ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

# POULTRY SCIENCE

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International Journal of Poultry Science 8 (10): 946-951, 2009
ISSN 1682-8356


# Protein and Amino Acid Supplementation Levels for Broilers in Pre-Starter Ration

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**Abstract:** Performance, ether extract, dry matter and protein digestibility and enzymatic activities of broilers fed different Crude Protein (CP) and amino acid levels in pre-starter ration were evaluated. 560 MPK male day-old chicks were raised until 21 days of age in heated brooders and allotted in a complete randomized design with eight treatments and five replicates each: A. 20% CP non-supplemented; B. 20% CP supplemented with Met, Lys and Thr; C. 20% CP supplemented with Met, Lys, Thr and Trp for 22% CP; D. 20% CP supplemented with Met, Lys, Thr and Trp for 24% CP; E. 22% CP non-supplemented; F. 22% CP supplemented with Met, Lys and Thr; G. 22% CP supplemented with Met, Lys, Thr and Trp for 24% CP; H. 22% CP supplemented with Met, Lys, Thr and Trp for 26% CP: All diets attended amino acid requirements of Rostagno *et al.* (2000) for corn-soybean rations with 2.900 Kcal/kg ME from 1-7 days. From 8-21 days, broilers received the same starter ration with 21.1% CP and 2900 Kcal/kg ME. The weight gain, feed intake and feed-to-gain ratio for total period and digestibility index and retention for Dry Matter (DM), CP (CP) and Ether Extract (EE) and digestibility parameters were measured from 4-7 days. In the $4^{th}$, $7^{th}$, $10^{th}$ and $14^{th}$ days of age, the pancreas of four birds was collected to amylase and tripsin analysis. The means were calculated and the Tukey test ($p<0.05$) was used to compare treatments. Low protein diets supplemented to attend 22 or 24% CP amino acid levels, were indicated for better results in performance, nutrient digestibility and retention. Amylase and Trypsin activities were affected with amino acid supplementation and reduced with birds age.

**Key words:** Enzyme activity, nutrient digestibility and retention, performance

## INTRODUCTION

The reduction of protein level is an economic and metabolic advantage to broilers (Deschepper and De Groote, 1995) and the 4-ration feeding system is becoming important to broiler industry in Brazil (Penz and Vieira, 1998). White Leghorn males fed ad libitum early in the pos-hatching period enhanced intestinal function because of the development of mucosa (Baranyiová and Holman, 1976). The enterocyte development in broilers can be enhanced by the presence of feed and the digestion process can mature earlier (Moran, 1985, Uni *et al*., 1998).

The perfect function of enzymes provides adequate digestion and as the villous and crypt cells become mature, the absorption occurs in an amount that permits an efficient utilization of nutrients. High levels of protein increased trypsin and chymotrypsin concentration (Austic, 1985). The maximum enzyme activity occurred at eight days of age for amylase and lipase and at 11 days for trypsin in pancreas and at 11 for lipase and 17 days for trypsin and amylase in intestine (Nitsan *et al*., 1991). This digestive adaptation requires a specific diet for the first seven or ten days after hatch. The 48 h feed restriction after hatch reduced enzyme activity in chicks (Sklan and Noy, 2000) and this is correlated to chick live weight and intestinal weight and resulted in lower nutrient availability.

The fat absorption is low in the first week because of bile salts low recovering taxes (immature enterohepatic cycle) (Maiorka, 2002). Amino acid supplementation can enhance performance until 21 days of age (Tafuri *et al*., 1984; Edmonds *et al*., 1985). It is important to observe the balance between essential and non-essential amino acids to guarantee adequate performance and nitrogen balance and best use of ingested protein (Deschepper and De Groote, 1995). The levels of protein between 22 and 24% seems adequate to pre-starter rations (Rocha *et al*., 2003; Stringhini *et al*., 2001) when fed from 1-7 days os age (Araújo *et al*., 1999, Stringhini *et al*., 2003). Then, this experiment was carried out to evaluate performance, ether extract, dry matter and protein digestibility, biometrical measures and enzymatic activities of broilers fed different crude protein levels supplemented with amino acid from 1-7 days.

## MATERIALS AND METHODS

This experiment was carried out in the experimental research station located at the Federal University of Goiás, Goiânia, Brazil during September and October 2001. In heated brooders, 560 MPK male day-old chicks were raised until 21 days of age. The brooders were equipped with feeders and waterers in a shape of 0.33 x 0.50 m in a total of 40 experimental units. A complete randomized design was used, with eight treatments and

five replicates each and the treatments were: A. Basal ration with 20% crude protein, non-supplemented; B. Basal ration with 20% crude protein, supplemented with methionine, lysine and threonine to attend the Brazilian Tables requirements (Rostagno *et al*., 2000); C Basal ration with 20% crude protein supplemented with methionine, lysine, threonine and thryptophan to attend the amino acid requirements for 22% CP ration using Brazilian Tables (Rostagno *et al*., 2000) recommendations; D. Basal ration with 20% crude protein supplemented with methionine, lysine, threonine and thryptophan to attend the amino acid requirements for 24% CP ration using Brazilian Tables (Rostagno *et al*., 2000) recommendations; E. Basal ration with 22% crude protein non-supplemented; F. Basal ration with 22% crude protein supplemented with methionine, lysine and threonine to attend the requirements proposed by Brazilian Tables (Rostagno *et al*., 2000); G. Basal ration with 22% crude protein supplemented with methionine, lysine, threonine and thryptophan to attend the amino acid requirements for 24% CP ration using Brazilian Tables (Rostagno *et al*., 2000) recommendations; H. Basal ration with 22% crude protein supplemented with methionine, lysine, threonine and thryptophan to attend the amino acid requirements for 26% CP ration using Brazilian Tables (Rostagno *et al*., 2000) recommendations.

The corn-soybean based rations (Table 1) were formulated with 2.900 Kcal/kg of Metabolizable Energy (ME) and birds were fed from 1-7 days. After 8 days, the broilers received the same starter ration with 21,1% CP and 2900 Kcal/kg of ME, until 21 days of age as proposed by Brazilian Tables (Rostagno *et al*., 2000).

In the experiment, the performance were analyzed at 1$^{st}$, 4$^{th}$, 7$^{th}$, 10$^{th}$, 14$^{th}$, 18$^{th}$ and 21$^{st}$ days of age, measuring the daily mortality and died birds weight and then weight gain, feed intake and feed-to-gain ratio were calculated. The digestibility parameters were measured from 4-7 days in a total excreta collection method and the digestibility index were calculated after laboratory analysis for Dry Matter (DM), Crude Protein (CP) and Ether Extract (EE) contents of rations and excreta samples (Silva and Queiroz, 2002) and retention of DM, CP and EE, calculated as a ratio of total intake of DM, CP and EE and weight gain from 4-7 days.

In the 4th, 7th, 10th and 14th days of age, the pancreas of four birds were collected to amylase and trypsin analysis in the Laboratory of Enzyme Technology of the Biological Institute of the Federal University of Goiás. Pancreas after defrosting was homogenized with buffer Tris-HCl 50 mM, pH 8.0, containing $CaCl_2$ 50 mM, in proportion of 1:20 (weight:volume). The crude extract was centrifuged at 14,000 g for 30 min under refrigeration at 4°C, filtered, sampled and stored at-20°C to determine amylase and trypsin activity according to Lima *et al*. (2003). The trypsin activity was analyzed by absorbency and each enzyme unit was considered the amount to increase absorbency from 0.1 at 280 nm (Kunitz, 1947). The amylase activity was measured by the amount of enzyme that is necessary to hydrolyze 0,1 mg of starch in a minute, as proposed by Fuwa (1954). The protein concentration of the pancreas was determined using bovine serum albumin (BSA-Sigma) following Bradford (1976) methodology. The means were calculated and the Tukey test ($p<0.05$) was used to compare treatments.

## RESULTS AND DISCUSSION

For mean weight gain, statistical difference ($p<0.05$) was observed from 1-10 days of age and the best results were observed for 22% CP supplemented with methionine and lysine (Table 2). In literature, lysine levels in starter ration affected weight gain and feed-to-gain ration (Noy and Sklan, 2002 and Kidd *et al*., 1998) and the best results were obtained to higher levels of lysine used (115 % of NRC (1994) requirements). But in experiments conducted with lysine levels 5% below and 5, 10 and 15% above Brazilian Tables recommendations (Rostagno *et al*., 2000), no effect in broiler performance in the starter phase (1-21 days) was observed (Vasconcelos *et al*., 1993).

In this experiment, for 1-14 days and 1-21 days of age, feed intake increased as the level of protein and amino acid supplementation increased (Table 3).

Nutrition in the first week must consider the vitelin sac and the feed utilization ability (Noy and Sklan, 2002). But, a little is known about feed regulation in the post-hatch period. When fat (3, 7 e 11%) and protein (18, 23 e 28%) levels were evaluated in the first week, feed efficiency increased with the protein levels and the feed intake was depressed for high protein and constant amino acid levels. In this experiment, feed-to-gain ratio (Table 4) was better ($p<0.05$) from 1-7 days of age as the supplementation increased, on both protein levels, maybe reflecting the best amino acid balance in rations to this age. Similar results for performance were obtained testing reduced protein levels in ration (20%) with supplementation of essencial amino acids (methionine, lysine, threonine, triptophan and arginine) and best results for performance for higher relations between sulfur amino acids and lysine (100 Lys:76 Met+Cys) (Stringhini *et al*., 2001).

The digestibility of dry matter and ether extract was not affected by treatments, but for protein, as the level of supplementation increased the values were higher ($p>0.05$) in the same protein level (Table 5). Despite of not being observed any statistical difference, the ether extract values increased in numeric values, maybe related to the inefficiency to recover the bile salts (Serafim and Nesheim, 1970), but its efficiency decreased as the supplementation levels were higher (Cançado and Baião, 2002).

Table 1: Ration composition and nutritional values for pre-starter and starter experimental rations

| | Pre starter (1-7d) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Ingredients | A | B | C | D | E | F | G | H | Starter 8-21d |
| Corn | 59.18 | 58.99 | 58.99 | 58.98 | 55.79 | 55.50 | 55.67 | 55.72 | 58.74 |
| Soybean meal | 31.87 | 31.40 | 31.27 | 30.90 | 37.50 | 37.22 | 36.58 | 35.89 | 37.10 |
| Dic. Phosphate | 1.921 | 1.926 | 1.928 | 1.931 | 1.883 | 1.887 | 1.892 | 1.899 | 1.97 |
| Limestone | 1.089 | 1.090 | 1.091 | 1.092 | 1.061 | 1.061 | 1.064 | 1.066 | 0.952 |
| DL-Met 99 | - | 0.295 | 0.297 | 0.302 | - | 0.228 | 0.236 | 0.245 | 0.092 |
| L-Lys HCl | - | 0.332 | 0.338 | 0.406 | - | 0.128 | 0.205 | 0.421 | - |
| L-Threonine | - | 0.058 | 0.096 | 0.184 | - | - | 0.092 | 0.184 | - |
| L-Thryptophan | - | - | 0.037 | 0.074 | - | - | 0.037 | 0.077 | 0.272 |
| Common Salt | 0.459 | 0.461 | 0.461 | 0.462 | 0.448 | 0.449 | 0.450 | 0.452 | 0.40 |
| Vitamin Suppl.[1] | 0.400 | 0.400 | 0.400 | 0.400 | 0.400 | 0.400 | 0.400 | 0.400 | |
| Mineral Suppl.[1] | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 | 0.476 |
| Vegetal oil | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | - |
| Inert [2] | 3.703 | 3.987 | 4.053 | 4.196 | 1.859 | 2.077 | 2.311 | 2.586 | |
| Total | 100.0 | 100.0 | 100.00 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| Calculated nutritional values | | | | | | | | | |
| CP (%) | 20.00 | 20.00 | 20.00 | 20.00 | 22.00 | 22.10 | 22.00 | 22.00 | 21.10 |
| ME (Kcal/kg) | 2850 | 2850 | 2850 | 2850 | 2850 | 2850 | 2850 | 2850 | 2900 |
| Linoleic acid (%) | 1.749 | 1.743 | 1.743 | 1.741 | 1.709 | 1.703 | 1.703 | 2.482 | 2.696 |
| Lysine (%) | 1.067 | 1.307 | 1.307 | 1.350 | 1.216 | 1.307 | 1.350 | 1.653 | 1.200 |
| Met + Cys (%) | 0.641 | 0.926 | 0.926 | 0.926 | 0.705 | 0.926 | 0.926 | 0.878 | 0.794 |
| Threonine (%) | 0.775 | 0.823 | 0.858 | 0.939 | 0.865 | 0.859 | 0.939 | 1.018 | 0.860 |
| Tryptophan (%) | 0.267 | 0.264 | 0.300 | 0.334 | 0.303 | 0.301 | 0.334 | 0.368 | 0.296 |
| Calcium (%) | 0.988 | 0.988 | 0.988 | 0.988 | 0.988 | 0.988 | 0.988 | 0.988 | 0.966 |
| Available P (%) | 0.466 | 0.466 | 0.466 | 0.466 | 0.466 | 0.466 | 0.466 | 0.466 | 0.484 |
| Sodium (%) | 0.224 | 0.224 | 0.224 | 0.224 | 0.224 | 0.224 | 0.224 | 0.224 | 0.154 |

[1]Vitamin and Mineral Supplement mixtures made by Nutron Alimentos, Campinas, SP, Brazil, [2] Inert: ground rice hulls

Table 2: Mean weight gain for broilers fed pre-starter rations (1-7 days) containing different levels of crude protein and amino acid supplementation

| | Period | | | | |
|---|---|---|---|---|---|
| Treatments | 1-7 | 1-10 | 1-14 | 1-18 | 1-21 |
| 20% CP non-supplemented | 70.7 | 174.4$^{ab}$ | 262.6 | 389.1 | 495.6 |
| 20% CP +Met | 81.5 | 183.1$^{ab}$ | 262.8 | 390.9 | 505.5 |
| 20% CP+Met+Lys+Thr to 22% CP | 73.3 | 158.4$^{b}$ | 232.8 | 348.7 | 427.8 |
| 20% CP+Met+Lys+Thr+Trp to 24% CP | 84.1 | 181.5$^{ab}$ | 269.1 | 401.6 | 500.3 |
| 22% CP non-supplemented | 76.8 | 181.6$^{ab}$ | 269.1 | 411.2 | 519.8 |
| 22% CP +Met | 85.9 | 192.2$^{a}$ | 279.2 | 403.7 | 514.1 |
| 22% CP +Met+Lys+Thr+Trp to 24% CP | 80.9 | 180.6$^{ab}$ | 261.1 | 362.2 | 481.9 |
| 22% CP +Met+Lys+Thr+Trp to 26% CP | 85.1 | 183.2$^{ab}$ | 270.9 | 403.6 | 515.8 |
| CV (%) | 10.30 | 9.07 | 8.65 | 8.53 | 9.04 |

Letters followed by different letters indicates statistical difference (Tukey, 5%), CV = Coefficient of variation

Table 3: Mean feed intake for broilers fed pre-starter rations (1-7 days) containing different levels of crude protein and amino acid supplementation

| | Period | | | | |
|---|---|---|---|---|---|
| Treatments | 1-7 | 1-10 | 1-14 | 1-18 | 1-21 |
| 20% CP non-supplemented | 92.1 | 222.2 | 285.8$^{ab}$ | 484.5 | 670.0$^{ab}$ |
| 20% CP +Met | 96.3 | 230.8 | 244.1$^{b}$ | 451.8 | 649.9$^{ab}$ |
| 20% CP+Met+Lys+Thr to 22% CP | 78.0 | 194.9 | 272.1$^{ab}$ | 454.5 | 568.8$^{b}$ |
| 20% CP+Met+Lys+Thr+Trp to 24% CP | 91.7 | 220.1 | 268.2$^{ab}$ | 467.7 | 669.5$^{ab}$ |
| 22% CP non-supplemented | 91.2 | 222.5 | 270.8$^{ab}$ | 483.8 | 706.1$^{ab}$ |
| 22% CP +Met | 91.8 | 229.3 | 279.2$^{ab}$ | 491.6 | 719.3$^{ab}$ |
| 22% CP +Met+Lys+Thr+Trp to 24% CP | 90.3 | 220.7 | 326.4$^{a}$ | 528.0 | 736.1$^{a}$ |
| 22% CP +Met+Lys+Thr+Trp to 26% CP | 89.5 | 217.3 | 330.3$^{a}$ | 543.6 | 782.3a |
| CV (%) | 11.45 | 8.71 | 13.55 | 10.36 | 9.93 |

Letters followed by different letters indicates statistical difference (Tukey, 5%), CV = Coefficient of variation

Table 4: Mean feed-to-gain ratio for broilers fed pre-starter rations (1-7 days) containing different levels of crude protein and amino acid supplementation

| | Period | | | | |
|---|---|---|---|---|---|
| Treatments | 1-7 | 1-10 | 1-14 | 1-18 | 1-21 |
| 20% CP non-supplemented | 1.321$^{a}$ | 1.309 | 1.167 | 1.285 | 1.408 |
| 20% CP +Met | 1.204$^{ab}$ | 1.334 | 1.001 | 1.215 | 1.356 |
| 20% CP+Met+Lys+Thr to 22% CP | 1.102$^{b}$ | 1.278 | 1.253 | 1.360 | 1.416 |
| 20% CP+Met+Lys+Thr+Trp to 24% CP | 1.106$^{b}$ | 1.275 | 1.071 | 1.224 | 1.408 |
| 22% CP non-supplemented | 1.242$^{ab}$ | 1.298 | 1.102 | 1.228 | 1.425 |
| 22% CP +Met | 1.123$^{b}$ | 1.266 | 1.132 | 1.268 | 1.460 |
| 22% CP +Met+Lys+Thr+Trp to 24% CP | 1.145$^{b}$ | 1.265 | 1.349 | 1.464 | 1.628 |
| 22% CP +Met+Lys+Thr+Trp to 26% CP | 1.103$^{b}$ | 1.267 | 1.336 | 1.423 | 1.605 |
| CV (%) | 5.97 | 4.02 | 10.20 | 4.68 | 5.59 |

Letters followed by different letters indicates statistical difference (Tukey, 5%), CV = Coefficient of variation

Table 5: Mean digestibility percentages and retention for dry matter (MS), crude protein (%) and Ether Extract (EE), per gram of weight gain, for broilers fed pre-starter rations (1-7 days) containing different levels of crude protein and amino acid supplementation

| | Digestibility (%) | | | Retention (g/g weight gain) | | |
|---|---|---|---|---|---|---|
| Treatments | DM | EE | CP | DM | EE | CP |
| 20% CP non-supplemented | 66.4 | 64.6 | 49.2$^{c}$ | 946.6 | 69.4$^{a}$ | 22.5 |
| 20% CP +Met | 68.4 | 66.9 | 59.7$^{a}$ | 871.7 | 57.5$^{b}$ | 24.8 |
| 20% CP+Met+Lys+Thr to 22% CP | 69.0 | 83.0 | 60.6$^{a}$ | 822.8 | 57.6$^{b}$ | 24.1 |
| 20% CP+Met+Lys+Thr+Trp to 24% CP | 65.3 | 75.9 | 57.3$^{a}$ | 822.5 | 44.7$^{c}$ | 22.1 |
| 22% CP non-supplemented | 66.6 | 82.9 | 53.5$^{bc}$ | 839.4 | 58.6$^{b}$ | 22.7 |
| 22% CP +Met | 66.6 | 79.8 | 58.6$^{a}$ | 820.1 | 42.4$^{c}$ | 26.0 |
| 22% CP +Met+Lys+Thr+Trp to 24% CP | 68.1 | 80.4 | 58.2$^{ab}$ | 830.9 | 49.4$^{bc}$ | 25.9 |
| 22% CP +Met+Lys+Thr+Trp to 26% CP | 70.0 | 82.1 | 58.8$^{ab}$ | 840.0 | 46.7$^{c}$ | 24.5 |
| CV (%) | 4.66 | 22.9 | 4.06 | 8.48 | 8.41 | 7.29 |

Letters followed by different letters indicates statistical difference (Tukey, 5%), CV = Coefficient of variation

Table 6: Mean amylase and trypsin activity for broilers, in different ages, fed pre-starter rations (1-7 days) containing different levels of crude protein and amino acid supplementation

| | Amylase, Unit per mg of protein | | | | Trypsin, Unit per mg of protein | | | |
|---|---|---|---|---|---|---|---|---|
| | Age, days | | | | | | | |
| Treatments | 4 | 7 | 10 | 14 | 4 | 7 | 10 | 14 |
| 20% CP non-supplemented | 21.1$^{b}$ | 23.5$^{a}$ | 7.6$^{bc}$ | 12.2$^{a}$ | 25.8$^{a}$ | 10.2$^{a}$ | 3.0$^{a}$ | 1.8$^{b}$ |
| 20% CP +Met | 18.9$^{b}$ | 9.1$^{bc}$ | 12.0$^{a}$ | 5.9$^{cd}$ | 22.6$^{b}$ | 4.2$^{b}$ | 1.4$^{b}$ | 2.4$^{a}$ |
| 20% CP+Met+Lys+Thr to 22%CP | 27.1$^{a}$ | 9.1$^{c}$ | 10.3$^{ab}$ | 4.0$^{d}$ | 12.3$^{c}$ | 1.4$^{c}$ | 2.1$^{bc}$ | 1.3$^{d}$ |
| 20% CP+Met+Lys+Thr+Trp to 24% CP | 26.4$^{a}$ | 12.0$^{b}$ | 9.2$^{abc}$ | 6.6$^{cd}$ | 6.9$^{d}$ | 1.9$^{d}$ | 1.0$^{e}$ | 1.7$^{c}$ |
| 22% CP non-supplemented | 19.7$^{b}$ | 11.6$^{bc}$ | 8.2$^{bc}$ | 10.7$^{ab}$ | 6.3$^{de}$ | 2.2$^{d}$ | 1.6$^{cd}$ | 1.0$^{e}$ |
| 22% CP +Met | 21.4$^{b}$ | 11.9$^{bc}$ | 9.7$^{abc}$ | 5.1$^{cd}$ | 5.6$^{e}$ | 3.6$^{d}$ | 1.1$^{e}$ | 1.7$^{c}$ |
| 22% CP +Met+Lys+Thr+Trp to 24% CP | 29.5$^{a}$ | 10.0$^{bc}$ | 11.5$^{a}$ | 8.5$^{bc}$ | 4.6$^{f}$ | 1.8$^{de}$ | 1.8$^{bc}$ | 1.3$^{d}$ |
| 22% CP +Met+Lys+Thr+Trp to 26% CP | 27.7$^{a}$ | 10.5$^{bc}$ | 7.3$^{c}$ | 7.4$^{bcd}$ | 4.1$^{f}$ | 2.3$^{e}$ | 1.2$^{e}$ | 1.4$^{d}$ |
| CV (%) | 5.13 | 8.42 | 10.85 | 14.63 | 2.93 | 4.96 | 9.49 | 2.40 |

Letters followed by different letters indicates statistical difference (Tukey, 5%), CV = Coefficient of variation

This is in agreement with literature (Deschepper and De Groote, 1995) when fed rations containing 21% CP from 1-21 days, supplemented with essential amino acids, best performance and protein retention were observed. The reduction in ability for digestion and absorption of some nutrients may be explained by the fact that intestine mucosa in not complete mature during the first 7 days (Batal and Parsons, 2002).

The enzyme activities were affected by treatments in all ages studied (Table 6). For amylase, the higher activity was obtained at four days of age ($p<0.01$). This was observed at 7 days and the higher activity was obtained with the low protein level and non-supplemented group. This can be related to the lower level related to requirement suggested by Brazilian Tables (Rostagno *et al*., 2000) for the first week of age (21.9%PB).

Few experiments focused the enzyme activity and nutrition in pre-starter phase. High protein levels didn't affect amylase and trypsin activity in the starter phase (1 -21 days of age) (Macari *et al*., 2002), but when the amount of ration ingested in relation to the enzyme activity was analyzed, higher activities for tripsin and amylase were observed (Noy and Sklan, 1997), fact that helps to justify the data obtained in this experiment.

When a 48 h fasting period is applied, the activity of these enzymes were reduced (Noy and Sklan, 2002).

Sakomura *et al.* (2004), studying the effect of broiler age on enzimatic activity and nutrient digestibility of soybean meal and full-fat soybean, observed that amylase and pancreatic trypsin increased linearly with broiler age and the same occured with pancreas development. But, in this research, maybe because of the focus on the protein and amino acid supplementation, the effect was the opposite, the enzyme activities increased in the first days of age, reducing until 14 days of age. Minafra (2007) evaluated the activity of pancreatic amylase and observed reduction from seven to 21 days of age, supplementing ?-amylase obtained from *Cryptococcus flavus* and *Aspergillus niger* HM2003 in pre-starter and starter rations.

Monteiro *et al.* (2006) evaluated the pancreatic amylase of broilers and the effect of electrolyte balance and protein level in diet and determined that birds fed 20% crude protein showed enzymatic activities (U/mg of protein) higher than 23% CP diets, which reflects the same effect obtained in this experiment.

**Conclusion:** Low protein diets supplemented to attend 22 or 24%PB amino acid levels, were indicated for better results obtained in performance, nutrient digestibility, nutrient retention. Enzymatic activities of pancreatic amylase and tripsin were affected by protein levels and reduced with birds age. Ideal protein concept can be applied normally to pre-starter phase, but maturity of gastrointestinal tract must be considered.

## ACKNOWLEDGEMENT

The authors thanks to CNPq for financial support and Abatedouro Sao Salvador and Granja Planalto for feed and chicks, respectively.

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