytic activity, respectively, in 40% feed-restricted chickens (Susbilla *et al.*, 2003).

As for intestinal enzymes, Fassbinder-Orth and Karasov (2006) detected a decrease in maltase activity. However, Pinheiro *et al.* (2004) observed an increase in sucrase activity but not in maltase activity 1 d following 70% *ad libitum* feed

restriction from 7 to 14 d of age, whereas Palo *et al.* (1995) observed an increase in intestinal maltase and sucrase activity 7 d after 4 d, 20% *ad libitum* feed restriction.

The different levels of restriction used in studies make it difficult to draw a conclusion regarding the effect of feed restriction on enzyme activity. Moreover, it is important to consider the stage of development during which the feed restriction is imposed to the chickens, given that animal age can influence enzymatic levels (Iji *et al.*, 2001; Sklan and Noy, 2003; Yegani and Korver, 2008) and metabolic rate (Christensen *et al.*, 2012). Duarte *et al.* (2011) studied the effect of feed restriction on gene expression and showed that variations occur according to animal age. The mRNA abundance of aminopeptidase, but not that of maltase, increased in chickens that were feed-restricted in the starter and finisher periods, while the mRNA abundance of the sucrase-isomaltase complex was higher only in chickens that were feed-restricted in the finisher periods (Duarte *et al.*, 2011).

Another relevant question is whether animals of different ages can show the same capacity to recover the enzyme activity after refeeding. Most studies agree that gastrointestinal organ weight is reduced by feed restriction but is

Received: June 2, 2013, Accepted: December 30, 2013

Released Online Advance Publication: February 25, 2014

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recovered by refeeding. Realimentation after feed restriction can improve feed efficiency and compensatory growth (Jang *et al.*, 2009), and it is possible that this response can be attributed to the recovery of enzyme activity. Therefore, it is imperative to know the physiological changes that underlie feed restriction and subsequent realimentation to better understand animal nutrition and health. In particular, it is important to know which changes in the gastrointestinal tract are responsible for processing dietary nutrients necessary for self-maintenance and growth (Gilbert *et al.*, 2008).

The present study compares the effects of feed restriction and subsequent realimentation on the activity of pancreatic chymotrypsin, trypsin, amylase and lipase and intestinal sucrase and maltase of broiler chickens between starter (from 7 to 17 d of age) and finisher (from 35 to 45 d of age) periods. In addition, the effects on gastrointestinal organ weight were also measured.

Materials and Methods

Birds and Diets

A total of 64 male broiler chicks (Cobb) were obtained from a commercial hatchery (Frigorífico Mabella Ltda, Tatuí, Brazil) on the day of hatching. The chicks were housed individually in metal batteries in an environmentally controlled room with constant illumination and controlled temperature (22°C to 31°C, according to age). Water and food were offered *ad libitum* during the experiment, except for feed-restriction periods. Two diets (Table 1) were formulated according to Rostagno *et al.* (2005) and offered during the starter (from 1 to 14 d of age) and grower-finisher periods (from 35 to 45 d of age). All procedures were approved by the University Ethics Committee for Animal Research.

The effect of feed restriction (for 7 d) and refeeding (for 3 d) on enzyme activity and organ weight was assessed in chickens at two different ages: 7 and 35 d of age. For each age, 32 birds were divided into two groups: feed-restricted (*n*

Table 1. Composition of experimental diets (%)

	Starter ¹ diet 1 to 14 d	Grower-Finisher ² diet 15 to 45 d
Ingredients		
Soybean meal	34.30	30.70
Corn	59.34	62.03
Calcium calcite	0.90	0.89
Dicalcium phosphate	1.80	1.68
Soybean oil	2.30	3.29
Salt	0.47	0.47
L-Lysine	0.20	0.25
L-Threonine	0.05	0.05
DL-Methionine	0.24	0.24
Mineral-vitamin mix ¹	0.40	0.40
Calculated analysis		
ME (kcal/kg)	3,002.97	3,101.60
Crude protein (%)	20.80	19.43
Crude fiber (%)	2.88	2.73
Calcium (%)	0.89	0.85
Available phosphorus (%)	0.44	0.42
Methionine (%)	0.53	0.51
Methionine + cysteine (%)	0.81	0.78
Lysine (%)	1.16	1.11

¹ Mineral and vitamin mix (1 to 14 d) supplied per kilogram of diet: Cu, 1.25 mg; Fe, 62.62 mg; I, 0.025 mg; Mn, 67.5 mg; Se, 0.225 mg; Zn, 68.75 mg; folic acid 1.25 mg; pantothenic acid, 12.5 mg; BHT (butylated hydroxytoluene) 2.5 mg; biotin, 0.125 mg; choline chloride, 750 mg; niacin, 37.5 mg; vitamin A, 12,500 IU; vitamin B₁, 2.5 mg; vitamin B₁₂, 25 mg; vitamin B₂, 5 mg; vitamin B₆, 5 mg; vitamin D₃, 2,500 IU; vitamin E, 25 mg; vitamin K₃, 2.5 mg; monensin, 125 mg.

² Mineral and vitamin mix (15 to 45 d) supplied per kilogram of diet: Cu, 5 mg; Fe, 25.05 mg; I, 0.01 mg; Mn, 27 mg; Se, 0.09 mg; Zn, 55 mg; folic acid 1 mg; pantothenic acid, 10 mg; BHT 2.0 mg; biotin, 0.1 mg; choline chloride, 600 mg; niacin, 30 mg; vitamin A, 10,000 IU; vitamin B₁, 2 mg; vitamin B₁₂, 20 mg; vitamin B₂, 4 mg; vitamin B₆, 4 mg; vitamin D₃, 2,000 IU; vitamin E, 20 mg; vitamin K₃, 2 mg; monensin, 100 mg.

=16), 30% of *ad libitum* intake, and control ($n=16$, fed *ad libitum*). Half of the birds in each group ($n=8$) were sacrificed to evaluate the effects of feed restriction (d14 or d42) and the other half ($n=8$) were assessed for the effects of refeeding (d17 or d45).

These chickens were also evaluated for changes in the genetic expression of intestinal enzymes and transporters. These results were published elsewhere (Duarte *et al.*, 2011). The changes in the body weight of the animals, considered to be an indicator of the feeding restriction effect, were presented in that paper in a table. We consider this repetition to be inappropriate because the evaluation of body weight was not a primary goal in any of the previous papers.

Organ Weight

After feed restriction (d14 and d42) and the realimentation period (d17 and d45), animals were sacrificed by cervical dislocation to collect the gastrointestinal organs (proventriculus, gizzard, small intestine, large intestine and pancreas). The organs were cleaned by flushing them with ice-cold saline solution (NaCl, 0.9%), dried with filter paper, and weighed.

Enzyme Analysis

The pancreas and jejunum were frozen in liquid nitrogen and stored in freezer at -80°C until being assayed. The activity of the pancreatic enzymes was measured after the pancreas was homogenized (1:20 wt/vol) in 50 mmol/l Tris-HCl buffer, pH 8.0, containing 50 mmol/l CaCl_2 . For trypsinogen determination (Kakade *et al.*, 1974), enterokinase was added to the homogenate and allowed to convert the enzyme into trypsin. Trypsin (E.C. 3.4.21.4) activity was then measured from the hydrolysis of p-nitroaniline from benzoyl-DL-arginine-p-nitroanilide (DL-BAPNA) at pH 8.2. Units are expressed as nanomoles of p-nitroanilide released per minute per milligram of protein. A similar method (Erlanger *et al.*, 1966) was used for the determination of chymotrypsin (E.C. 3.4.21.1), with BAPNA replacing N-glutaryl-L-phenylalanine-p-nitroanilide (GPNA). The reaction was stopped with 3% acetic acid solution. Amylase (E.C. 3.2.1.1) was measured by the iodometric method (In vitro Diagnostica, Itabira, Brazil) modified by Caraway (1959) and the activity was expressed as amylase units (AU) per microgram of protein. One amylase unit is the amount of enzyme that hydrolyzes 10 mg of starch in 30 min. Lipase (E.C. 3.1.1.3) activity was measured using the BALB-DNTP method (In vitro Diagnostica). Accordingly, lipase hydrolyzes the thioester producing a thioalcohol that reacts with nitrobenzoic acid, yielding a yellow anion. The color intensity is proportional to the enzyme concentration. The enzyme activity was expressed in International Units (IU) per microgram of protein, which was measured according to Bradford (1976).

To measure the activity of intestinal disaccharidases by the Dahlqvist method (Dahlqvist, 1964), the jejunum was dissected lengthwise and the mucosa was scraped off with a glass microscope coverslip. The obtained tissue was mechanically homogenized after the addition of 4 parts of ice-cold deionized water. Maltase (E.C. 3.2.1.20) and sucrase (E.C.

3.2.1.10) activities were assayed by incubating aliquots of the homogenates with the appropriate substrate in malate buffer at pH 6.4. Released glucose was measured using the glucose-oxidase method (Laborlab, Guarulhos, Brazil). Enzyme activity is expressed in units per milligram of protein.

Statistical Analyses

Values are given as the means $\pm$ SE. Differences between experimental groups and their respective controls for each age were analyzed using Student's t-test in GraphPad Prism 5.0 (GraphPad Software, Inc, La Jolla, CA, USA). The significance level was set at $P < 0.05$.

Results

Organ Weight

The organ weights (Table 2) of feed-restricted chickens was lower than those in the control groups, regardless of age ($P < 0.05$). Upon refeeding, the organ weights of older chickens increased ($P < 0.05$) and reached those of the control group, while the organ weights of younger chickens remained lower.

When the organ weights were normalized with respect to body weight (Table 3), the proventriculus of feed-restricted animals was heavier at the starter (d14) and finisher periods (d42) than that of the control group. The gizzard ($P < 0.05$) of feed-restricted animals was heavier than that of the control group at the starter period. However, the small intestine relative weight was lower in feed-restricted chickens at the finisher period compared to the control group. The relative weights of the pancreas and large intestine were not affected by feed restriction at either age ($P > 0.05$).

Upon refeeding, the relative weights of all organs of animals feed-restricted during the finisher period (d45) increased and exceeded the values observed in the control group ($P < 0.05$). In the starter period (d17), the same relationship were observed with the gizzard, proventriculus and small intestine, which were heavier than in the control group ($P < 0.05$).

Pancreatic Enzymes

Trypsin and amylase activity were lower ($P < 0.05$, Table 4) in chickens that were feed-restricted from d7 to 14. The pancreatic enzymes were not affected in animals feed-restricted from d35 to 42. There was no difference between refeed and control groups at both starter and finisher periods. The lipase and chymotrypsin activities were not affected by feeding regimen at any age ($P > 0.05$).

Intestinal Enzymes

The disaccharidase of younger chickens was not affected ($P > 0.05$, Table 4) either by feed restriction (d14) or realimentation (d17). In the older animals, the sucrase activity was lower ($P < 0.05$) in feed-restricted chickens (d42) compared to that of the control group, and after refeeding (d45), the disaccharidase activity was similar to that of the control group ($P > 0.05$).

Discussion

Feed restriction has been widely studied during the starter phase of broiler development because this practice reduces

Table 2. Organ weight (g) of chickens at 7 and 35 d of age subjected to feed-restriction (7d) or to feed restriction (7d) followed by refeeding (3d)

	Restriction effect		Refeeding effect	
	Restricted (7d)	Control-7 Ad libitum (7d)	Restricted+ Refed (7d+3d)	Control-10 Ad libitum (10d)
7 d of age	d14		d17	
Proventriculus	1.53±0.05	2.30±0.14*	2.76±0.11	3.46±0.08 †
Gizzard	6.62±0.14	9.49±0.27*	9.80±0.27	13.22±0.69 †
Small Intestine	9.46±0.29	16.85±1.40*	21.13±0.97	28.40±1.05 †
Large Intestine	1.52±0.12	2.81±0.32*	2.86±0.15	4.18±0.18 †
Pancreas	0.79±0.06	1.46±0.09*	1.69±0.17	2.16±0.11 †
35 d of age	d35		d42	
Proventriculus	5.55±0.19	6.74±0.23*	7.45±0.36	7.11±0.37
Gizzard	26.75±0.99	34.53±3.01*	36.47±2.30	32.64±1.67
Small Intestine	32.04±1.21	49.86±1.83*	52.78±3.57	50.72±3.24
Large Intestine	9.43±0.54	12.88±0.47*	14.36±0.76	14.07±0.66
Pancreas	3.12±0.13	4.00±0.16*	4.62±0.42	4.54±0.24

Data are means±SE

Control-7 and Control-10 indicate the control animals fed ad libitum by 7 and 10 d, respectively.

* Indicates a significant difference between Restricted and Control-7 groups and † indicates a significant difference between Restricted+refed and Control-10 groups (Student's *t*-test, $P<0.05$).

Table 3. Relative organ weight (g/100 g of body weight) of chickens at 7 and 35 d of age subjected to feed-restriction (7d) or to feed restriction (7d) followed by refeeding (3d)

	Restriction effect		Refeeding effect	
	Restricted (7d)	Control-7 Ad libitum (7d)	Restricted+ Refed (7d+3d)	Control-10 Ad libitum (10d)
7 d of age	d14		d17	
Proventriculus	0.95±0.03	0.73±0.02*	0.90±0.03	0.74±0.02 †
Gizzard	4.09±0.25	3.08±0.17*	3.20±0.09	2.82±0.14 †
Small Intestine	5.84±0.14	5.33±0.22	6.87±0.19	6.07±0.21 †
Large Intestine	0.95±0.08	0.89±0.08	0.93±0.05	0.89±0.03
Pancreas	0.49±0.03	0.47±0.02	0.55±0.05	0.46±0.02
35 d of age	d35		d42	
Proventriculus	0.30±0.01	0.27±0.01*	0.35±0.01	0.27±0.01 †
Gizzard	1.46±0.06	1.39±0.12	1.72±0.07	1.25±0.05 †
Small Intestine	1.74±0.05	2.01±0.06*	2.49±0.12	1.94±0.09 †
Large Intestine	0.51±0.03	0.52±0.02	0.68±0.03	0.54±0.02 †
Pancreas	0.17±0.01	0.16±0.01	0.21±0.01	0.17±0.01 †

Data are means±SE

Control-7 and Control-10 indicate the control animals fed ad libitum by 7 and 10 d, respectively.

* Indicates a significant difference between Restricted and Control-7 groups and † indicates a significant difference between Restricted+refed and Control-10 groups (Student's *t*-test, $P<0.05$).

metabolic disorders and reproductive problems, and controls excess weight gain (Camacho *et al.*, 2004). During this phase, the gastrointestinal tract develops rapidly compared to the rest of the body, while enzyme and absorption activities in the intestinal epithelium also increase swiftly (Uni *et al.*, 1998). Although feed restriction can be applied after the first

two weeks of life to improve productive efficiency, decrease carcass fat content, and reduce mortality rates (Urdaneta-Rincon and Leeson, 2002), there is a shortage of literature on the effects of this practice on the gastrointestinal tract of growth-phase chickens. Given that the development of gastrointestinal organs and enzymatic levels change depend-

Table 4. Pancreatic and intestinal enzyme activity of chickens at 7 and 35 d of age subjected to feed-restriction (7d) or to feed restriction (7d) followed by refeeding (3d)

	Restriction effect		Refeeding effect	
	Restricted (7d)	Control-7 Ad libitum (7d)	Restricted+ Refed (7d+3d)	Control-10 Ad libitum (10d)
7 d of age	d14		d17	
Trypsin (nmol/mg protein)	46.3±5.0	75.0±13.8*	112.1±5.3	123.8±22.7
Chymotrypsin (nmol/mg protein)	2.6±0.2	3.0±0.8	4.4±0.4	4.4±1.3
Amylase (AU/μg protein)	3.1±0.4	4.8±0.6*	3.8±0.7	2.4±0.5
Lipase (IU/μg protein)	1.5±0.2	1.5±0.1	1.7±0.1	2.2±0.3
Sucrase (U/mg protein)	1.9±0.2	2.3±0.2	2.3±0.2	2.2±0.2
Maltase (U/mg protein)	3.9±0.7	4.0±0.5	3.4±0.4	3.0±0.2
35 d of age	d35		d42	
Trypsin (nmol/mg protein)	197.2±14.2	173.1±11.8	169.5±5.1	200.1±27.2
Chymotrypsin (nmol/mg protein)	4.2±0.7	5.9±0.7	4.0±0.8	5.7±1.2
Amylase (AU/μg protein)	3.7±1.0	4.6±1.3	3.6±0.5	2.8±0.2
Lipase (IU/μg protein)	2.3±0.4	2.5±0.2	1.5±0.4	1.0±0.2
Sucrase (U/mg protein)	2.3±0.1	3.2±0.4*	2.3±0.4	2.1±0.2
Maltase (U/mg protein)	3.6±0.4	4.8±0.8	2.9±0.7	3.5±0.5

Data are means±SE

Control-7 and Control-10 indicate the control animals fed ad libitum by 7 and 10 d, respectively.

* Indicates a significant difference between control and restricted groups. (Student's *t*-test, *P*<0.05)

ing on age (Iji *et al.*, 2001; Sklan and Noy, 2003; Yegani and Korver, 2008), it is very important to consider the stage of development during which feed restriction is imposed on chickens.

Thus, the innovation of the present study was to show the age-dependent sensitivity of digestive enzymes to malnutrition in chickens and the reversal of decreased enzyme activity within 3 d of refeeding. The enzyme activity observed herein also cannot be explained by diet alone (Chotinsky *et al.*, 2001) and supports the fact that each digestive enzyme has its own sensitivity to malnutrition (Susbilla *et al.*, 2003). It was demonstrated that feed-restriction in younger birds causes a decrease in the activities of pancreatic trypsin and amylase, but not in the activities of chymotrypsin, lipase and intestinal sucrase. In older animals, there is a decrease only in intestinal sucrase.

The decrease in pancreatic enzyme activity in younger animals is supported by the report by Palo *et al.* (1995) on

chickens that were feed-restricted for 7 d, which could be attributed to a decrease in protein synthesis, given that fasting inhibited pancreatic protein synthesis in mice (Sans *et al.*, 2004). Moreover, protein restriction inhibited the response of acinar cells to cholecystokinin (Prost and Belleville, 1991), which plays a role in stimulating enzyme secretion in the pancreas. A similar response was observed in mice that were fed a protein-restricted diet for 4 d, and a decrease in pancreas weight and pancreatic cell atrophy was observed under these conditions (Crozier *et al.*, 2009). Although chickens of both ages showed a decrease in pancreas weight, there was a larger reduction in younger animals – approximately 50% compared with 22% in older animals – thus contributing to the large difference in pancreatic enzymes between age groups.

Our findings regarding maltase activity are supported by Fassbinder-Orth and Karasov (2006), in which the jejunal and ileal maltase activities of young animals were not

affected by feed restriction. Maltase activity results were also correlated with the response of mRNA expression previously observed in our laboratory (Duarte *et al.*, 2011), which suggests that this gene can have a pre-transcriptional regulation (Gal-Garber *et al.*, 2000). With regard to intestinal sucrase, our results in younger chickens were different from Pinheiro *et al.* (2004), who observed an increase in enzyme activity in 14 d of age chickens that were 30% feed-restricted for 7 d. This difference could be attributed to a difference between the level and duration of feed restriction employed by Pinheiro *et al.* (2004). Sucrase activity appeared to be of post-transcriptionally regulated, which also occurs with other enzymes and transporters (Ihara *et al.*, 2000; Duarte *et al.*, 2011). In fact, comparing our results with the results of Duarte *et al.* (2011) on mRNA expression, it is possible to conclude that the results are independent of the mRNA level.

It is worth mentioning that on the third day of refeeding, the activities of all enzymes was not different from those of the control. In their study, Crozier *et al.* (2009) verified that after 4 d of receiving a normoproteic diet, protein-restricted mice showed a reversal of the decreased concentration of amylase and chymotrypsin. The restoration of enzyme activity could occur because pancreatic cell death did not occur after feed restriction, and instead, only cell atrophy or a decrease in protein synthesis in the pancreas occurred (Sans *et al.*, 2004). Pancreatic cells were not evaluated in this study, but it is possible that there was no atrophy, given that after 3 d of refeeding, the pancreas weight was similar to the control in younger chickens and higher than the control in older animals. The recovery can be explained by an increase in pancreatic protein synthesis as shown by Sans *et al.* (2004). However, these authors did not show a related increase in the mRNA levels of the digestive enzymes, which was most likely due to the shorter refeeding time (2 h of refeeding). Moreover, for intestinal activity, the recovery can be explained by the replacement of the enterocytes used in enzyme expression during the refeeding period, as indicated by Yamauchi and Tarachai (2000), who showed a rapid recovery of villus height through increased epithelial cell area and cell mitosis after 1 d of refeeding in chickens.

Although there are discrepancies in the literature concerning the effects of feed restriction on the gastrointestinal system, the observed reduction in organ weights at both ages is an expected response given the trophic effects of nutrients (Fassbinder-Orth and Karasov 2006; Wijten *et al.*, 2010) and the reduction in the basal metabolic rate in response to feed restriction (Christensen *et al.*, 2012). A decrease in metabolic rate could lead to a reduction in the energy required to maintain gastrointestinal turnover. In fact, feed restriction affects intestinal villus height, cell area, cell proliferation, and mitosis rate (Shamoto and Yamauchi 2000).

It is well known that adequate development of the digestive system, especially of the small intestine, is essential for food digestion and absorption and, consequently, for animal nutrition. Thus, the reduction of gastrointestinal

organs observed in this study may have contributed to the decrease in body weight observed in chickens that were feed restricted during the starter and finisher periods (Duarte *et al.*, 2011).

However, age influenced the organ response after refeeding. In fact, although it is known that the recovery of the small intestine is rapid in response to refeeding (Yamauchi *et al.*, 1996), only older feed-restricted animals could reach the same absolute organ weights as those of the control group. This different response may be caused by a lower basal metabolic rate and maintenance requirements typical of old chickens (Zubair and Leeson 1994; Tona *et al.*, 2004). In addition, feed restriction shows more deleterious effects when it is applied in the first week post-hatch (Uni *et al.*, 2003; Tabedian *et al.*, 2010). The feed-restriction of chickens at 6 weeks of age caused an increase in the jejunal villus height, which was regarded as an adaptive strategy to maximize nutrient uptake once feeding was reestablished (Thompson and Applegate 2006).

Other results from feed restriction were found when the organ weights were normalized relative to body weight. The relative weight of the proventriculus in starter and finisher periods and the weight of the gizzard in starter periods were higher than those of the control, showing that these organs were less affected by feed restriction than body weight.

Hypertrophy of the gizzard was also observed in broilers that were 30% feed-restricted from 7 to 14 d (Lázaro *et al.*, 2004). According to Govaerts *et al.* (2000), birds that are feed-restricted can give priority to the development of supply organs, such as the proventriculus and gizzard, at the expense of the growth of demand tissues, such as the breast and thigh.

The relative weight of the large intestine did not change in response to feed restriction in both ages. Lázaro *et al.* (2004) also did not find differences in the length of ceca between the control and 30% feed-restricted chickens from 4 to 14d. Similarly, chickens fed a low-protein diet did not show in a change in weight of the large intestine (Buwjoom *et al.*, 2010). However, the large intestine in rats that were feed restricted exhibited a susceptibility to acute changes in nutrient supply, with lower protein content and lower rates of protein synthesis (Merry *et al.*, 1991). However, these authors showed that the large intestine is less responsive than the small intestine to acute changes in nutrient supply. Changes in this organ could also indicate the existence of an inflammatory process caused by pathogen adhesion. However, reports concerning the effects of feed restriction on the large intestine in chickens are scarce.

In conclusion, this study showed that the changes caused by feed restriction are fast and reversible given that enzymatic activity and organ weight were similar to those of the control groups after 3 days of *ad libitum* feeding. Our data contributed to the knowledge of the adaptive changes of chickens to malnutrition and showed that the response to feed restriction varies according to age.

Acknowledgments

Grants from National Council for Scientific and Tech-

nological Development (CNPq/Brazil).

References

- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72: 248–254. 1976.
- Buwjoom T, Yamauchi K, Erikawa T and Goto H. Histological intestinal alterations in chickens fed low protein diet. *Journal of Animal Physiology and Animal Nutrition*, 94: 354–361. 2010.
- Camacho MA, Suárez ME, Herrera JG, Cuca JM and García-Bojalil CM. Effect of age of feed restriction and microelement supplementation to control ascites on production and carcass characteristics of broilers. *Poultry Science*, 83: 526–532. 2004.
- Caraway WT. A stable starch substrate for the determination of amylase in serum and other body fluids. *American Journal of Clinical Pathology*, 32: 97–99. 1959.
- Chotinsky D, Toncheva E and Profirov Y. Development of disaccharidase activity in the small intestine of broiler chickens. *British Poultry Science*, 42: 389–393. 2001.
- Christensen K, Thaxton YV, Thaxton JP and Scanes CG. Change in body temperature during growth and in response to fasting in growing modern meat type chickens. *British Poultry Science*, 53: 531–537. 2012.
- Crozier SJ, D'Alecy LG, Ernst SA, Ginsburg LE and Williams JA. Molecular mechanisms of pancreatic dysfunction induced by protein malnutrition. *Gastroenterology*, 137: 1093–1101. 2009.
- Dahlqvist A. Method for assay of intestinal disaccharidases. *Analytical Biochemistry*, 7: 18–25. 1964.
- Duarte CRA, Vicentini-Paulino MLM, Buratini Jr. J, Castilho ACS and Pinheiro DF. Messenger ribonucleic acid abundance of intestinal enzymes and transporters in feed-restricted and refed chickens at different ages. *Poultry Science*, 90: 863–868. 2011.
- Erlanger BF, Edel F and Cooper AG. The action of chymotrypsin on two new chromogenic substrates. *Archives of Biochemistry and Biophysics*, 115: 206–210. 1966.
- Fassbinder-Orth CA and Karasov WH. Effects of feed restriction and realimentation on digestive and immune function in the Leghorn chick. *Poultry Science*, 85: 1449–1456. 2006.
- Gal-Garber O, Mobjeesh SJ, Sklan D and Uni Z. Partial sequence and expression of the gene for and activity of the sodium glucose transporter in the small intestine of fed, starved and refed chickens. *Journal of Nutrition*, 130: 2174–2179. 2000.
- Gilbert ER, Wong EA and Webb Jr. KE. Peptide absorption and utilization: Implications for animal nutrition and health. *Journal of Animal Science*, 86: 2135–2155. 2008.
- Govaerts T, Room G, Buyse J, Lippens M, De Groote G and Decuyper E. Early and temporary quantitative food restriction of broiler chickens. 2. Effects on allometric growth and growth hormone secretion. *British Poultry Science*, 41: 355–362. 2000.
- Ihara T, Tsujikawa T, Fujiyama Y and Bamba T. Regulation of PepT-1 peptide transporter expression in the rat small intestine under malnourished conditions. *Digestion*, 61: 59–67. 2000.
- Iji PA, Saki A and Tivey DR. Body and intestinal growth of broiler chicks on a commercial starter diet. 2. Development and characteristics of intestinal enzymes. *British Poultry Science*, 42: 514–522. 2001.
- Jang IS, Kang SY, Ko YH, Moon YS and Sohn SH. Effect of qualitative and quantitative feed restriction on growth performance and immune function in broiler chickens. *Asian-Australasian Journal of Animal Science*, 22: 388–395. 2009.
- Kakade ML, Rackis JJ, McGhee JE and Puski G. Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. *Cereal Chemistry*, 51: 376–381. 1974.
- Lázaro R, Latorre MA, Medel P, Gracia M and Mateos GG. Feeding regimen and enzyme supplementation to rye-based diets for broilers. *Poultry Science*, 83: 152–160. 2004.
- Merry BJ, Goldspink DF and Lewis SE. The effects of age and chronic restricted feeding on protein synthesis and growth of the large intestine of the rat. *Comparative Biochemistry and Physiology – Part A: Comparative Physiology*, 98A: 559–562. 1991.
- Palo PE, Sell JL, Piquer FJ, Soto-Salanova MF and Vilaseca L. Effect of early nutrient restriction on broiler chickens. 1. Performance and development of the gastrointestinal tract. *Poultry Science*, 74: 88–101. 1995.
- Pinheiro DF, Cruz VC, Sartori JR and Vicentini-Paulino MLM. Effect of early feed restriction and enzyme supplementation on digestive enzyme activities in broilers. *Poultry Science*, 83: 1544–1550. 2004.
- Prost J and Belleville J. Age and protein restriction followed by balanced refeeding affect pancreatic digestive enzyme outputs and turnover times in rats. *Journal of Nutrition*, 121: 2044–2054. 1991.
- Rostagno HS, Albino LFT, Donzele JL, Gomes PC, Oliveira RF, Lopes DC, Ferreira AS and Barreto SLT. Tabelas brasileiras para aves e suínos: Composição de alimentos e exigências nutricionais. 2nd ed. Universidade Federal de Viçosa. (Viçosa). 2005.
- Sans MD, Lee SH, D'Alecy LG and Williams JA. Feeding activates protein synthesis in mouse pancreas at the translational level without increase in mRNA. *American Journal of Physiology – Gastrointestinal and Liver Physiology*, 287: G667–G675. 2004.
- Shamoto K and Yamauchi K. Recovery responses of chick intestinal villus morphology to different refeeding procedures. *Poultry Science*, 79: 718–723. 2000.
- Sklan D and Noy Y. Functional development and intestinal absorption in the young poult. *British Poultry Science*, 44: 651–658. 2003.
- Susbilla JP, Tarvid I, Gow CB and Frankel TL. Quantitative feed restriction or meal-feeding of broiler chicks alter functional development of enzymes for protein digestion. *British Poultry Science*, 44: 698–709. 2003.
- Tabedian SA, Samie A, Pourreza J and Sadeghi GH. Effect of fasting or post-hatch diet's type on chick development. *Journal of Animal and Veterinary Advances*, 9: 406–413. 2010.
- Thompson KL and Applegate TJ. Feed withdrawal alters small-intestinal morphology and mucus of broilers. *Poultry Science*, 85: 1535–1540. 2006.
- Tona K, Onagbesan OM, Bruggeman V, Mertens K, Yego Y and Decuyper E. Comparison of feed intake, blood metabolic parameters, body and organ weights of growing broilers originating from dwarf and standard broiler breeder lines. *International Journal of Poultry Science*, 3: 422–426. 2004.
- Uni Z, Ganot S and Sklan D. Posthatch development of mucosal function in the broiler small intestine. *Poultry Science*, 77: 75–82. 1998.
- Uni Z, Smirnov A and Sklan D. Pre- and posthatch development of goblet cells in the broiler small intestine: effect of delayed access to feed. *Poultry Science*, 82: 320–327. 2003.

- Urdaneta-Rincon M and Leeson S. Quantitative and qualitative feed restriction on growth characteristics of male broiler chickens. *Poultry Science*, 81: 679–688. 2002
- Wijtten PJ, Hangoor E, Sparla JK and Verstegen MW. Dietary amino acid levels and feed restriction affect small intestinal development, mortality, and weight gain of male broilers. *Poultry Science*, 89: 1424–1439. 2010.
- Yamauchi K, Kamisoyama H and Isshiki Y. Effects of fasting and refeeding on structures of the intestinal villi and epithelial cells in White Leghorns hens. *British Poultry Science*, 37: 909–921. 1996.
- Yamauchi K and Tarachai P. Changes in intestinal villi, cell area and intracellular autophagic vacuoles related to intestinal function in chickens. *British Poultry Science*, 41: 416–423. 2000.
- Yegani M and Korver DR. Factors affecting intestinal health in poultry. *Poultry Science*, 87: 2052–2063. 2008.
- Zubair AK and Leeson S. Effect of varying period of early nutrient restriction on growth compensation and carcass characteristics of male broilers. *Poultry Science*, 73: 129–136. 1994.