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Activity of Digestive Enzymes in Chicken's Small Intestine and Caeca: Effect of Dietary Protein and Carbohydrate Content

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ABSTRACT

Digestion is a mediating factor between the animals and their environment, one of the variables related to the efficiency in extracting energy from nutrients is rate of hydrolysis. Phylogenetical and functional hypothesis has been proposed linking dietary flexibility and enzyme lability. Species belong to Parvclass Galloanserae, studied until now, did not modulate aminopeptidase-N activity but they did modulate disaccharidases activities. Additionally, peptide hydrolysis has been demonstrated in avian caeca, but not in chickens. Finally, dietary proteins are essential for chicken growth in the first stages of development, but little information is available in chickens beyond 42 days of life. Chickens beyond that age were fed for 15 days either a high protein (D_{HP} = 49.72% protein and 11.92% carbohydrates) or a high starch diet (D_{HS} = 52.82% carbohydrates and 10.49% protein). Aminopeptidase-N, maltase and sucrase, were assessed in chicken's small intestines and caeca. Body mass of D_{HP} birds was 37.5% higher than body mass of D_{HS} birds, at the end of the trial. Aminopeptidase-N and sucrase did not change, but maltase exhibited higher activity in D_{HS} than in D_{HP} birds. The lack of aminopeptidase-N modulation and its relatively high activity in caeca, together with a modulation of maltase, contribute and give apparent support to the functional hypothesis. Surprisingly, a high quantity of protein resulted important for growth in chickens after 42 days of life. Also it is important to notice that a casein diet has been demonstrated as a high digestible meal for chickens, so the last data may be of interest for poultry industry.

Key words: Chickens, caeca, dietary carbohydrates, dietary proteins, enzyme digestion, growth pattern, phenotypic flexibility

INTRODUCTION

Digestion is a mediating factor between the animals and their environment. The efficiency of the digestive system in extracting energy from nutrients is directly related to three variables: (1) Digesta retention time, (2) Rates of hydrolysis, fermentation and absorption and (3) Digestive tract surface area and volume. Enterocyte membrane bound enzymes hydrolyse the food molecules that

arrive to the small intestine (McWhorter *et al.*, 2009). Aminopeptidase-N (EC 3.4.11.2) also known as leucine-aminopeptidase and amino-oligopeptidase (Vonk and Western, 1984), is an intestinal dipeptidase that hydrolyses oligopeptides into amino acids and accounts for most peptidase activity of the brush border membrane (Maroux *et al.*, 1973). Carbohydrates, as maltose and small oligosaccharides are broken down to monosaccharides by enzyme complexes located in the brush border of intestinal cells, the maltase-glucoamylase (EC 3.2.1.20) and the sucrase-isomaltase (EC 3.2.1.48) (Noren *et al.*, 1986). In this study they will be called sucrase and maltase, respectively. Sucrase catalyzes sucrose hydrolysis into its constituent sugars. In addition, this enzyme complex catalyzes maltose hydrolysis, a disaccharide derived from the hydrolysis of starch and glycogen. Maltose is also hydrolyzed by a specific enzyme, maltase (Semenza and Auricchio, 1989; Martinez Del Rio, 1990; Martinez Del Rio *et al.*, 1995).

Intestinal disaccharidase activities have been studied in chickens fed diets with variable protein and carbohydrate contents and it has been found that the activities of these enzymes are modulated by the availability of their substrates (Siddons, 1972; Biviano *et al.*, 1993). As for peptide hydrolysis, we failed to find information for aminopeptidase-N activity in chickens beyond 42 days old.

An interesting pattern of plasticity of the intestinal enzyme activities in birds has emerged. Birds with vestigial caeca belonging to the Parvclass Passerae modulate the aminopeptidase-N activity, whereas disaccharidase activities remain constant and conversely, most bird species with functional caeca of the Parvclass Galloanserae did not exhibit an apparent flexibility of their aminopeptidase-N activity but they did modulate disaccharidases activities according to the amount of disaccharides present in the diet (Martinez Del Rio *et al.*, 1995; Afik *et al.*, 1995; Ciminari, 1997; Sabat *et al.*, 1998; Levey *et al.*, 1999; Caviedes-Vidal *et al.*, 2000; Ciminari *et al.*, 2003; Ciminari *et al.*, 2005; Foye and Black, 2006; Brzek *et al.*, 2010). To date, two hypotheses have been proposed to explain this intestinal modulation pattern (Caviedes-Vidal *et al.*, 2000). The first, conceived under a phylogenetical framework, proposes that differences in the modulation mechanisms of intestinal enzymes between Passerae and Galloanserae reflect differences in phylogenetical history. The second hypothesis, posed under a functional approach, proposes that birds having vestigial non-functional caeca modulate intestinal peptidases, while birds with developed functional caeca do not. Caviedes-Vidal *et al.* (2000) supported this last hypothesis arguing that a small intestinal escape of amino nitrogen, as peptides, to functional caeca could support microbial growth in these organs. In addition, the small intestine of birds with vestigial non-functional caeca has probably been selected to extract the maximum available amino nitrogen rather than excreting it as waste.

Several functions have been attributed to the avian caeca, including fiber digestion, recycling of urinary nitrogen, osmoregulation, water and solutes absorption, vitamin synthesis and protein digestion, but the quantitative significance of these functions is still unclear (McWhorter *et al.*, 2009; Svihus *et al.*, 2013). To our knowledge, peptide hydrolysis in the cecae has not yet been demonstrated for chickens, although the surface of the caeca has numerous villi (Strong *et al.*, 1989) and is similar to the jejunum surface (Ferrer *et al.*, 1991) as well as caecal active sugar and amino acid uptakes have been described in these birds (Obst and Diamond, 1989; Moreto *et al.*, 1991). For these reasons digestion and final nutrient extraction may occur in the caeca.

There are essential amino acids that are required for synthesis of indispensable proteins. Birds given diets supplemented with these amino acids have greatly increased the rate of protein synthesis and hence the rate of poultry growth and egg-laying. Experiments on chicken growth

have been carried out by feeding birds from 7-28 days old on diet with different protein content (from 0-70%) for 10 days. In these studies those birds fed on a high protein diet gained more weight than those on a low protein diet (Imondi and Bird, 1967; Davis and Austic, 1997; Rosebrough *et al*., 2002). In most of the studies related to the influence of the dietary protein content on the growth pattern and performed in chickens, they were used birds from hatch to four or six weeks old of age, very little information is available after that age. Only one study showed that chickens fed on a 52.5% carbohydrate-25% protein diet from day 1 to 73 grew up at higher rates and achieved higher masses than birds fed on a 76% protein-carbohydrate-free diet (Biviano *et al*., 1993).

Therefore, the goals of this study were: (1) To assess the modulation of the small intestine aminopeptidase-N, maltase and sucrase in chickens fed diets differing protein and carbohydrate content, (2) To evaluate the magnitude and importance of protein and carbohydrate breakdown activity in the caeca and (3) To investigate the growth pattern of chickens beyond 42 days of life [the common slaughter age of broiler chickens (Leterrier *et al*., 1998) and up to 57 days under the effect of a protein-rich diet.

Based on previous observations, we predicted that: (1) Chickens do not modulate the activity of aminopeptidase-N, but they will modulate maltase and sucrase activities when exposed to diets with an increase in their specific enzyme substrates, (2) The caeca would reached peptidase and carbohydrase activities founded in the small intestine distal section and (3) In chickens over 42 days of age, growth rate would not differ significantly between high-protein and high-starch diet.

MATERIALS AND METHODS

Animal care and housing: Thirteen hatch-day Cobbs chickens were obtained from Forrajera San Luis (San Luis, Argentina). The birds were housed alone in individual cages (0.50×0.30×0.35 m) in our animal room under constant environmental conditions (room temperature: 25.2±0.3°C, relative humidity: 50±9%, photoperiod: 14:10 h light: dark) with *ad libitum* water and food. A commercial diet was used (Ganave® Alimentos Pilar S.A., Argentina), with the following composition:

- Total protein: 20%
- Total fat: 4%
- Crude fiber: 3.9%

Animal maintenance, trial protocols and sample collection followed protocols reviewed by the Animal Care Committee of the Facultad de Química, Bioquímica y Farmacia of the Universidad Nacional de San Luis.

Diet acclimation: On day 42 chickens were randomly divided into two groups, six birds were fed with a 49.72% protein, 11.92% carbohydrate diet (D_{HP}) and seven birds with a 10.49% protein, 52.82% carbohydrate diet (D_{HS}). Casein and corn starch were used as sources of protein and carbohydrate, respectively. These semi-synthetic and isocaloric diets (ME D_{HP}=15.47 kJ g^{-1} and ME D_{HS}=16.03 kJ g^{-1}) were formulated according to Caviedes-Vidal *et al*. (2000), based on Murphy and King (1982) (Table 1). Food and water were offered *ad libitum* for 15 days and body mass was monitored.

Table 1: Composition of the semi-synthetic diets fed to chickens

	Diets	
Semi-synthetic diets	*Low protein (D_{HS})	High protein (D_{HP})
Chemical composition (% w/w)		
Proteins	10.49	49.72
Carbohydrates	52.82	11.92
Lipids	8.34	9.17
Components (% w/w)		
Casein[a]	12.3	60.3
Corn oil[b]	8	8
Corn starch[c]	62	14
Salt mixture[d]	5.5	5.5
Sodium bicarbonate	1	1
Choline chloride	0.2	0.2
Vitamin mixture[e]	1	1
Cellulose[f]	5	5
Ground silica sand	5	5
Totals	100	100
Metabolizable energy content (kJ g^{-1})[¥]	16.03	15.47

*Caviedes-Vidal *et al*. (2000), High starch diet modified from Murphy and King (1982). [¥]Estimation based on metabolizable energy prediction equations and data for poultry for foodstuffs (NRC, 1994) and values provided by diet component manufacturers. [a]Concentrado de proteínas de leche en polvo (Milkaut® S.A., Argentina), [b]Aceite Mazola® (Aceitera General Deheza S.A., Argentina) [c]Maizena® (Unilever S.A., Argentina), [d]Salt mixture Fox-Briggs (Fox and Briggs, 1960) and H_3BO_3 (9×10^{-4} g), $Na_2MoO_4.2H_2O$ (9×10^{-4} g), $CoSO_4.2H_2O$ (1×10^{-4} g), Na_2SeO_3 (2×10^{-5}), [e]Vitamin mixture AIN 76 and [f]Celufil-hydrolysed USB corp

Sample collection: Experiment was terminated when chickens were 57 days old. Briefly, birds were anesthetized using ethyl ether and the entire gastrointestinal tract was removed and chilled in ice-cold avian saline solution as done by Caviedes-Vidal and Karasov (1996). The small intestine was separated from the rest of the gastrointestinal tract and extraneous tissues were removed. The content was flushed out with cold avian saline and the intestine was measured for length and weighed. Immediately after, 10 cm-long segments from the proximal, medial and distal parts (relative to the pylorus) of the small intestine were cut. Pieces were weighed and rapidly frozen and stored at -140°C. The two caeca were processed exactly as the small intestines and were divided into three portions: the neck section (closest to the small intestine), the medium section and the end section (the fundus).

Sample preparation: Intestinal and caeca segments were thawed at 4°C and homogenized for 30 sec using a Fisher Scientific homogenizer in 350 mM mannitol in 1 mM Hepes/KOH (pH 7), using 10 mL g^{-1} tissue. The activity of membrane-bound enzymes was measured in whole tissue homogenates (Martinez Del Rio, 1990).

Enzyme assays

Disaccharidases assay for intestinal and caecal enzymes: Disaccharidase activities, maltase (E.C. 3.2.1.20) and sucrase (E.C. 3.2.1.48), were determined in the small intestine and caeca homogenates. The colorimetric method developed by Dahlqvist (1984) and modified by Martinez Del Rio (1990) was used. Briefly, aliquots of 40 μL of tissue homogenate appropriately diluted were incubated with 40 μL of 56 mM sugar (maltose or sucrose) solutions in 0.1 M maleate/NaOH pH 6.5. After a 10 min incubation at 40°C, there was added 1 mL of Glicemia

Enzimática study reagent (glucose-oxidase 1000 U mL, peroxidase 120 U mL, 26 mM L^{-1} 4-aminophenazone, 55 M L^{-1} phenol, 0.92 M L^{-} Tris buffer pH 7.4; Wiener® Laboratorios, Argentina). The mixture was allowed to stand at room temperature and after 20 min the absorbance was read at 505 nm in a Spectronic 21D spectrophotometer. Enzyme activity was determined using a glucose standard curve.

Aminopeptidase-N assay: Aminopeptidase-N (E.C. 3.4.11.2) was assayed using L-alanine-p-nitroanilide as a substrate (Maroux *et al.*, 1973). Aliquots of 10 µL of the tissue homogenate were added to 1 mL assay solution, made of 2.0 mM L-alanine-p-nitroanilide in 0.2 M phosphate buffer (NaH_2PO_4/Na_2HPO_4, pH 7). The reaction was incubated during 10 min at 40°C and then, stopped with 3 mL of chilled 2 M acetic acid. Absorbance was measured at 384 nm and activity was determined using a p-nitroanilide standard curve.

Protein measurement: The protein concentration in our samples was estimated using the commercial Wiener® Lab Proti 2 Assay (EDTA/Cu reagent; Wiener® Laboratorios, Argentina). Absorbance was read at 540 nm and the serum standard from the kit was used as standard.

Standardization: On the basis of absorbance standards constructed for glucose and p-nitroanilide, standardized intestinal activities were calculated. Enzyme activities were calculated as summed (total) hydrolysis activity (µmol min^{-1}), specific activity per unit intestinal (or caeca) wet mass (µmol min^{-1} g wet $tissue^{-1}$), specific activity per g of protein (µmol min^{-1} g $protein^{-1}$) and specific activity per nominal surface area (µmol min^{-1} cm^{-2}). Although we made all those calculations, we present the data in µmol min^{-1} g wet $tissue^{-1}$, because of their advantages in using the mass of the tissue instead of the amount of enzyme protein, that allows "scaling up" the measurements done in the test tube to the whole organ and hence estimating its capacity to perform a certain function (Martinez Del Rio, 1990; Karasov and Martinez Del Rio, 2007). The summed hydrolysis activity of the entire small intestine and caeca were calculated by multiplying activity per gram tissue in each region by 1/3 of the small intestine and caeca total mass and summed over the three regions.

Data analysis: Results are given as Means±SEM and n is the number of individuals (n = 6 for D_{HP} birds and n = 7 for D_{HS} birds). A standard least-square method was used to estimate parameters of linear regressions. ANOVA-RM was used to examine the effect of the diets and the gut regions on enzyme activities and a paired t-test was used to assess statistical differences for all other comparisons. Data were tested for normality (Kolmogorov-Smirnov test) and homogeneity of the variance (Levene test). When data did not meet these assumptions, a nonparametric test was used (Mann-Whitney U-test). Lengths, nominal surface areas, masses and summed enzymes activities of the small intestines and caeca were normalized by body mass to test the effect of animal size on these parameters. None of these parameters were correlated to body mass ($p>0.05$), probably because the range of body mass in each group was too small. Thus, absolute values were normalized by dividing them by the body mass. Significance level for all tests was set at $p<0.05$. Kinetic parameters were determined by fitting the kinetic data by non-linear curve fitting (Wilkinson, 1992) to the equation:

$$\text{Activity} = \frac{\text{Vmax} \times \text{concentration}}{\text{Km}^* + \text{concentration}}$$

RESULTS

Growth: All chickens followed the same growth trajectories during the first period (day 1-41) when fed on the same diet (commercial starter diet). However, when birds were separated into two groups and fed both experimental diets (D_{HP} and D_{HS}) for 15 days, growth rates differed. Birds fed on D_{HP} achieved higher mass on day 57 than those fed on D_{HS} [D_{HP}: 1764.83±81.61 g vs. D_{HS}: 1283.14±102.92 g; $F_{1,11}$ =12.280 $p<0.01$] (Fig. 1). Body growth was adequately described by logistic equations ($r^2>0.99$).

Gut morphology: Intestinal and caeca measurements (mass, length and nominal surface area), were significantly larger in birds fed on a D_{HP} diet than in birds fed on a D_{HS} diet.

As previously mentioned, birds on D_{HP} were heavier than those on D_{HS} Thus, to further investigate if the observed differences were due to a body size effect, body mass standardized values were contrasted. None of these standardized parameters exhibited a significant difference between diet groups (Fig. 2; intestine mass t=-0.957 p = 0.359, length t=-2.066, p = 0.063 and nominal surface area t=-1.722 p = 0.113; caeca mass t=-0.033, p = 0.974, length t=-1.157, p = 0.272 and nominal surface area t=0.0486, p = 0.962). This finding suggests that the differences of the absolute values are related to the animals' body size and not to a specific response of the small intestine and caeca to the diets.

Intestinal enzymes

Specific activity intra diet comparisons with positional effect: All three enzymes exhibited a significant positional effect along the small intestine (Fig. 3a-c). Maltase and sucrase medial small intestine activities were higher than proximal and distal activities, regardless of the diet (Table 2, Tukey HSD, $p<0.01$). Aminopeptidase-N medial activities were significantly higher than distal activities in both treatments (Tukey HSD, $p<0.05$) and proximal activity did not differ from the other two (Table 2). Interaction between diet and position was not significant for all enzymes ($p>0.5$).

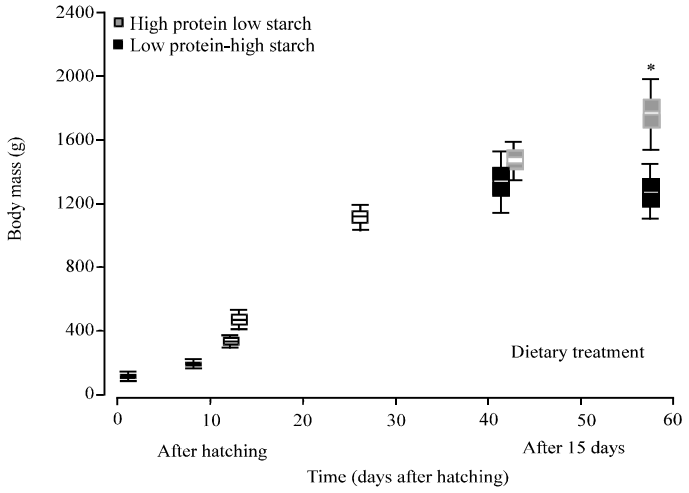

Fig. 1: Beginning on day 42 after hatch, chickens were fed on a high protein-low starch or a low protein-high starch diet for 15 days. *At the end of the experiment bird groups reached significant differences in body mass ($p<0.01$). Symbols represent Means±SEM

Table 2: Summary of repeated measure ANOVAs for enzyme activities in small intestine and caeca

		Enzyme activity					
		Maltase		Sucrase		Aminopeptidase-N	
Effect on organ	df*	F	p	F	p	F	p
Small intestine							
Diet	1,10	6.30	<0.05	2.60	0.14	0.34	0.57
Position	2,20	47.57	<0.001	71.29	<0.001	9.55	<0.01
Diet×position	2,20	0.50	0.61	0.23	0.79	0.37	0.69
Ceca							
Diet	1,23	0.63	0.44	1.95	0.18	0.06	0.80
Position	2,46	6.90	<0.01	5.59	<0.01	7.09	<0.01
Diet×position	2,46	0.97	0.39	0.07	0.93	0.34	0.72

*df: Degrees of freedom in the ANOVA

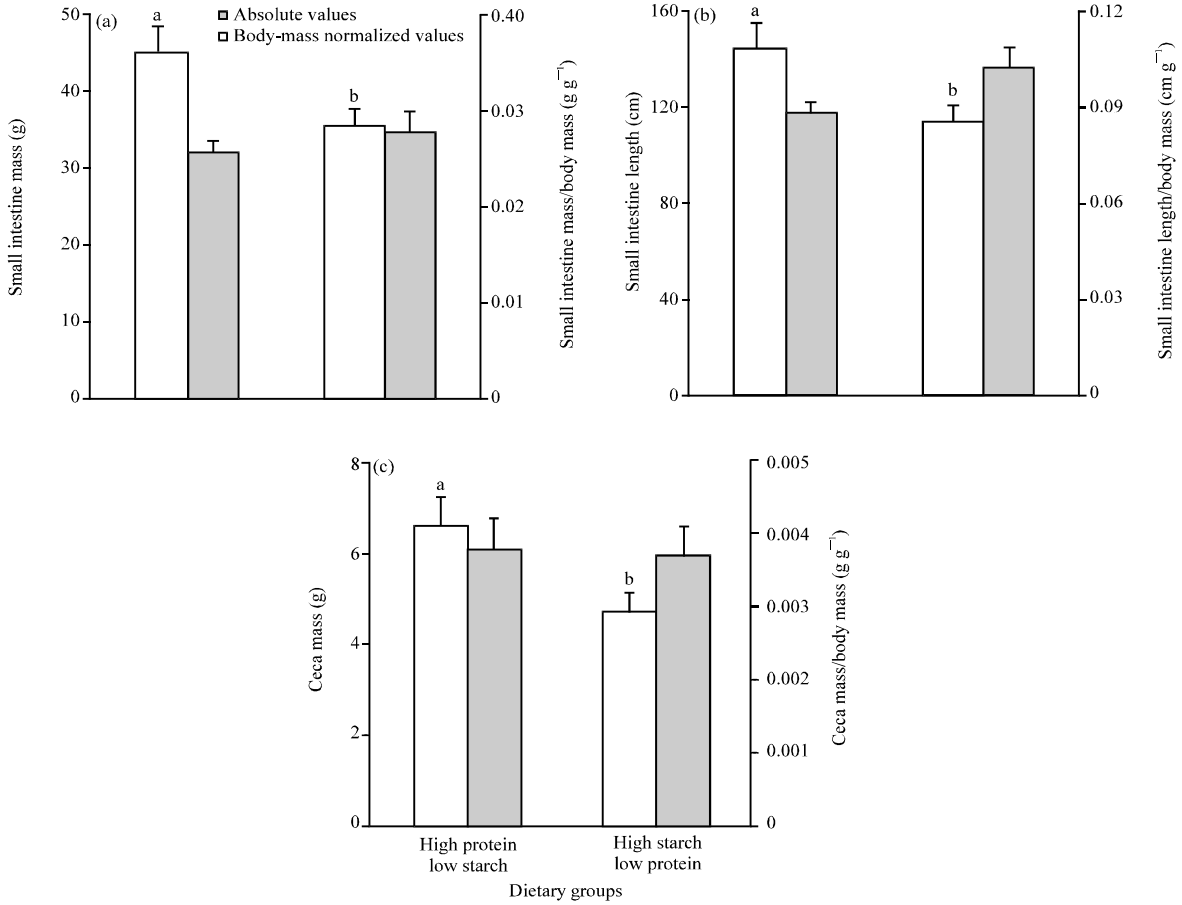

Fig. 2(a-c): Absolute and body mass-normalized values of chickens after 15 days of treatment with a high-protein-low starch or a low protein-high starch diets. Bars are Means±SEM. Dissimilar characters above error bars reflect means differing significantly ($p<0.05$)

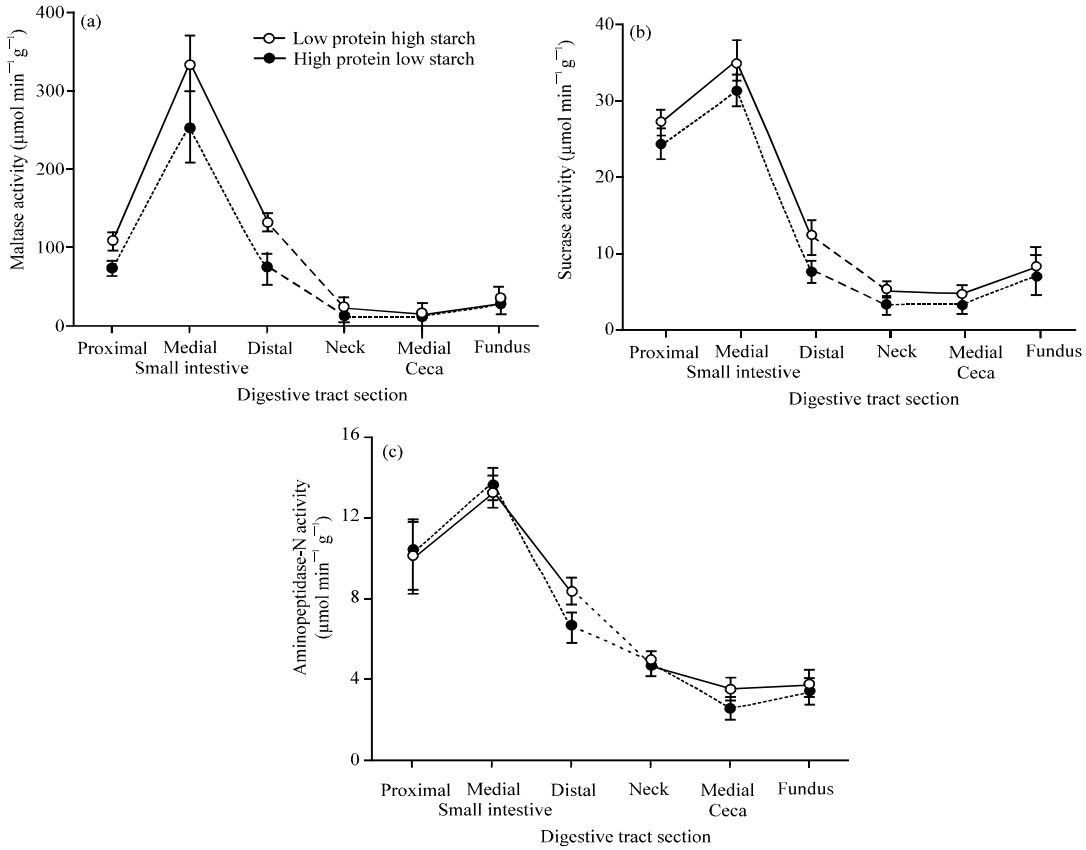

Fig. 3(a-c): Variation in brush border enzyme activities of (a) Maltase, (b) Sucrase and (c) Aminopeptidase-N as a function of the position along the small intestine and caeca in chickens after 15 days of treatment with a high-protein-low starch or a low protein-high starch diets. Statistical comparisons results are in Table 2

Inter diet comparisons with diet effect: The second prediction was held for maltase and aminopeptidase activities but not for sucrase at the level of specific activities. Maltase showed a dietary effect in the expected direction since chickens provided with D_{HS} had significantly greater activities of this enzyme when compared to birds fed on D_{HP} (Table 2, Fig. 3a). Significantly greater activity was only detected in the proximal (49%>) and distal (79.5%>) small intestine regions of D_{HS} birds ($D_{HS}>D_{HP}$, $p<0.05$) while in the medial region no differences were observed ($D_{HS}=D_{HP}$, $p=0.180$). On the contrary, sucrase did not show the expected differences between diets in any intestinal segment (Table 2, Fig. 3b). Aminopeptidase-N activity, as predicted, remained constant in chickens treated with both diets (Table 2, Fig. 3c).

Summed activity: Summed hydrolysis activities of the intestinal protease and carbohydrases exhibited a dissimilar response between dietary groups in this study. Aminopeptidase-N was significantly higher for D_{HP} than for D_{HS} birds ($t=2.847$ $p=0.0159$) (Fig. 4c). Conversely, no effect of summed hydrolysis rates of maltase ($t=-1.240$ $p=0.240$) and sucrase ($t=1.474$ $p=0.168$) was observed (Fig. 4a, b). However, these results may be due to a body size effect rather than to the outcome of a specific dietary substrate ingestion, since body mass normalized maltase summed hydrolysis rates were higher for chickens fed on D_{HS} than on D_{HP} ($t=-3.386$ $p=0.006$).

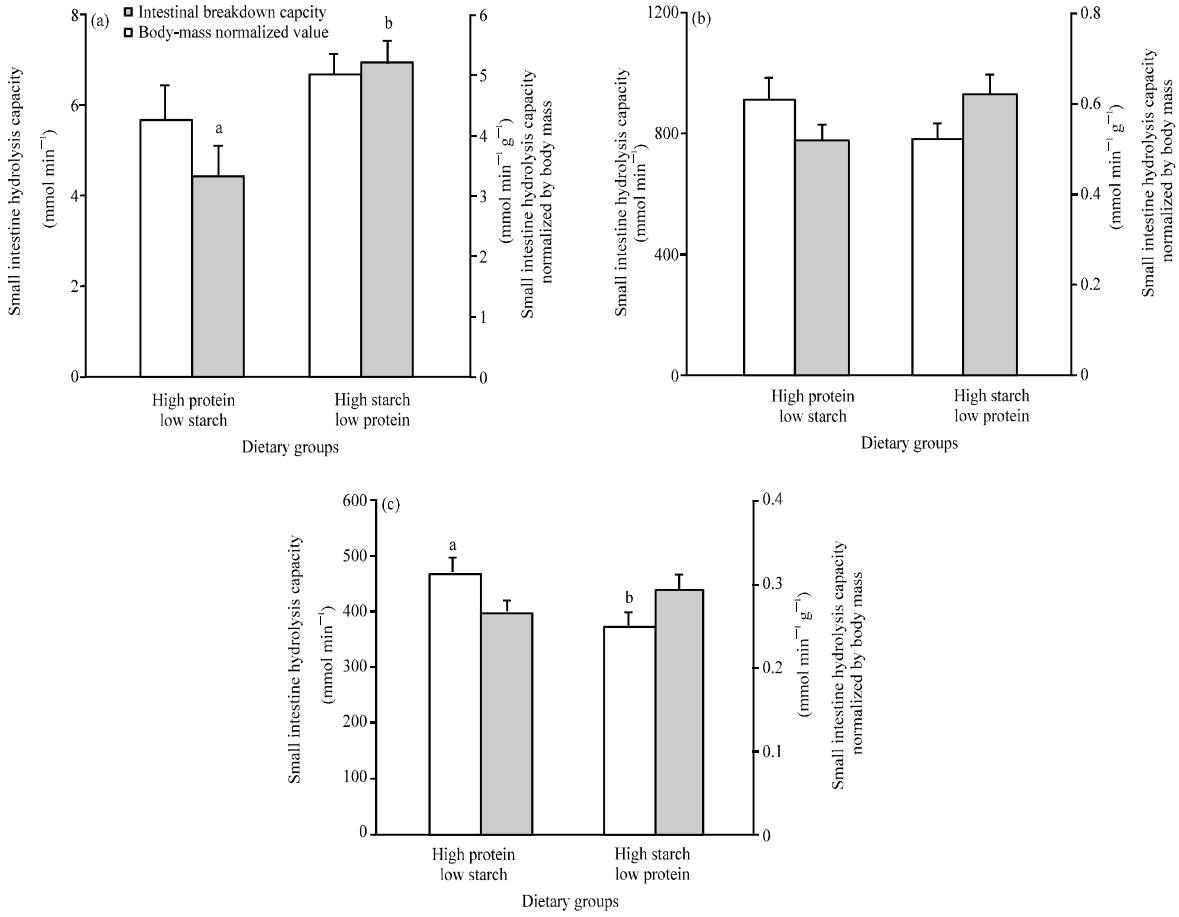

Fig. 4(a-c): Non-transformed values of summed intestinal breakdown capacity and normalized by body mass for (a) Maltase (b) Sucrase and (c) Aminopeptidase-N of chickens after 15 days of treatment with a high-protein-low starch or a low protein-high starch diet. Bars are Means±SEM. Dissimilar characters above error bars reflect means differing significantly ($p<0.05$)

No differences were observed for sucrase ($t=-1.720$ $p=0.113$) and aminopeptidase-N ($t=-1.186$ $p=0.261$) (Fig. 4b, c).

Protein content: The small intestine protein content did not differ significantly between diets in any of the small intestine regions and averaged 0.233±0.0138 g g^{-1} wet tissue. Intestinal protein content was not correlated with intestinal mass ($F_{1,32} = 4.019$ $p>0.05$).

Caecal enzymes

Specific activity intra diet comparisons with positional effect: There were significant differences among the activities of the different sections of the caeca for the three enzymes assayed (Table 2, Fig. 3a-c). Aminopeptidase-N for chickens on D_{HP} exhibited a higher activity in the neck than in the medial section and the fundus breakdown rate was not different from the other two caeca sections (Tukey HSD $p<0.05$). Whereas for D_{HS} chickens, no differences

Table 3: Small intestine, caeca and total hydrolysis capacities of aminopeptidase-N, sucrase and maltase of chickens treated either with a high protein-low starch (D_{HP}) or a low protein-high starch (D_{HS}) diets

	Aminopeptidase-N (mmol min^{-1})		Sucrase (mmol min^{-1})		Maltase (mmol min^{-1})	
Parameters	D_{HP}	D_{HS}	D_{HP}	D_{HS}	D_{HP}	D_{HS}
Small intestine	0.47	0.37	0.91	0.78	5.66	6.71
Caeca	0.022	0.019	0.029	0.029	0.11	0.11
Total hydrolysis capacity	0.49	0.39	0.944	0.81	5.77	6.81
Caeca contribution (%)	4.53	4.93	3.18	3.60	1.92	1.56

were observed along the different sections of the caeca for aminopeptidase-N activity. Maltase and sucrase activities of the D_{HS} dietary group were higher in the fundus than in the medial section and neck (Tukey HSD $p<0.05$) and no differences were detected for the D_{HP} group.

Giving support to the third prediction, the contrast of the different caeca and small intestine segments between dietary groups of the specific aminopeptidase-N activity revealed that the values obtained for the neck section were about half of the proximal and distal small intestine activities and around 30% of the medial intestine section hydrolysis rate (Fig. 2c). On the contrary, carbohydrases activities did not hold the prediction since activities in the caeca were much lower than in the small intestine: (1) The neck and medial caeca sections exhibited 10 times and 20 times lower maltase activity than in the proximal and medial region of the small intestine, respectively while in the fundus, maltase achieved half of the activity assayed in the small intestine distal section, (2) Sucrase activities of the neck and medial sections of the caeca were 10 times smaller than in the proximal and medial small intestine and, in the fundus, the activity reached similar values than in the distal small intestine (Fig. 3a, b).

Inter diet comparisons with diet effect: No differences were apparent in the activities of the three enzymes between the dietary groups (Fig. 3, Table 2).

Protein content: No differences were observed for summed hydrolysis rates between the dietary groups for maltase ($t=0.0629$, $p=0.951$), sucrase ($t=0.0566$, $p=0.958$) and aminopeptidase-N ($U=28.0$, $p=0.317$). However, as previously noted for the small intestine, this lack of difference between treatments may be due to a body size effect. Thus, body size normalized summed activity rates were tested and again we failed to find significant differences among the dietary groups (aminopeptidase-N, $U=14.0$ $p=0.317$; maltase, $U=13.0$ $p=0.253$; sucrase, $t=-0.807$ $p=0.436$). The caeca contribution to the total enzyme hydrolysis capacity is limited in both dietary groups (Table 3).

Summed activity: Caeca protein content did not change between diets in any of the caeca regions and the average was 0.239 ± 0.0102 g g^{-1} caeca. Caeca protein content was not significantly correlated with caeca mass ($F_{1,72} = 0.047$ $p=0.829$).

DISCUSSION

Growth: This is the first study where chickens raised on a high protein diet beyond 42 days of life, reached heavier body masses than birds fed on a high starch diet (Fig. 1). Nevertheless, our results suggest that only a high protein content in the diet is not enough to achieve an adequate

growth, dietary carbohydrates must be present in the diet too. Biviano *et al.* (1993) raised chickens on a carbohydrate-free diet (0%) and a high protein level (76.5%) and they gained smaller body masses than those fed on a high carbohydrate diet (52.5%) with a low content in proteins (25%). In another study chickens from 42-56 days old fed on diets with protein levels higher than those fed the control group generally did not increase final body weight but generally improved feed utilization and decreased carcass fat content (Cabel and Waldroup, 1991).

Gut morphology: Changes in the size of the gastrointestinal tract have been related to incremental feeding rates, cold exposure, low quality diet (high fiber) or reproduction (Karasov and Martinez Del Rio, 2007). In our study, relative small intestine and caeca masses, lengths and nominal surface areas of chickens were not affected by the high protein treatment or the high starch diet (Fig. 2a-c). In other words, the ability to process diets with dissimilar composition does not rely on morphological adjustments of the gut in chickens. Our birds compensated the lack of gut size variation when challenged with the contrasting diets, by displaying a high fixed aminopeptidase-N enzyme activity level and an adequate amount of flexibility of the tissue specific maltase activity.

Diet effect

Specific activities: Aminopeptidase-N wasn't assayed in chickens beyond 42 days old before and as for sucrase, they did not show significantly different levels between birds fed on the two diets (Fig. 3a-c). Maltase showed the expected positive correlation between dietary substrate and enzyme activity. These results support the prediction based on the observed pattern for most Galloanserae studied to date (except for domestic ducks and wild turkeys) that carbohydrase activity is modulated according to the level of carbohydrate available in the diet and that aminopeptidase-N remains constant. The ability to up-regulate an intestinal carbohydrase and the limited ability to up-regulate an intestinal protease is expected to occur in a domestic animal such as the chicken that has been exposed to high carbohydrate food for centuries. In addition, it is important to notice that our aminopeptidase-N activity values are much higher than those reported for bird species that modulate this enzyme (Sabat *et al.*, 1998; Levey *et al.*, 1999; Caviedes-Vidal *et al.*, 2000; Ciminari *et al.*, 2005). Therefore, these elevated aminopeptidase-N constitutive levels in chickens suggest that these birds have an adequate biochemical machinery to afford the digestion of protein-rich diets.

Sucrase activity was not affected by any of the diets (D_{HP} and D_{HS}). However, this result was expected given that sucrose may not be an important constituent of the diet in a granivorous bird. Similar results were obtained in Chickens (Siddons, 1972), Snow geese (Ciminari *et al.*, 1999) and Canada geese (Ciminari *et al.*, 1998).

Summed hydrolysis rates: Modulation of the hydrolytic and transport activity of the intestine's brush border may involve changes in absorption and hydrolysis rates due to altered densities of transporters and hydrolases per unit intestinal mass and/area ("specific modulation") or, may involve changes in intestinal mass and/or area ("non-specific modulation", Karasov and Diamond, 1983). We only found specific modulation in one of the intestinal enzyme activity studied in maltase and we didn't find changes in small intestine lengths, nominal surface area and masses, consequently we only found non-specific modulation in maltase (Fig. 4b-c).

Caeca enzyme activities

Contribution of the caeca to the total hydrolysis rate of peptides and carbohydrates: Results show the importance of the caeca in recovering the peptides and less the carbohydrates, who reached to this organ and may be broken down by the animal membrane bound enzymes, aminopeptidase-N and the disaccharidases maltase and sucrase. Besides, even though the contribution of the caeca proteolytic capacity to the total protein degradation is scant (4.5-5%, Table 3), the specific activity measured is considerable. This high rate of membrane bound proteolysis activity is in agreement with the uptake level of amino acids observed by several researchers in chicken caeca (Obst and Diamond, 1989; Calonge *et al*., 1990; Planas *et al*., 1990; Moreto *et al*., 1991) which was similar to that of the jejunum. Similar high specific peptidase activities in the caeca were measured for Canada and Snow geese (Ciminari, 1997). Nitsan and Alumot (1963) also reported a high proteolytic activity in the caeca of chickens fed on a raw soybean diet. These authors suggested that when raw soybean is fed, protein digestion is inhibited in the small intestine. Therefore, the undigested protein reaches the caeca where it is digested and absorbed through their walls or through the colon walls.

Caeca contribution to the total hydrolysis of carbohydrates is smaller than that of peptides (1.5-3.5%). These results are consistent with a modest contribution of the caeca to the total gut capacity to absorb sugars (Obst and Diamond, 1989). As for the specific sucrase activity of the caeca, the levels measured were very low in the neck and medial section, but in the fundus, this enzyme exhibited similar levels to those found in the distal small intestine. The relatively high levels of sucrase found in the fundus are in agreement with the idea that considerable amounts of polysaccharides not digested by intestinal carbohydrases are hydrolyzed to disaccharides by microbes (Jorgensen *et al*., 1996) and also this is consisten with the types of material that enter the caeca, like finely-ground particles and/or soluble, low molecular weight, non-viscous molecules (Svihus *et al*., 2013).

However, these activity levels pose an intriguing question about the functional significance of this enzyme and use of the disaccharides breakdown products by the animal, since according to (Planas *et al*., 1987), the sugar active transport ability of the fundus is null for chickens. One possible explanation might be that caeca paracellular absorption pathway plays an important role in sugar absorption as it occurs for the small intestine of birds (Caviedes-Vidal, 2003, 2007). Future research on passive absorption of peptides and sugars in the caeca of galliforms would be of interest to test this hypothesis.

Dietary modulation patterns in birds: Our results contribute and give apparent support to the functional hypothesis (Caviedes-Vidal *et al*., 2000) that, in the Galloanserae, a small escape of amino nitrogen as peptides from the intestine to the caeca can support microbial growth whereas the small intestine of the passerines, seems to have been selected to extract the maximum available amino nitrogen, rather than excreting it as waste. Additionally, in the chicken's caeca a small amount of proteins and carbohydrates digestion takes place. On the other hand, our findings are not enough to reject hypothesis that the observed lack of intestinal protease dietary modulation has a phylogenetical component because chickens belong to the Parvclass Galloanserae and they have an important caeca. Thus, even though our observations are an important contribution to enhance the amount of species tested, especially in the galliforms group, a definitive answer would require testing Passerae species having functional caeca and Galloanserae species lacking caeca.

Chickens raised on a high protein diet: It must be underscore that the utilization of a semisynthetic diet using casein as a protein source has some advantages when compared to commercial poultry diets, most of which are based on maize, wheat or barley. These commercial foods have considerable levels of fibers which reduce the level of digestibility and increase the amount of wastes (Jozefiak *et al*., 2004). Maize is the most frequent grain used in poultry diets among the above-mentioned cereals and even though it represents an excellent source of metabolizable energy for poultry, protein content of maize is both quantitatively and qualitatively poor (Cowieson, 2005). Peptide digestibility was very high in chicks fed on a dextrose-casein diet compared with corn-soy bean meal and corn-canola meal diets (Batal and Parsons, 2002). These results are consistent with the observation that casein is highly digestible even immediately after hatch (Sulistiyanto *et al*., 1999).

CONCLUSION

The observation that chickens notably improved their growth when fed on a high protein diet (49.72%) after day 42 (usually the slaughter day) achieving significant higher body masses (37.5% more at day 57) may be of interest for poultry industry.

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