Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X Oct - Dec 2006 / v.8 / n.4 / 233 - 241

Influence of Delayed Placement and Dietary Lysine Levels on Small Intestine Morphometrics and Performance of Broilers

■ Author(s)

Franco JRG[1]
Murakami AE[1]
Natali MRM[2]
Garcia ERM[1]
Furlan AC[1]

[1] Programa de Pós Graduação em Zootecnia– PPZ, Universidade Estadual de Maringá.
[2] Departamento de Ciências Morfofisiológicas – DCM, Universidade Estadual de Maringá – UEM.

■ Mail Address

JRG Franco
Universidade Estadual de Maringá – UEM
Avenida Colombo, 5790. Bloco 32. Zona 07
87.020-900. Maringá, PR, Brasil

E-mail: zegallifranco@hotmail.com
aemurakami@uem.br

■ Keywords

Abdominal fat, intestinal mucosa, ideal protein, yolk sac, villi.

■ Acknowledgments

The authors are grateful to Fundação de Apoio ao Desenvolvimento Científico and Tecnológico do Paraná, Fundação Araucária, Brazil, for financially supporting this research project.

Arrived: February / 2006
Approved: August / 2006

ABSTRACT

This experiment studied the influence of delayed placement (HI) and digestible lysine level (DL) on the morphometrics of the intestinal mucosa and on the performance of broilers. A total number of 1,705 Cobb 500 male chicks were used in a completely randomized experimental design in a factorial arrangement with four HI (12, 24, 36 and 48h), and two DL level in the starter diet (1.143 and 1.267%), with four replicates and 55 birds per experimental unit. The amino acids methionine-cystine, threonine, and tryptophan were balanced according to the ideal protein (IP) concept. Small intestine morphometrics was evaluated using histology slides of the duodenum and jejunum. There was no interaction between HI and DL levels for any of the studied parameters. The 1.143% level of DL promoted better performance results at 21 and 42 days of age, as well as higher duodenum and jejunum crypt depth, and duodenum villi height at 21 days of age. HI negatively influenced the morphometrics of the small intestine during the starter phase, and the performance of broilers up to 42 days of age. There was no effect of the treatments on yolk sac utilization or abdominal fat percentage. It was concluded that the use of 1.143% DL and HI of 12 hours promoted better development of the small intestine mucosa up to 21 days of age, and broiler performance at market age.

INTRODUCTION

Broilers have an outstanding genetic potential for growth and meat production; however, in order to realize this potential, they must be fed to meet their nutritional requirements. In addition, they must be able to digest the ingested feed, and to absorb the nutrients contained in it. These processes are directly correlated to the development of the gastrointestinal tract, particularly of the small intestine.

The maturation of the small intestine is essential to optimize broiler growth, as digestion and absorption rates are directly influenced by cell proliferation and differentiation rates, as the higher the villi and their density, the larger is the area of surface for digestion and absorption (Boleli *et al.*, 2002).

Immediately after hatching, broiler chicks are submitted to drastic nutritional changes, from a lipid-rich nutrition from the yolk sac to a carbohydrate-rich exogenous diet. According to Noy & Sklan (1997), the ingested nutrients stimulate intestinal development, and therefore, feed supply tends to increase intestinal absorptive surface, as well as the potential to assimilate nutrients, and consequently, bird growth.

However, after hatching, broiler chicks remain in the hatchery until they are sexed, and vaccinated. They are then transported to the farms to be housed. During this period, chicks are deprived from feed and

water, resulting in a significant growth reduction, with consequences in the short and in the long term (Noy & Sklan, 1997).

From hatching until housing, chicks are nourished by the yolk sac, which is inside the abdomen (Noy *et al.*, 1996). There are indications that nutrient supply from the yolk sac is not enough to support the rapid growth of the newly hatched chick of housing, and therefore, feed supply, takes a long time (Gonzales *et al.*, 2003).

In addition to hatching-delayed placement, nutrition during the starter phase of broiler production, particularly the adequate amino acid balance in the diet, has a great influence on the productive performance of these birds. As to amino acids, lysine is of great concern, as this is one of the main components of muscle proteins.

Formulating diets based on the ideal protein concept provides a more adequate balance among amino acids in the diet. In this concept, amino acids are expressed as a ratio to a reference amino acid, which is usually lysine (Emmert & Baker, 1997). In addition, the use of diets based on ideal protein concept allows the reduction of crude protein levels in broiler diet, which reduces feed cost and nitrogen excretion, a major environmental pollutant.

However, the formulation of practical broiler diets balancing all amino acids according to the ideal protein concept is still unfeasible due to the high cost of some synthetic amino acids, and to the difficulty in the determination of amino acid composition in feedstuffs. This has led nutritionists to apply the ideal protein concept in practical broiler diets only for the first limiting amino acids (methionine+cystine, lysine, threonine, and tryptophan).

This study aimed at evaluating the influence of delayed placement (HI) and digestible lysine level (DL) in starter diets formulated according to ideal protein concept on intestinal mucosa morphometrics and performance of broilers.

MATERIAL AND METHODS

The experiment was carried out at the poultry house of Iguatemi Experimental Farm and at the Animal Histology Lab of the State Unversity of Maringa, PR, Brazil. A total number of 1,760 male Cobb broiler chicks, with an average weight of 43.5 g at hatching, derived from 47-week-old broiler breeders, was used.

After removal from the hatcher, chicks were wing-sexed, vaccinated for Marek disease, and transported to the experimental house. Chicks were randomly distributed to the treatments, weighed, and kept in cardboard boxes up to the determined delayed placements. During this period, chicks had no access to water or feed. Total period from the removal from the hatchery until the first experimental group was housed was 12 hours.

Light was continuously provided during the first week, and 20 hours of light and four hours of dark were used daily until the end of the experiment. House temperature and relative humidity were monitored. Chicks were vaccinated via eye drop for Newcastle and Infectious Bursal diseases on the seventh day.

A completely randomized experimental design, with a factorial arrangement 4 x 2 (delayed placements of 12, 24, 36, and 48h x digestible lysine levels in the starter diets of 1.143 and 1.267%) was used. Therefore, there were eight treatments with four replicates and 55 birds per experimental unit.

After housing, birds were fed the experimental diets up to 21 days of age with the experimental diet, and with a conventional diet up to 42 days of age, as shown in Table 1. In the starter phase (1 to 21 days), diets had the same nutrient levels, but different digestible lysine (1.143 and 1.267%), and therefore different digestible methionine+cystine, threonine, and tryptophan levels, according to the ideal protein standard proposed by Rostagno *et al.* (2000) for digestible amino acids. During the grower phase (22 to 42 days), all birds received a single feed, formulated on ideal protein basis and containing 1.045% digestible lysine.

Bird mortality was daily recorded, allowing flock livability to be determined as a function of the studied treatments. Possible causes were determined by necropsy.

During the first 48 hours after hatching, birds were weighed at 12 and 48 hours. Broilers were then weighed on days 21 and 42, when feeds were also weighed to determine feed intake, feed conversion ratio, and European Production Index (EPI), calculated as EPI=(daily weight gain [g] x livability [%]/(feed conversion ratio [g/g]) x 100.

Abdominal fat percentage was determined by weighing the adipose tissue around the vent, the bursa of Fabricius, gizzard, proventriculus, and adjacent abdominal muscles in two birds per experimental unit (eight birds per treatment). It was expressed as a percentage of live weight at 21 and 42 days.

Intestinal mucosa morphometrics used the small intestine (from the distal portion of the duodenal loop to Meckel's diverticulum) randomly removed from fie birds per treatment on days 7, 21, and 42.

Table 1 – Percentage and calculated composition of experimental diets during starter (1 to 21 days) and grower (22 to 42 days) periods.

Ingredients, %	Starter		Grower
Corn, grain	67.850	68.550	73.050
Soybean meal, 45%	27.700	26.800	22.200
Dicalcium phosphate	1.860	1.860	1.650
Limestone	1.020	1.020	0.960
Soybean oil	0.210	——-	0.720
L-Lysine HCL, 78%	0.393	0.581	0.433
DL-Methionine, 98%	0.287	0.386	0.259
L-Threonine, 98%	0.060	0.155	0.053
L-Trytophan, 98%	——-	0.028	0.026
Vitamin supplement[1,2]	0.100	0.100	0.100
Mineral supplement[3]	0.50	0.50	0.50
Salt	0.460	0.460	0.390
Antioxidant	0.010	0.010	0.010
Total	100.000	100.000	100.000
Calculated values			
Metabolizable energy, kcal/kg	3.000	3.000	3.100
Crude protein, %	19.000	19.000	17.000
Calcium, %	0.960	0.960	0.875
Available phosphorus, %	0.450	0.450	0.406
Digestible lysine, %	1.143	1.267	1.045
Digestible methionine + cystine, %	0.812	0.900	0.742
Met+Cys/Lys ratio	71	71	71
Digestible tryptophan, %	0.199	0.220	0.193
Tryptophan/lysine ratio	17	17	18
Digestible threonine, %	0.675	0.748	0.596
Threonine/lysine ratio	59	59	57
Sodium, %	0.222	0.222	0.192
Chloride, %	0.311	0.312	0.272

1- Starter Vitamin Mixture (Content per kg of the premix): Vit. A 7,000,000 UI; Vit. D3 2,200,000 UI; Vit.E 11,000 mg; Vit. K3 1,600 mg; Vit. B1 2,000 mg; Vit. B2 5,000 mg, Vit. B12 12,000 mcg; Niacin 35,000 mg; Pantothenic Acid 13,000 mg; Folic Acid 800 mg; Antioxidant 100,000. 2 - Grower Vitamin Mixture (Content per kg of the premix): Vit. A 6,000.000UI; Vit. D3 2,000,000 UI; Vit.E 10,000 mg; Vit. K3 1,000 mg; Vit. B1 1,400 mg; Vit. B2 4,000 mg, Vit. B12 10,000 mcg; Niacin 30,000 mg; Pantothenic Acid 11,000 mg; Folic Acid 600 mg; Antioxidant 100,000. 3 - Mineral Mixture (Content per kg of the premix): Iron 10,000 mg as $FeSO_4 \ddagger H_2O$; Copper 16,000 mg as $CuSO_4$; Iodine 2,400 mg as $Ca(IO_3)_2$; Zinc 100,000 mg as $ZnSO_4$; Manganese 40,000 mg as $MnSO_4 \ddagger H_2O$; Seleniun 400 mg as $NaSe_2O_3$.

Birds were sacrificed by neck dislocation, and fragments measuring approximately 4 cm of the duodenum and the jejunum were collected. Collected samples were rinsed with saline solution, fixed in Bouin solution for 12-24 hours, dehydrated in graded alcohol series, cleared in xylol, and embedded in paraffin to obtain 5 µm-thick transversal histological cuts. Slides were stained by Hematoxylin-Eosin method, as described by Beçak & Paulete (1976).

Morphometrics analyses included the measurement of 30 villi height, and 30 crypt depth per animal, with the aid of the software Image Pro-Plus 4.1, Média Cibertecnics, and final magnification of 10X (optical) per 2X (objective) coupled to an Olympus Bx 40 microscope.

In order to evaluate treatment effects on yolk sac utilization, eight birds per treatment (two birds per experimental unit) were randomly removed at 12, 24, 36, and 48 hours after hatching, and their yolk sacs, if present, were removed and weighed.

Statistical analysis of the yolk sac weights removed within the first 48 hours of the chick live considered only one group 12 hours after hatching (12-hour fasting); three experimental groups 24 hours after hatching (24-hour fasting, and fed 12 hours after hatching with 1.143 or 1.267% DL); five experimental groups 36 hours after hatching (36-hour fasting, and four groups fed 12 and 24 hours after hatching with 1.143 or 1.267% DL); seven experimental groups 48 hours after hatching (48-hour fasting, and the groups fed 12, 24, and 36 hours after hatching with 1.143 or 1.267% DL). After 48 hours, when the experimental diets were fed to the last group, lysine levels started to be considered for all experimental groups.

Until 48 hours after hatching, a model identity analysis was carried out to evaluate the effects of housing, and digestible lysine levels, comparing models with or without fasting. When the effect of fasting was significant, the test of Tukey (5%) was used to compare means.

The statistical analysis of data was performed with the aid of the software Saeg (1998). After analysis of variance, degrees of freedom referring to delayed placements were exploded in polynoms, and determination coefficients (R^2) were calculated considering the sum of squares of the regression as a function of total square sum.

RESULTS

Performance and yolk sac utilization

Average house temperatures were, respectively, 17°C in the morning (8:00 am), and 19.47°C in the afternoon (5:00 pm). Air relative humidity, monitored at the same times, was 70.50% in the morning, and 64.26% in the afternoon.

As to chick weight at 12 to 48 hours after hatching, a 6.32% (43.5g to 40.75g) reduction was observed in average weight, with a 2.75-g loss.

Results of yolk sac utilization during the first 48 hours after hatching are presented in Table 2. There was no effect of treatments ($P>0.05$) on this parameter.

Performance results during the starter phase (1 to 21 days) and total period (1 a 42 days) are presented in Tables 3 and 4, respectively. There was no interaction between digestible lysine levels and delayed placements on the parameters analyzed in both phases.

Table 2 – Influence of delayed placement and lysine levels in feeds based on ideal protein concept on yolk sac average weight of broilers (12 to 48 hours after hatching).

Delayed placement (hours)	DigestibleLysine (%)	Assessment Age[1] (hours)			
		12	24	36	48
				g	
12	1.143	4.10	3.06	3.04	1.99
	1.267		3.67	2.88	1.10
24	1.143		3.02	2.60	1.80
	1.267			2.78	2.10
36	1.143			2.42	1.95
	1.267				2.32
48	1.143				1.94
	1.267				
Mean		4.10	3.25	2.74	1.58
CV (%)		22.15	25.88	27.15	32.62

(P≥0.05) Non-significant. 1 - hours after hatching.

Table 3 – Influence of delayed placement and lysine levels in feeds based on ideal protein concept ono desempenho dos frangos de corte no período inicial (1 a 21 days).

Parameters	Body weight (g)	Feed intake (g)	Feed conversion ratio (g/g)	Abdominal fat (%)
Delayed placement (hours)				
12	747.85 (100.0)[1]	1099.85	1.584	1.73
24	728.26 (97.38)	1064.48	1.534	1.61
36	708.66 (94.76)	1029.10	1.543	1.70
48	689.07 (92.14)	993.74	1.554	1.55
Digestible Lysine (%)				
143	735.47	1076.91	1.547	1.66
1.267	698.19	1017.45	1.562	1.64
Mean	716.83	1047.18	1.555	1.65
CV (%)	2.86	3.04	2.81	11.8
Analysis of Variance				
Lysine level	*	*	NS	NS
Delayed placement	Linear[2]	Linear[3]	NS	NS
Interaction	NS	NS	NS	NS

* Significant (P≤0,05); NS = Non-significant. 1 - Numbers between parentheses are relative to the 12-hour delayed placement. 2 - Y = 767.442 – 1.63276X; R^2 = 0.36. 3 - Y = 1139.73 – 3.058X; R^2 = 0.50.

During the starter phase (1 to 21 days, Table 3), digestible lysine levels influenced (P<0.05) live weight and feed intake. Birds fed diets containing 1.143% were heavier and ate more than those fed 1.267% digestible lysine.

There was no effect of delayed placement on feed conversion ratio and abdominal fat percentage at 21 days; however, as delayed placement increased, and consequently feed and water fasting increased, there was a linear reduction (P<0.05) in live weight (Y = 767.442 – 1.63276X) and feed intake (Y = 1139,73 – 3,058X).

Performance results for the entire experimental period (1 to 42 days) and abdominal fat percentage at 42 days are shown in Table 4. There was an effect (P<0.05) of lysine levels on body weight and production factor, with birds fed 1.143% digestible lysine presenting the best results. On the other hand, there was no effect on feed intake, feed conversion ratio, livability, and abdominal fat.

As to delayed placement effect on performance at 42 days (Table 4), there was a linear and reducing effect (P<0.05) on live weight (Y = 2592.49 – 3.3212X) and production factor (Y = 330.904 – 0.535065X). However, as delayed placement increased, there was a quadratic effect (P<0.05) on feed intake (Y = 4836.04 – 24.94X + 0.30891X^2; Figure 1) on feed intake, with the lowest intake obtained at a delayed placement of 40.4 hours.

Morphometrics of the Small Intestine Mucosa

Mean villi height and crypt depth at the duodenum and jejunum of broilers with 7, 21, and 41 of age are shown in Table 5. These parameters were different (P<0.05) in both segments as a function of the evaluated ages.

Villi height and crypt depth increased as the birds aged; however, each structure behaved differently in each segment as a function of age. In the duodenum,

Table 4 - Influence of delayed placement and lysine levels in feeds based on ideal protein concept on broiler performance (1 to 42 days).

Parameters	Body weight (g)	Feed intake (g)	Feed conversion ratio(g/g)	Production factor	Livability (%)	Abdominal fat (%)
Delayed placement (hours)						
12	2552.64 (100.0)[1]	4581.24	1.813	324.48	95.75	1.991
24	2512.78 (98.44)	4415.41	1.807	318.06	96.57	1.905
36	2472.93 (96.88)	4338.55	1.788	311.64	95.25	1.780
48	2433.07 (95.32)	4350.65	1.812	305.22	95.25	1.983
Digestible Lysine (%)						
1.143	2520.91	4448.07	1.797	320.71	96.00	1.97
1.267	2463.10	4385.21	1.813	308.76	95.38	1.86
Mean	2492.01	4416.64	1.805	314.74	95.69	1.92
CV (%)	2.09	2.33	1.33	4.97	3.75	16.06
Analysis of Variance						
Lysine level	*	NS	NS	*	NS	NS
Delayed placement	Linear[2]	Quadrático[3]	NS	Linear[4]	NS	NS
Interaction	NS	NS	NS	NS	NS	NS

* Significant (P≤0,05); NS = Non-significant 1 - Numbers between parentheses are relative to the 12-hour delayed placement. 2 - Y = 2592.49 - 3.3212X; R^2 = 0.36; 3 - Y = 4836.04 – 24.94X + 0.30891X^2; R^2 = 0.47; 4. Y = 330.904 – 0.535065X; R^2 = 0.19.

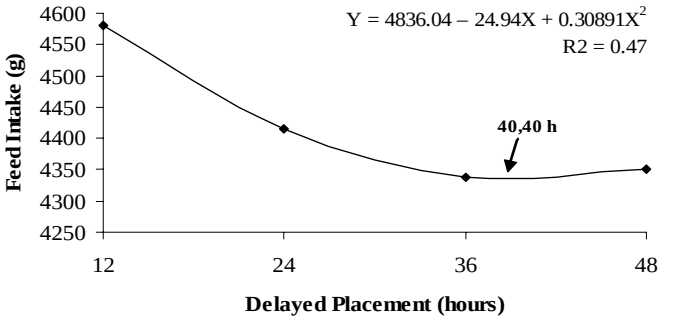

Figure 1 – Effect of delayed placement on broiler feed intake (42 days of age).

Table 5 - Villi height and crypt depth in the duodenum and jejunum of broilers with 7, 21, and 42 days of age.

Age (days)	Villi height		Crypt depth	
	Duodenum	Jejunum	Duodenum	Jejunum
			µm	
7	770.12b	384.63c	153.65b	88.41b
21	1366.25a	735.49b	155.72b	119.28a
42	1374.68a	1082.08a	182.47a	134.40a
Mean	1170.35	734.07	163.95	114.03
CV (%)	14.90	18.31	23.13	25.19

a,b - Means followed by different letter in the same column are different by the test of Tukey (P<0.05).

villi height increased up to 21 days of age, whereas in the jejunum, it continued up to 42 days. Duodenum crypt depth started increasing when birds were 21 days of age, and in the jejunum, this increase was observed only until 21 days of age.

The morphometrics evaluated on days 7, 21, and 42 are presented in Table 6. There was no interaction between delayed placements and digestible lysine levels in none of the studied ages.

At seven days of age, there was no effect of digestible lysine levels on duodenum or jejunum villi height, but an increase (P<0.05) in crypt depth in both segments as digestible lysine level increased (Table 6).

Delayed placement had a negative linear influence (P≤0.05) villi height in the duodenum (Y = 886.149 – 3.806X) and in the jejunum (Y = 431.66 – 1.54263X) at 7 days of age (Table 6). However, no effect of delayed placement on crypt depth was observed in either intestinal segment.

At 21 days of age (Table 6), digestible lysine levels influenced (P≤0.05) only villi height at the duodenum and crypt depth in the duodenum and the jejunum, with birds fed 1.143% digestible lysine presenting higher villi as compared to those fed 1.267% digestible lysine. However, no differences were observed in jejunum villi height, independent of dietary lysine level.

delayed placements did not influence jejunum villi height or crypt depth in the duodenum or the jejunum at 21 days of age, but there was a quadratic effect (Y = 1828.72 – 32.6592X + 0.482192X^2) on duodenum villi height as delayed placement increased (Table 6 and Figure 2). The lowest villi height was observed for the 33.87-hour delayed placement.

At 42 days of age (Table 6), there was no effect of digestible lysine levels or delayed placements on villi height or crypt depth in the duodenum or jejunum in the experimental birds.

DISCUSSION

The yolk sac utilization results (Table 2), independent of delayed placement and, therefore, of feeding time, are different from those fund by Noy *et al.*, (1996), but are consistent with the results of Gonzales *et al.* (2003).

Table 6 – Influence of delayed placement and digestible lysine levels in feeds based on ideal protein concept on the morphometrics of the mucosa of the duodenum and the jejunum of broilers with 7, 21, and 42 days of age.

Parameters	7 days				21 days				42 days			
	Duodenum		Jejunum		Duodenum		Jejunum		Duodenum		Jejunum	
	Villi height	Crypt depth*[1]	Villi height	Crypt depth	Villi height*[1]	Crypt depth	Villi height	Crypt depth*[1]	Villi height	Crypt[1] depth*	Villi height	Crypt depth*[1]
	µm											
Delayed placement, hours												
12	840.48	147.61	413.15	86.75	1506.24	164.25	774.16	120.03	1416.22	185.01	1125.51	179.56
24	794.81	170.68	394.64	96.48	1322.64	167.56	720.73	124.63	1364.44	180.93	1053.79	199.10
36	749.13	138.07	376.13	88.78	1277.91	131.06	692.59	109.31	1443.42	208.42	1172.85	192.06
48	703.46	163.35	357.61	84.05	1372.05	149.11	740.23	123.28	1262.40	152.39	954.13	158.45
Digestible Lysine, %												
1.143	774.53	138.63	383.33	82.47	1446.69	168.98	738.40	125.21	1385.56	183.76	1065.71	136.70
1.267	765.47	169.51	386.01	94.68	1289.35	142.25	724.48	113.38	1363.80	181.20	1087.43	132.69
Mean	770.00	154.07	384.67	88.58	1368.02	155.62	731.44	119.30	1374.68	182.48	1076.57	134.8
												wP:<M
CV (%)	10.20	21.32	16.02	16.13	10.424	17.01	18.94	15.04	13.81	20.75	15.14	27.84
Analysis of Variance												
Lysine level	NS	*	NS	*	*	*	NS	*	NS	NS	NS	NS
Delayed placement	Linear[2]	NS	Linear[3]	NS	Quadratic	NS	NS	NS	NS	NS	NS	NS
Interaction	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

* Significant (P≤0,05); NS = Non-significant; *1 - Crypt depth; 2 - Y = 886.149 – 3.806X; R2 = 0.26; 3 - Y = 431.66 – 1.54263X; R^2 = 0.12; 4.Y = 1828.72 – 32.6592X + 0.482192X^2; R^2 = 0.23.

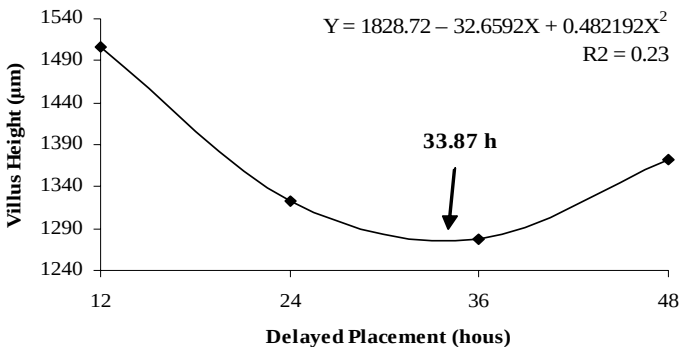

Figure 2 – Influence of delayed placement on villi height of the duodenum of 21-day-old broilers.

Although there was no difference among treatments on yolk sac weight during the fist 48 hours after hatching, chick average weight decreased during this period (from 4.1 g to 1.58 g). when the yolk sac weight loss is related to chick weight loss as feed and water fasting increased (from 12 to 48 hours after hatching), it is suggested that the nutrients available in the yolk sac were not sufficient to allow weight gain or even weight maintenance of chicks during this period. This possibly led birds to use their body reserves to supply the nutritional requirement for survival, which resulted in body weight reduction.

The negative effect of delayed placement on performance at 21 days (Table 3) and 42 days (Table 4) of age is consistent with the findings of Halevy *et al.* (2000) and Gonzales *et al.* (2000), who observed that long feed fasting between hatching and housing negatively influences broiler performance.

According to Noy & Sklan (1998), the earlier the birds have access to feed after hatching, the greater is their growth performance as compared to chicks submitted to long fasting periods.

However, the increase in feed intake after the 40.4-hour housing period observed for the entire experimental period (1 to 42 days; Table 4 and Figure 1) may indicate that birds housed later may have developed a compensatory mechanism in order to increase feed intake and nutrient utilization. However, despite this increase in feed intake, there was no improvement in weight gain, which demonstrates that adequate weight gain in the first hours after hatching is essential to optimize broiler final weight.

The higher weight gain of 21-day-old broilers fed 1.143% digestible lysine (Table 3) is consistent with the recommendation proposed by Rostagno *et al.* (2000) for the phase of 1 to 21 days of age, and by Rostagno *et al.* (2005) for average performance of male broilers for the phase of 8 to 21 days of age (1.1466% digestible lysine).

Although results showed that feeding 1.143% digestible lysine improved broiler performance at 21 days of age as compared to a 1.267% digestible lysine level, the use of higher lysine levels during the starter phase was associated to better performance during the finisher phase (Kidd *et al.*, 1998).

The lower weights of broilers fed 1.267% digestible lysine until 21 days of age (P≤0.05) may be related to the protein level used during the starter phase. The 19% protein level may have not been sufficient to provide the necessary amount of nitrogen for the synthesis of non-essential amino acids during the starter phase. When the dietary lysine level was increased, and consequently, of methionine+cystine, threonine, and tryptophan level, under the concept of ideal protein, this may have caused unbalance among essential and non-essential amino acids, causing worse performance. This possible unbalance was described by Shutte *et al.* (1997), who fed chicks diets based on corn and soybean meal, and observed that glycine+serine were limiting when dietary protein level was below 21%.

When unbalanced, amino acids can be catabolized and excreted as uric acid by birds, or be converted in lipids, and deposited as adipose tissue. In broilers, fat is mainly accumulated in the abdomen; however, no effects of dietary digestible lysine levels were observed on the abdominal fat percentage in 21-day-old broilers were observed in the present study (Table 3). This may indicate that the possible amino acids excess ingested by the broilers fed 1.267% digestible lysine may have been excreted.

The higher catabolism and excretion of excessive amino acids may have negatively influenced broiler performance during the starter phase as, according to Costa *et al.* (2001), the elimination of these amino acids require high energy use. Therefore, the energy that could be utilized for tissue accretion is deviated to nitrogen excretion.

The effect (P<0.05) of digestible lysine levels in the starter feeds on body weight and production factor at 42 days (Table 4) shows the importance of the starter diet on the final performance of broilers. Nevertheless, the improvement in body weight and production factor of birds fed 1.143% DL may be attributed to the low protein level used in the starter phase and to the unbalance between essential and non-essential amino acids as dietary lysine level increased, as previously discussed.

As to the analysis of intestinal mucosa morphometrics as a function of age (Table 5), the observed increases in duodenum villi height until 21 days of age, and in jejunum villi height until 42 days of age are justified as, according to Uni *et al.* (1998a), the duodenum starts to develop earlier than the jejunum. Moreover, these results demonstrate lack of synchronism in the development of different intestinal segments.

The reduction in duodenum and jejunum villi height observed at 7 days of age as a function of increase in delayed placements (Table 6) is consistent with the findings of Noy & Sklan (1997), who demonstrated significant reduction in villi height and in number of enterocytes per villi only after five to six days of fasting.

The negative effect of fasting immediately after hatching on small intestine villi height is possibly related to an increase in villi extrusion rate, leading to a reduction in height. In this case, according to Yamauchi *et al.* (1996), epithelial cells present lysosomal autophagic vacuoles, which are characteristic of cell death, after long periods of fasting, suggesting that fasting causes intracellular digestion.

The maintenance of the intestinal mucosa epithelium and of the support structures costs about 20% of the gross energy consumed by the animal (Mcbride & Kelly, 1990). Therefore, the higher the need of mucosal repair, the lower the energy available for weight gain in broilers (Macari & Maiorka, 2000).

Although no differences in crypt depth were observed as a function of the evaluated delayed placements, the "compensatory" increase in duodenum villi height after 33 hours (Table 6, Figure 2) can probably be attributed to higher cellular synthesis. This interference can be explained by the fact that enterocyte proliferation seems not to be limited to the crypt. According to Uni *et al.* (1998b), in layers, cells proliferate both in the crypts and in the intermediate third of small intestinal villi.

The quadratic behavior of duodenum villi height at 21 days of age (Figure 2) may indicate that delayed placements longer than 33 hours may lead the body to develop a mechanism to compensate the problems caused by fasting immediately after hatching. The "compensatory" increase in duodenum villi height would increase surface area of nutrient absorption, improving feed efficiency.

The effect of delayed placement on villi height on both studied intestinal segments seems to decrease as the bird ages, as no differences were found in morphometric parameters in 42-day-old broilers (Table 6). However, the possible improvement in nutrient absorption during the first few weeks after hatching influenced body weight at 42 days of age, with birds housed 12 hours after hatching presenting higher body weight as slaughter as compared to those housed 48 hours post-hatch.

Increased crypt depth in the duodenum and in the jejunum in 7-day-old broilers (Table 6) was observed with higher dietary lysine levels. This effect may have

also been caused by the consequent higher methionine+cystine, threonine, and tryptophan levels under the concept of ideal protein, which may have stimulated cell proliferation.

According to Maiorka *et al.* (2002), a trophic effect of amino acids on crypt depth can be considered. Trophic agents stimulate the development of the intestinal mucosa, that is, stimulate mitosis, and consequently, promote cell hyperplasia, resulting therefore in higher crypt and villi development. However, the higher villi in the duodenum, and the deeper crypts in the duodenum and jejunum at 21 days of age in birds fed 1.143% DL could be explained by the negative influence of amino acid unbalance on crypt development. This was demonstrated by Swatson *et al.* (2002), who studied the effects of protein level, amino acid balance, and feed intake on the structure of the intestinal mucosa of 10 to 24-day-old broilers. These authors observed higher crypt depth and villi height in birds fed balanced amino acid ratios as compared to unbalanced amino acid ratios.

According to Rostagno *et al.* (2005), broiler amino acid requirements are different as a function of age, and these requirements are higher in the first week of age. This indicates that amino acid unbalance can be higher or lower, depending on bird age, and consequently, nutritional requirements. Therefore, the supply of pre-starter feed containing 1.267% digestible lysine on ideal protein concept closely matched the birds requirements, whereas the maintenance of this level until broilers were 21 days of age may have contributed to amino acid unbalance.

Despite the influence of digestible lysine levels on crypt depth and villi height until 21 days of age, the supply of basal feed during the grower phase allowed the same intestinal development at 42 days of age (Table 6).

As previously shown, broilers fed 1.143% digestible lysine during the starter phase presented higher body weight and higher production index at the end of the rearing period. This results indicated that the optimization of the development pf the studied intestinal segments evaluated until 21 days of age have a significant influence on the productive parameters of broilers at market age. This is probably associated to the importance of nutrient absorption during this early period, in which broilers grow fast, and tissues and organs important for their development mature, thereby optimizing productive performance.

CONCLUSION

It was concluded that the supply of feed containing 1.143% digestible lysine, and based on ideal protein concept, until 21 days of age, improved both duodenal mucosa development until this age, and overall productive performance, independent from delayed placement.

On the other hand, delayed placement had a negative influence on broiler growth, with a 21-hour delayed placement promoting the highest development of the small intestine mucosa until 21 days of age, and the best productive performance until 42 days of age.

REFERENCES

Beçak W, Paulete J. Técnicas de citologia e histologia. Livros Técnicos e Científicos. Rio de Janeiro; 1976. 305p.

Boleli IC, Maiorka A, Macari M. Estrutura funcional do trato digestório. In: Macari M, editor. Fisiologia aviária aplicada a frangos de corte. Jaboticabal: FUNEP-UNESP; 2002. p.75-95.

Costa FGP, Rostagno HS, Albino LFT, Gomes PC, Toledo RS, Vargas Junior JG. Níveis dietéticos de proteína bruta para frangos de corte de 1 a 21 e 22 a 42 days of age. Revista Brasileira de Zootecnia 2001; 30(5):1498-1505.

Emmert JL, Backer DH. Use of the protein concept for precision formulation of amino acid levels in broiler diets. Journal Applied Poultry Research 1997; 6(4):462-470.

Gonzales E, Leandro NSM, Varoli Jr JC, Takita TS, Loddi MM. O tempo de jejum do neonato e a restrição alimentar precoce influenciando a produtividade de frangos de corte na idade de abate. Revista Brasileira e Ciência Avícola 2000; 2 supl. 2:4

Gonzales E, Kondo N, Saldanha ÉSPB, Loddi MM, Careghi C, Decuypere E. Performance and physiological parameters of broiler chickens subjected to fasting on the neonatal period. Poultry Science 2003; 82:1250–1256.

Halevy O, Geyra A, Barak M, Zehava U., David S. Early posthatch starvation decreases satellite cell proliferation and skeletal muscle growth in chicks. Journal of Nutrition 2000; 130:858-864.

Kidd MT, Kerr BJ, Halpin JP, Mcward GW, Quarles CL. Lysine levels in starter and grower-finisher diets affect broiler performance and cacass traits. Journal Applied Poultry Research 1998; 7:351-358.

Macari M, Maiorka A. Função gastrintestinal e seu impacto no rendimento avícola. In: Conferência Apinco de Ciência e Tecnologia Avícolas; 2000; Campinas, São Paulo. Brasil. Campinas: Fundação Apinco de Ciência e Tecnologia Avícolas; 2000. p.161-174.

Maiorka A, Boleli IC, Macari M. Desenvolvimento e reparo da mucosa intestinal. In: Macari M, editor. Fisiologia aviária aplicada a frangos de corte. 2.ed. Jaboticabal: FUNEP:FAPESP; 2002. p.113-123.

Mcbride BW, Kelly JM. Energy cost of absorption and metabolism in the ruminant portal-drained viscera and liver: a review. Journal of Animal Science 1990; 68:2997-3010.

Noy Y, Uni Z, Sklan D. Routes of yolk utilization in the newly-hatched chick. British Poultry Science 1996; 37:987-996.

Noy Y, Sklan D. Posthatch development in poultry. Journal Applied Poultry Research 1997; 6:344-354.

Noy Y, Sklan D. Metabolic responses to early nutrition. Journal Applied Poultry Research 1998; 7:437-451.

Rostagno HS, Albino LFT, Donzele JL, Gomes PG, Ferreira AS, Oliveira RF, Lopes DC. Tables brasileiras para aves e suínos; composição de alimentos e exigências nutricionais. 2 ed. Viçosa: Universidade Federal de Viçosa; 2000. 141p.

Rostagno HS, Albino LFT, Donzele JL, Gomes, PC, Oliveira RF, Lopes DC, Ferreira AS, Barreto SLT. Tables brasileiras para aves e suínos; composição de alimentos e exigências nutricionais. 3 ed. Viçosa: Universidade Federal de Viçosa; 2005. 186p.

Saeg - Sistema para Análises Estatísticas e Genéticas: versão 8.0. Viçosa: Fundação Arthur Bernardes. Central de Processamento de Dado; 1998. Apostila.

Shutte JB, Smink W, Pack M. Requirements of young broiler chicks for glycine plus serine. Archives Geflueglek 1997; 61:43-47.

Swatson HK, Gous R, Iji PA, Zarrinkalam R. Effect of dietary protein level, amino acid balance and feeding level on growth, gastrointestinal tract, and mucosal structure of the small intestine in broiler chickens. Animal Research 2002; 51:501–515.

Uni Z, Ganot S, Sklan D. Posthatch development of mucosal function in the broiler small intestine. Poultry Science 1998a; 77:75-82.

Uni Z, Platin R, Sklan D. Cell proliferation in chicken intestinal epithelium occurs both in the crypt and along the villus. Comparative Physiology. B. 1998b; 168(4):241-247.

Yamauchi K, Kamisoyama H, Isshiki Y. Effects of fasting and refeeding on structure of the intestinal villi and epithelial cells in white leghorn hens. British Poultry Science 1996; 37:909-921.