Effect of Early Feed Restriction and Enzyme Supplementation on Digestive Enzyme Activities in Broilers

D. F. Pinheiro,* V. C. Cruz,† J. R. Sartori,† and M. L. M. Vicentini Paulino*,[1]

*Department of Physiology, Institute of Bioscience, Paulista State University–Botucatu, São Paulo, Brazil; and †Department of Animal Improvement and Nutrition, School of Veterinary Medicine and Zootechniques, Paulista State University–Botucatu, São Paulo, Brazil

ABSTRACT The effect of feed restriction and enzymatic supplementation on intestinal and pancreatic enzyme activities and weight gain was studied in broiler chickens. Quantitative feed restriction was applied to chickens from 7 to 14 d of age. An enzyme complex mainly consisting of protease and amylase was added to the chicken ration from hatching to the end of the experiment. Birds subjected to feed restriction whose diet was not supplemented showed an increase in sucrase, amylase, and lipase activities immediately after the restriction period. Amylase, lipase, and chymotrypsin activities were higher in chickens subjected to feed restriction and fed a supplemented diet than in those only subjected to feed restriction. Trypsin activity increased after feed restriction and after supplementation, but there was no interaction between these effects. Early feed restriction had no effect on enzyme activity in 42-d-old chickens. Chickens subjected to early restriction and fed the supplemented diet presented higher sucrase, maltase, and lipase activities than nonsupplemented ones ($P < 0.05$). There was no effect of early feed restriction or diet supplementation on weight gain to 42 d. Percentage weight gain from 14 to 42 d of age was equivalent in feed-restricted and ad libitum fed birds. Feed-restricted broilers fed a supplemented diet showed a higher percentage weight gain than nonsupplemented birds. We conclude that enzymatic supplementation potentiates the effect of feed restriction on digestive enzyme activity and on weight gain.

(*Key words*: broiler, digestive enzyme, enzymatic supplementation, feed restriction)

2004 Poultry Science 83:1544–1550

INTRODUCTION

Birds subjected to feed restriction for short periods during the early growth phase show improvement of feed efficiency and reach a weight similar to that of birds fed ad libitum at the time of slaughter (Auckland and Morris, 1971; Plavnik et al., 1986; Plavnik and Hurwitz, 1991). Feed restriction has been used to reduce the incidence of ascites (Tottori et al., 1997), sudden death syndrome (Blair et al., 1993; Gonzales et al., 1998), and skeletal deformities (Lee and Leeson, 2001) that are undesirable effects observed in fully fed broilers.

The improvement in feed efficiency observed in feed-restricted chickens has been attributed to reduced overall maintenance requirements caused by a transient decrease in basal metabolic rate (Zubair and Leeson, 1994). However, the improved feed efficiency can also be related to higher feed intake and to the hypertrophy of the gastrointestinal tract that occurs after the restriction period, when the birds are fed ad libitum (Michael and Hodges, 1973; Lilja et al., 1985; Yu and Robinson, 1992; Zubair and Leeson, 1994).

Improved digestive efficiency cannot be attributed only to morphological changes in the gastrointestinal tract. In fact, the digestive process is highly dependent on endogenous enzyme activity (Osman, 1982; Pubols, 1991). Thus, we hypothesized that there would be changes in enzyme activity in broilers subjected to feed restriction, which could explain, at least in part, the improvement in feed efficiency.

Improved feed efficiency and hypertrophy of the gastrointestinal tract can also be observed in chickens fed an enzyme-supplemented diet (Lima et al., 2002). Exogenous enzymes increase nutrient digestibility by breaking down the fiber in plant cell walls or by hydrolyzing proteins resistant to endogenous enzymes. This response has been observed with the addition of exogenous enzymes to diets that are high viscosity (Simbaya et al., 1996), as well as to corn- and soy-based diets, considered to be low viscosity diets (Zanella et al., 1999). Because the use of enzymes increases the availability of nutrients in the small intestine, this practice may induce increased enzyme activity. If so, this effect may potentiate the hypothesized effect of feed restriction on enzyme activity and, consequently, on weight gain.

Received for publication July 28, 2003.

Accepted for publication February 12, 2004.

[1]To whom correspondence should be addressed: mlvpauli@ibb.unesp.br.

The practice to feed exogenous enzymes to feed-restricted chickens could be a desirable feeding strategy that might produce birds with maximum lean body mass and final BW at a minimum feed intake. In addition, this practice might offer an economic advantage over a continuous ad libitum feeding regimen. However, little is known about the effect of feed restriction and enzymatic supplementation on the activity of digestive enzymes in broilers (Palo et al., 1995). Thus, the aim of this study was to determine the effect of feed restriction and enzymatic supplementation on activity of intestinal and pancreatic enzymes and on BW gain.

MATERIALS AND METHODS

Experimental Design

A total of 144 male broiler chicks (Ross line) were obtained from a commercial hatchery on the hatching day. They were housed in metal batteries in an environmentally controlled room with constant illumination and controlled temperature. The birds were randomly allocated to 24 pens (49 × 42 × 58 cm), each containing 6 chicks. Six blocks, 4 experimental units within a block, and 6 replicates per treatment were used. The experiment consisted of a 2 × 2 factorial arrangement of treatments, with 2 feed conditions (feed restriction or no feed restriction) and 2 levels of enzyme supplementation (with or without addition of enzymes to the diet). All procedures were approved by the University Ethics Committee for Animal Research.

Two groups of birds were subjected to feed restriction from 7 to 14 d of age. The other 2 groups received feed ad libitum from d 1 to 42. Chicks in all treatments were fed meal diets (Table 1) formulated according to Rostagno et al. (2000). The daily food amount supplied to the feed-restricted birds was equivalent to 70% of the quantity consumed by the birds fed ad libitum on the previous day. After the period of restriction, the broilers were fed ad libitum until the end of the experiment.

One group of feed-restricted chickens and one group of ad libitum fed chickens received enzyme supplementation in the diet for the duration of the experiment. The enzyme complex used was a blend of dried *Aspergillus oryzae* fermentation extract and dried *Bacillus subtilis* fermentation product that contained bacterial and fungal amylase and protease. The enzyme activity was 25,000 proteolytic activity of casein per gram (PC) for protease and 5,000 Sandstedt Kneen Blish (SKB) per gram for amylase. The enzymatic preparation was supplied in the ration at 600 ppm.

Sample Collection and Enzyme Analysis

The effects of treatments were determined at 14 and 42 d of age. For this, one chick chosen randomly from each pen was weighed and killed by cervical dislocation at the appropriate age. The birds had been starved for 8 h to permit intestinal emptying. The pancreas and a 10-cm segment of the small intestine immediately distal to the pancreas, free of residual food, were removed and frozen in liquid nitrogen. Samples were stored in liquid nitrogen until required for assay.

The samples were thawed and homogenized before being assayed for intestinal sucrase (EC 3.2.1.48) and maltase (EC 3.2.1.20), and for pancreatic lipase (EC 3.1.1.3), amylase (EC 3.2.1.1), trypsin (EC 3.4.4.4), and chymotrypsin (EC 3.4.4.5).

The activity of the pancreatic enzymes was determined after the whole organ was homogenized (1:20, wt/vol) in 50 m*M* Tris-HCl buffer, pH 8, containing 50 m*M* $CaCl_2$. For trypsinogen determination (Kakade et al., 1974), enterokinase was added to the homogenate and allowed to convert the enzyme into trypsin. Trypsin 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*-nitroaniline released per minute per milligram of protein. A similar method was used for the determination of chymotrypsin (Erlanger et al., 1966), with BAPNA replacing N-glutaryl-*L*-phenylalanine-*p*-nitroanilide (GPNA). The reaction was stopped with 3% acetic acid solution. Amylase was determined by the iodometric method[2] and the activity was expressed as amylase units per milligram of protein (AU/mg of protein). One amylase unit is the amount of enzyme that will hydrolyze 10 mg of starch in 30 min. Lipase activity was obtained by a colorimetric method.[3] Accordingly, lipase hydrolyzes the thioester, producing a thioalcohol that reacts with nitrobenzoic acid, yielding a yellow anion. The color intensity is proportional to enzyme concentration. The enzyme activity was expressed as International Units (IU) per milligram of protein.

To determine the activity of intestinal enzymes (Dahlqvist, 1964), the mucosa was removed by gentle scraping with a glass microscope coverslip and homogenized after the addition of 4 parts of ice-cold deionized water. The disaccharidases sucrase and maltase were assayed by incubating aliquots of the homogenates with the appropriate substrate in malate buffer at pH 6.4. Released glucose was determined by the glucose-oxidase method.[2] Enzyme activity was expressed as units per milligram of protein, which was determined by the method of Lowry et al. (1951).

Statistical Analysis

Data were analyzed in a factorial arrangement with 2 levels of feed condition (ad libitum and restricted) and 2 levels of enzyme addition (with and without enzyme addition). When there was no interaction effect, i.e., the F value for R × S was not significant, the isolated effect of both feed condition and enzyme supplementation (R and S values in the tables) was taken into account and it was possible to conclude whether feed restriction and

[2]CELM, Sao Paulo, Brazil.
[3]In vitro Diagnostica, Barbacena, Brazil.

TABLE 1. Composition and calculated dietary analysis

Ingredients and composition (%)	Starter 1 to 14 d	Grower 15 to 35 d	Finisher 36 to 42 d
Soybean meal	33.50	28.00	24.90
Corn	61.00	66.50	69.00
Calcium calcite	1.00	0.90	0.85
Dicalcium phosphate	2.00	1.90	1.60
Soy oil	1.10	1.50	2.60
Salt	0.35	0.35	0.35
L-Lysine	0.30	0.30	0.20
DL-Methionine	0.25	0.20	0.15
Mineral mix[1]	0.10	0.05	0.05
Vitamin mix[2]	0.40	0.30	0.30
Total	100.00	100.00	100.00
Calculated analysis			
ME (kcal/kg)	3,028.42	3,106.78	3,202.50
Crude protein (%)	21.74	19.54	18.14
Crude fiber (%)	2.70	2.56	2.46
Calcium (%)	0.99	0.91	0.81
Available phosphorus (%)	0.48	0.46	0.40
Methionine (%)	0.57	0.50	0.43
Methionine + cysteine (%)	0.94	0.84	0.75
Lysine (%)	1.36	1.21	1.05

[1]Mineral mix supplied the following per kilogram of diet: Cu, 70 mg; Fe, 50 mg; I, 1.25 mg; Mn, 60 mg; Se, 0.2 mg; Zn, 50 mg.

[2]Vitamin mix (1 to 14 d) supplied the following per kilogram of diet: vitamin A, 7,500 IU; vitamin D_3, 2,500 IU; vitamin E, 15 mg; vitamin K_3, 1.2 mg; vitamin B_{12}, 12.5 μg; thiamine, 1.5 mg; riboflavin, 5.5 mg; pyridoxine, 2 mg; niacin, 35 mg; calcium pantothenate, 10 mg; folic acid, 0.6 mg; biotin, 0.06 mg; choline chloride, 350 mg; methionine, 1,550 mg; coccidiostat, 100 mg; antioxidant, 20 mg.
Vitamin mix (15 to 35 d) supplied the following per kilogram of diet: vitamin A, 6,000 IU; vitamin D_3, 2,000 IU; vitamin E, 12 mg; vitamin K_3, 0.8 mg; vitamin B_{12}, 12 μg; thiamine, 1 mg; riboflavin, 4.5 mg; pyridoxine, 1.5 mg; niacin, 30 mg; calcium pantothenate, 10 mg; folic acid, 0.55 mg; biotin, 0.04 mg; choline chloride, 130 mg; methionine, 1,550 mg; coccidiostat, 20 mg; antioxidant, 20 mg.
Vitamin mix (36 to 42 d) supplied the following per kilogram of diet: vitamin A, 10,000 IU; vitamin D_3, 2,000 IU; vitamin E, 10 mg; vitamin K_3, 1 mg; vitamin B_{12}, 10 μg; riboflavin, 5 mg; niacin, 18 mg; calcium pantothenate, 10 mg; choline chloride, 200 mg; methionine, 1,200 mg; coccidiostat, 60 mg; antioxidant, 10 mg.

enzyme supplementation influenced the studied variables independently of each other. When the F value for R × S was significant, showing that the effect of restriction differed as a function of enzyme supplementation, ANOVA was followed by the Tukey test. The Tukey test compared the effect of feed condition on supplemented and nonsupplemented birds and the effect of supplementation on feed-restricted and ad libitum fed birds. The *P* value for all comparisons, except from the Tukey test, is shown in the tables. The level of significance for the Tukey test was $P < 0.05$.

RESULTS

Pancreatic Enzymes

There was interaction between feed restriction and enzyme supplementation on the activity of all pancreatic enzymes, except trypsin, immediately after feed restriction (R × S values, Table 2). Amylase and lipase activity increased immediately after feed restriction in birds whose diet was not supplemented. This response was still higher when the broilers were fed a supplemented diet. Chymotrypsin activity was higher in broilers whose diet was restricted and supplemented (Table 2). Supplementation did not contribute to the effect of feed restriction on trypsin activity ($P = 0.3001$, Table 2). The mean value for trypsin activity was 180.55 nmol/mg of protein in feed-restricted broilers and 58.1 nmol/mg of protein in ad libitum fed broilers. This difference was significant ($P = 0.0001$, Table 2). Mean trypsin activity was 129.35 nmol/mg of protein in birds fed the supplemented diet and 109.3 nmol/mg of protein in birds whose diet was not supplemented. This difference was significant ($P = 0.0011$, Table 2).

At 42 d, there was interaction between feed restriction and enzyme supplementation for amylase and lipase ($P = 0.0173$ and $P = 0.0498$, respectively). The effect of restriction on amylase activity was observed only in chickens fed the supplemented diet (Table 3). Lipase activity was higher only in nonrestricted birds fed the supplemented diet. In both conditions, i.e., restricted and ad libitum feeding, lipase activity was higher when the diet was supplemented (Table 3). Neither enzyme supplementation nor feed restriction had an effect on trypsin or chymotrypsin activity.

Intestinal Enzymes

There was interaction between feed restriction and enzyme supplementation on sucrase ($P = 0.0065$) and maltase ($P = 0.0620$) activity immediately after the feed restriction period (Table 2). Feed restriction induced an increase in sucrase activity only in chickens fed a nonsupplemented diet. Conversely, maltase activity was

TABLE 2. Effect of 30% feed restriction (from 7 to 14 d of age) and enzyme supplementation on intestinal and pancreatic enzyme activity in broilers (n = 6) at 14 d of age[1]

Enzymes added	Feed restriction	Amylase (UA/mg of protein)	Lipase (IU/mg of protein)	Trypsin (nmol/mg of protein)	Chymotrypsin (nmol/mg of protein)	Sucrase (U/mg of protein)	Maltase (U/mg of protein)
Yes	No	60.9 ± 1.5^{bA}	25.9 ± 0.7^{bA}	70.8 ± 2.5	2.9 ± 0.2^{bA}	21.8 ± 0.4^{aB}	21.5 ± 0.4^{bB}
	Yes	226.5 ± 2.9^{aA}	78.5 ± 2.7^{aA}	187.9 ± 5.6	11.2 ± 0.5^{aA}	20.2 ± 1.1^{aB}	30.4 ± 0.5^{aB}
No	No	61.5 ± 1.8^{bA}	22.5 ± 2.8^{bA}	45.4 ± 2.1	2.9 ± 0.1^{aA}	29.8 ± 0.9^{bA}	41.0 ± 1.5^{aA}
	Yes	158.3 ± 12.4^{aB}	52.6 ± 3.2^{aB}	173.2 ± 4.3	3.9 ± 0.2^{aB}	36.6 ± 0.7^{aA}	42.5 ± 1.6^{aA}
ANOVA	R[2]	0.0001	0.0001	0.0001	0.0001	0.0686	0.0125
	S[3]	0.0022	0.0008	0.0011	0.0001	0.0001	0.0001
	R × S	0.0019	0.0063	0.3001	0.0001	0.0065	0.0620

[a,b; A,B]Lowercase superscripts (a and b) indicate comparison between feed-restricted and ad libitum fed chickens for each diet supplied, with and without enzyme added. Uppercase superscripts (A and B) indicate comparisons between animals fed a supplemented and a nonsupplemented diet in each feeding condition, i.e., restricted and ad libitum. These differences were calculated by the Tukey test when the *F*-value for interaction was found to be significant. Means not followed by the same letter (a,b or A,B) differ significantly by the Tukey test ($P < 0.05$).

[1]Data are expressed as mean ± SEM. R = feed restriction; S = supplementation.

[2]A significant R-value indicates that there was a difference between the means for restricted and nonrestricted animals, independently of supplementation. The means are not indicated in the table.

[3]A significant S value indicates that there was a difference between the means for supplemented and nonsupplemented chickens, regardless of restriction. The means are not indicated in the table.

higher in feed-restricted birds receiving a supplemented diet (Table 2).

At 42 d, there was interaction between feed restriction and enzyme supplementation for sucrase ($P = 0.0001$) and maltase ($P = 0.0001$) activity (Table 3). Sucrase and maltase activity was higher in early-restricted birds fed a supplemented diet. In birds whose diet was not supplemented there was a decrease in enzymatic activity in response to feed restriction (Table 3).

BW and Feed Consumption

In 14-d-old chickens, BW and cumulative feed intake for ad libitum fed birds were higher than feed-restricted birds ($P = 0.0001$). In 42-d-old chickens, BW for ad libitum fed birds was also higher than feed-restricted birds ($P = 0.0612$). There was no difference in the cumulative feed intake from d 14 to 42 between feed-restricted and ad libitum fed birds. Body weight and feed intake did not change in response to diet supplementation (Table 4, S values).

There was interaction between feed restriction and enzyme supplementation for percentage weight gain ($P = 0.0925$). Early feed-restricted birds fed a supplemented diet had a higher percentage weight gain from 14 to 42 d than nonsupplemented birds. There was also an increase in weight gain when early-restricted chickens received a supplemented diet (Table 4).

DISCUSSION

The results of our experiment show adaptation of digestive enzymes to feed restriction in broilers, characterized by an increase in the activity of sucrase, amylase, lipase, and trypsin immediately after the restriction period in birds fed a nonsupplemented diet. The ability of

TABLE 3. Effect of 30% feed restriction (from 7 to 14 d of age) and enzyme supplementation on intestinal and pancreatic enzyme activity in broilers (n = 6) at 42 d of age[1]

Enzymes added	Feed restriction	Sucrase (U/mg of protein)	Maltase (U/mg of protein)	Amylase (UA/mg of protein)	Lipase (IU/mg of protein)	Trypsin (nmol/mg of protein)	Chymotrypsin (nmol/mg of protein)
Yes	No	15.9 ± 0.9^{bB}	23.2 ± 2.8^{bA}	98.5 ± 6.6^{bA}	36.2 ± 1.3^{aA}	80.1 ± 5.4	4.1 ± 0.1
	Yes	19.2 ± 0.4^{aA}	39.5 ± 1.7^{aA}	128.1 ± 5.3^{aA}	27.9 ± 1.2^{bA}	89.9 ± 4.9	4.2 ± 0.2
No	No	20.3 ± 0.6^{aA}	30.1 ± 0.6^{aA}	119.0 ± 4.3^{aA}	20.8 ± 0.9^{aB}	91.1 ± 6.4	4.4 ± 0.1
	Yes	10.8 ± 0.5^{bB}	17.4 ± 0.9^{bB}	104.3 ± 6.2^{aA}	19.4 ± 0.9^{aB}	87.6 ± 5.5	4.6 ± 0.4
ANOVA	R[2]	0.0012	0.4513	0.3796	0.0089	0.7167	0.6011
	S[3]	0.0187	0.0064	0.8484	0.0001	0.6204	0.9597
R × S		0.0001	0.0173	0.0498	0.4507	0.3303	

[a,b; A,B]Lowercase superscripts (a and b) indicate comparison between feed-restricted and ad libitum fed chickens for each diet supplied, with and without enzyme added. Uppercase superscripts (A and B) indicate comparisons between animals fed a supplemented and a nonsupplemented diet in each feeding condition, i.e., restricted and ad libitum. These differences were calculated by the Tukey test when the F value for interaction was found to be significant. Means not followed by the same letter (a,b or A,B) differ significantly by the Tukey test ($P < 0.05$).

[1]Data are expressed as mean ± SEM. R = feed restriction; S = supplementation.

[2]A significant R-value indicates that there was a difference between the means for restricted and nonrestricted animals, independently of supplementation. The means are not indicated in the table.

[3]A significant S value indicates that there was a difference between the means for supplemented and nonsupplemented chickens, regardless of restriction. The means are not indicated in the table.

TABLE 4. Effect of 30% feed restriction (from 7 to 14 d) and enzymatic supplementation on BW (g), percentage weight gain (%) and feed intake (g) in broilers[1]

Enzymes added	Feed restriction	Feed intake (g)		BW (g)		Weight gain (%)
		1 to 14 d	14 to 42 d	14 d	42 d	14 to 42 d
Yes	No	410.6 ± 4.7	2,564.3 ± 99.0	362.5 ± 5.5	2,233.3 ± 17.4	521.6 ± 12.8^{bA}
	Yes	353.9 ± 4.3	2,504.1 ± 89.6	314.9 ± 1.6	2,168.3 ± 36.8	588.7 ± 11.1^{aA}
No	No	406.6 ± 3.8	2,599.9 ± 92.4	354.9 ± 4.4	2,220.0 ± 37.6	527.8 ± 14.8^{aA}
	Yes	345.9 ± 4.2	2,735.2 ± 85.9	321.1 ± 3.8	2,081.7 ± 44.6	544.5 ± 6.8^{aB}
ANOVA	R	0.0001	0.9276	0.0001	0.0612	0.0092
	S	0.3683	0.3153	0.8913	0.3353	0.1953
	R × S	0.7582	0.4583	0.2055	0.4768	0.0925

[a,b; A,B]Lowercase superscripts (a and b) indicate comparison between feed-restricted and ad libitum-fed chickens for each diet supplied, with and without enzyme added. Uppercase superscripts (A and B) indicate comparisons between animals fed a supplemented and a nonsupplemented diet in each feeding condition, i.e., restricted and ad libitum. These differences were calculated by the Tukey test when the F value for interaction was found to be significant. Means not followed by the same letter (a,b or A,B) differed significantly by the Tukey test ($P < 0.05$).

[1]Data are expressed as mean ± SEM. R = feed restriction; S = supplementation.

[2]A significant R-value indicates that there was a difference between the means for restricted and nonrestricted animals, independently of supplementation. The means are not indicated in the table.

[3]A significant S value indicates that there was a difference between the means for supplemented and nonsupplemented chickens, regardless of restriction. The means are not indicated in the table.

the digestive tract to respond to changes in physiological needs has been demonstrated in mammals (Ferraris and Diamond, 1997) and birds (Dykstra and Karasov, 1992; Biviano et al., 1993), mainly with respect to nutrient absorption and organ structure. In fact, birds subjected to feed restriction increased intestinal absorption, and the gastrointestinal tract organs seemed to be spared the effect of restriction on weight that affects other organs and the BW. These responses depend on animal age and on the duration of the restriction period (Casirola et al., 1997; Ferraris et al., 2001).

The responses observed in the present study partially agree with those reported by Palo et al. (1995). These authors determined the activities of pancreatic and intestinal enzymes in broilers subjected to feed restriction either from 7 to 14 d of age or from 11 to 14 d, and observed an increase of pancreatic enzymes at 14 d of age, immediately after the restriction period. However, the intestinal enzyme activities only increased at 21 d of age, 7 d after the resumption of feeding. In our study, and in that of Palo et al. (1995), the increase in enzyme activity caused only by feed restriction could not be observed at 42 d of age. These results may indicate the occurrence of adaptation of the enzymatic response to restriction, or that such response may present a peak followed by a decrease before the final phase. However, the higher enzymatic response may have contributed to the higher percentage weight gain observed in birds subjected to feed restriction.

The increase in enzyme activity can be part of the digestive adaptation observed after feed restriction, which, according to Zubair and Leeson (1994), is considered one of the factors contributing to growth compensation. These investigators observed hypertrophy of the digestive tract associated with higher feed intake relative to BW in chickens subjected to early feed restriction. They did not observe reduction of maintenance requirements during refeeding, which, according to a number of investigators, could explain a higher efficiency in energy retention after the restriction period (Harris and Martin, 1984; De Boer et al., 1986). The higher enzyme activity may have been stimulated by the presence of feed in the gastrointestinal tract for a longer time, provoked by a slower rate of feed passage through the gastrointestinal tract (Barash et al., 1992).

In feed-restricted broilers fed a supplemented diet, we observed an increase in pancreatic enzyme activity immediately after the restriction period. Comparison of the two groups of restricted birds, supplemented and nonsupplemented, showed that supplementation caused a higher response of all pancreatic enzymes except trypsin. The effect of supplementation and the effect of restriction, alone, could not provoke an increase in enzyme activity in the final phase, with the exception of lipase, which was increased in ad libitum fed chickens receiving the supplemented diet. However, an increase in amylase, sucrase, and maltase activities could be observed in birds subjected to both feed restriction and a supplemented diet.

These responses may have been influenced by an increase of substrate in the gastrointestinal tract caused by the action of exogenous enzyme. The increased response in digestive enzymes supports the hypothesis that birds modulate specific enzymes according to substrate levels, rather than maintaining high enzyme activity constantly (Karasov and Hume, 1997). The higher enzyme activity observed in birds subjected to early feed restriction and fed a supplemented diet may have contributed to the higher weight gain because it is known that enzymes play a rate-determining role in providing the substrates for growth (Uni, 1999).

In the present study, supplementation did not induce a significant increase in BW in birds fed ad libitum. The difference between our results and those reported by Zanella et al. (1999) can be attributed to different amounts of supplemental enzymes, because in our study the enzyme proportion used was 600 g per ton of ration, whereas in the study of Zanella et al. (1999), the proportion was 1,000 g per ton. In fact, a high correlation has been demonstrated between enzyme concentration in the diet and weight gain in chickens (Marquardt et al., 1996). However, enzyme supplementation induced an 8% improvement in BW in chickens subjected to early feed restriction, a response coherent with the enzymatic response mainly if we consider that the enzymes that act on carbohydrates were augmented during the final phase. The weight gain by feed-restricted birds and birds fed a supplemented diet shows that the compensatory weight gain attained by feed-restriction (Auckland and Morris, 1971; Plavnik and Hurwitz, 1985, 1989, and 1991; Plavnik et al., 1986; Fontana et al., 1992) may be higher if the diet is supplemented.

We may conclude that enzyme supplementation improved the activity of digestive enzymes and BW gain in birds subjected to feed restriction, indicating a potentiation between the effects of diet supplementation and feed restriction.

ACKNOWLEDGMENTS

The authors thank Denise Rangel Silva Sartori for her help during the experiments, Sheila Zambello de Pinho for assistance with the statistical analyses, Alice Eiko Murakami and Antonio Celso Pezzato for valuable advice, and Gilson Luis Volpato for suggestions on the manuscript.

REFERENCES

Auckland, J. N., and T. R. Morris. 1971. Compensatory growth in turkeys: Effect of undernutrition on subsequent protein requirements. Br. Poult. Sci. 12:41–48.

Barash, I., N. Zafira, and I. Nir. 1992. Metabolic and behavioral adaptation of light-bodied chicks to meal feeding. Br. Poult. Sci. 33:271–278.

Biviano, A. B., C. Martínez del Rio, and D. L. Phillips. 1993. Ontogenesis of intestinal morphology and intestinal disaccharidases in chickens (*Gallus gallus*) fed contrasting purified diets. J. Comp. Physiol. B. 163:508–518.

Blair, R., R. C. Newberry, and E. E. Gardinen. 1993. Effects of lighting pattern and dietary tryptophan supplementation on growth and mortality in broilers. Poult. Sci. 72:495–502.

Casirola, D. M., B. Rifkin, W. Tsai, and R. P. Ferraris. 1997. Adaptation of intestinal nutrient transport to chronic caloric restriction in mice. Am. J. Physiol. 271:G192–G200.

Dahlqvist, A. 1964. Method for assay of intestinal disaccharidases. Anal. Biochem. 7:447–454.

DeBoer, J. O., A. J. van Es, L. C. Roovers, J. M. van Raaij, and G. A. J. Hautvast. 1986. Adaptation of energy metabolism of overweight women to low-energy intake, studied with whole-body calorimeters. Am. J. Clin. Nutr. 44:585–595.

Dykstra, C. R., and W. H. Karasov. 1992. Changes in gut structure and function of house wrens (*Troglodytes aedon*) in response to increased energy demands. Physiol. Zool. 65:422–442.

Erlanger, B. F., F. Edel, and A. G. Cooper. 1966. The action of chymotrypsin on two new chromogenic substrates. Arch. Biochem. Biophys. 115:206–210.

Ferraris, R. P., Q. X. Cao, and S. Prabhakaram. 2001. Chronic but not acute energy restriction increases intestinal nutrient transport in mice. J. Nutr. 131:779–786.

Ferraris, R. P., and J. Diamond. 1997. Regulation of intestinal sugar transport. Physiol. Rev. 77:257–302.

Fontana, E. A., W. D. Weaver, Jr., B. A. Watkins, and D. M. Edenbow. 1992. Effect of early feed restriction on growth, feed conversion and mortality in broiler chickens. Poult. Sci. 71:1296–1305.

Gonzales, E., J. Buyse, M. M. Loddi, T. S. Takita, J. R. Sartori, and E. Decuypere. 1998. Performance, incidence of metabolic disturbances and endocrine variables of food-restricted male broiler chickens. Br. Poult. Sci. 77:1646–1653.

Harris, R. S. B., and R. J. Martin. 1984. Recovery of body weight from below *set point* in mature female rats. J. Nutr. 114:1143–1150.

Kakade, M. L., J. J. Rackis, and J. G. McGhee. 1974. Determination of trypsin inhibitor activity of soy products. A collaborative analysis of an improved procedure. Cer. Chem. 51:376–382.

Karasov, W. H., and I. D. Hume. 1997. Vertebrate gastrointestinal system. Pages 409–480 in Handbook of Comparative Physiology. W. Danstzler, ed. American Physiological Society, Bethesda, MD.

Lee, K. L., and S. Leeson. 2001. Performance of broilers fed limited quantities of feed or nutrients during seven to fourteen days of age. Poult. Sci. 80:446–454.

Lilja, C., I. Sperber, and H. L. Marks. 1985. Postnatal growth and organ development in Japanese quail selected for high growth rate. Growth 49:51–62.

Lima, A. C. F., M. Macari, J. M. Pizauro Júnior, and E. B. Malheiros. 2002. Atividade enzimática pancreática de frangos de corte alimentados com dietas contendo enzima ou probiótico. Rev. Bras. Cienc. Avic. 4:187–193.

Lowry, O. H., N. R. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.

Marquardt, R. R., A. Brenes, Z. Zhang, and D. Boros. 1996. Use of enzymes to improve nutrient availability in poultry feedstuffs. Anim. Feed Sci. Technol. 60:321–330.

Michael, E., and R. D. Hodges. 1973. Histochemical changes in the fowl intestine associated with enhanced absorption after restriction. Histochemie 36:39–44.

Osman, A. M. 1982. Amylase in chicken intestine and pancreas. Comp. Biochem. Physiol. 73:571–574.

Palo, P. E., J. L. Sell, F. J. Piquer, L. Vilaseca, and M. F. Soto-Salanova. 1995. Effect of early nutrient restriction on broiler chickens. 2. Performance and digestive enzyme activities. Poult. Sci. 74:1470–1483.

Plavnik, I., and S. Hurwitz. 1991. Response of broiler chickens and turkey poults to food restriction of varied severity during early life. Br. Poult. Sci. 32:343–352.

Plavnik, I., and S. Hurwitz. 1985. The performance of broiler chicks during and following a severe feeding restriction at an early age. Poult. Sci. 64:148–155.

Plavnik, I., and S. Hurwitz. 1989. Effect of dietary protein, energy, and feed pelleting on the response of chicks to early feed restriction. Poult. Sci. 68:1118–1125.

Plavnik, I., J. P. McMurtry, and R. W. Rosebrough. 1986. Effect of early feed restriction in broilers. Growth performance and carcass composition. Growth 50:68–76.

Pubols, M. H. 1991. Ratio of digestive enzymes in the chick pancreas. Poult. Sci. 70:337–342.

Rostagno, H. S., L. F. T. Albino, and J. L. Donzele. 2000. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. H. S. Rostagno, ed. UFV-Viçosa, Brazil.

Simbaya, J., B. A. Slominski, W. Guenter, A. Morgan, and L. D. Campbell. 1996. The effects of protease and carbohydrase supplementation on the nutritive value of canola meal for poultry: In vitro and in vivo studies. Anim. Feed Sci. Technol. 61:219–234.

Tottori, J. R., Y. Yamaguchi, M. Murakama, K. Sato, K. Uchida, and S. Tateyama. 1997. The use of feed restriction for mortality control of chickens in broilers farms. Avian Dis. 41:433–437.

Uni, Z. 1999. Functional development of the small intestine in domestic birds: Cellular and molecular aspects. Poult. Avian Biol. 10:167–179.

Yu, M. W., and F. E. Robinson. 1992. The application of short-term feed restriction to broiler chickens production: A review. J. Appl. Poult. Res. 1:147–153.

Zanella, I., N. K. Sakomura, F. G. Silversides, F. G. A. Figueiredo, and M. Pack. 1999. Effect of enzyme supplementation on broiler diets based on corn and soybeans. Poult. Sci. 78:561–568.

Zubair, A. K., and S. Leeson. 1994. Effect of varying period of early nutrient restriction on growth compensation and carcass characteristics of male broilers. Poult. Sci. 73:129–136.