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Intestinal and Pancreas Enzyme Activity of Broilers Exposed to Thermal Stress

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ABSTRACT

Thirty-two Hubbard broilers were raised under commercial husbandry to evaluate the effect of temperature exposure (26 and 35ºC) on the activity of digestive enzymes at different ages (23 and 46 days) and different diet energy levels (2,900 and 3,200 kcal ME/kg). Data were analyzed in a 2x2x2 factorial arrangement (energy/temperature/age) in a completely randomized design. Animals were slaughtered after four hours of heat exposure for sampling of an intestinal segment, to extract alkaline phosphatase, and pancreas, to extract amylase, lipase and trypsin. The activity of intestinal alkaline phosphatase was influenced by energy level and animal age. Energy level of diet and environmental temperature interfered on the activity of pancreatic amylase. Lipase activity was affected only by broiler age. There was no effect of treatments on pancreatic trypsin activity. It was concluded that the activity of the pancreatic enzymes can be regulated by different mechanisms under stress conditions induced by heat.

INTRODUCTION

At high temperatures, birds tend to lose appetite; and feed intake, growth rate, muscle and egg production are reduced (Dale & Fuller, 1980; Cowans & Michie, 1980; Temim *et al*. 1999, 2000; Geraert *et al*., 1996) and visceral fat increases in relation to carcass weight (Temim *et al*., 2000; Geraert *et al*., 1996). Feed intake and utilization are closely related to digestion and absorption, which are in turn affected by environmental temperature changes (Owen & Wiggs, 1971; Kitchin & Morris, 1971; Temim *et al*., 2000; Bonnet *et al*., 1997; Itoh *et al*., 2001). According to Bonnett *et al*. (1997), when environmental temperature increased from 22ºC to 32ºC there was a decrease in feed efficiency from 0.485 to 0.340 in poultry, indicating that the functional digestive tract adaptation could be related to compensatory adjustment on production rate and enzymatic contents of digestive secretions (Osman & Tanios, 1983). However, both the synthesis and the levels of pancreatic amylase, trypsin and lipase were shown to change due to diet modifications, induced by the presence of their respective substrates in gastrointestinal lumen (Brannon, 1990). Nevertheless, very few studies were related to putative thermal stress effects on the activity of digestive enzymes of poultry. The aim of this work was to evaluate alterations in the activity of intestinal alkaline phosphatase, pancreatic trypsin, amylase and lipase in birds fed diets containing two energy levels and submitted to thermal stress (35ºC/4h).

MATERIAL AND METHODS

The trial was conducted using 32 male Hubbard broilers, raised according to standard commercial husbandry techniques. Enzyme activity means were analyzed in a completely randomized design and a factorial scheme 2x2x2 (energy level x thermal stress x age).

Two corn and soybean-based diets were fed *ad libitum*, containing energy levels of 2,900 and 3,200 kcal ME/kg. Starter (0-28 days) and finisher (29-42 days) diets were formulated according to the appropriate nutritional requirements for birds in each phase and composition of diets is shown in Table 1.

At 23 and 46 days, four animals per treatment were submitted to thermal stress in a thermostatically controlled climate chamber (adjusted to keep environmental temperature at 35°C and a relative air humidity of 60%), during four hours, without food and water. Control animals were kept in cages at room temperature of 26ºC, without food and water during the same period of time.

Immediately after heat stress, control and stressed animals were slaughtered and pancreas and intestine (duodenum, jejunum and ileum) were collected. Tissues were immediately frozen in liquid nitrogen and stored at – 70ºC freezer for further analysis.

Pancreas was homogenized with an Ultra-Turrax in 500 mM Tris-HCl buffer (1:20 w/v) containing 50 mM $CaCl_2$, pH 8.0, at 4 °C. The homogenate was centrifuged at 14,000 x *g* for 30 min, at 4ºC. An aliquot of the supernatant was used for immediate lipase determination and the remaining supernatant was frozen in liquid nitrogen and stored at –70 °C freezer until the activity of the other enzymes was determined.

Pancreas α-amylase activity was measured by hydrolysis of starch followed by determination of the amount of maltose produced, in accordance to Bernfeld (1955). One unit of enzyme activity was defined and expressed as the amount of enzyme that produced one µmol of maltose/min, at 37°C.

Pancreas lipase activity was assessed by titration (Tietz & Fiereck, 1966), using as substrate the olive oil emulsion (SIGMA®) and the colipase excess extracted from poultry pancreas. One unit of enzymatic activity was defined and expressed as the quantity of enzyme that released one µmol of fatty acid per minute.

Activation of pancreas trypsinogen was accomplished by a pre-incubation period with 0.08 units of Enterokinase (SIGMA®) for 30 minutes. After the activation, trypsin activity was determined at 37°C in accordance to Kakade *et al*. (1974) using N-α-benzoyl-L-arginine-p-nitroanilide (L-BApNA, SIGMA®) as substrate. One unit of enzyme activity was defined and expressed as the amount of enzyme that released one mmol of *p*-nitroanilide/min, at 37°C.

The intestinal mucosa was homogenized in 500 mM Tris-HCl buffer containing 50 mM $CaCl_2$ (1:10 w/v), pH 8.0, at 4°C. Homogenate was centrifuged under refrigeration (4ºC) at 14,000 x *g* for 15 minutes. *p*-PNPPase (*p*-nitrophenyl-phosphatase) activity of intestinal alkaline phosphatase was discontinuously determined at 37°C, measuring the liberation of *p*-nitrophenolate ion (ε=17,600 M^{-1}. cm^{-1}, pH 13) at 410 nm (Pizauro *et al*., 1995). One unit of enzyme activity was defined and expressed as the

Table 1 – Composition of starter (1-28 d) and finisher (29-42 d) experimental diets.

Ingredients	Starter diet		Finisher diet	
(%)	2,900 kcal/kg	3,200 kcal/kg	2,900 kcal/kg	3,200 kcal/kg
Ground corn	56.123	48.853	63.050	55.780
Soybean meal	37.415	38.818	31.692	33.095
Soybean oil	1.462	7.329	0.258	6.125
Premix[1]	5.000	5.000	5.000	5.000
Total	100.000	100.000	100.000	100.000
Crude Protein (%)	22.260	22.260	20.250	20.250

1- Mineral and vitamin premix, levels/kg: 176,000 IU vit. A, 40,000 IU vit. D3, 500 mg vit. E, 100 mg vit. K3, 36mg vit.B1, 200 mg vit. B2, 50mg vit. B6, 560mcg vit. B12, 700mg niacin, 3mg biotin, 500mg pantothenic acid, 30mg Folic acid, 20g choline, 1,100mg iron, 300mg copper, 1,800mg manganese, 1,200mg zinc, 24mg iodine, 3mg sodium selenite, 20g DL-methionine, 380g dicalcium phosphate (304g in initial phase), calcium carbonate 176g (141 g in initial phase), 36g iodized salt, 2g nitrovin, 10g coccidicide, 200mg antibiotic and 1g BHT.

amount of enzyme that released one µmol of *p*-nitrophenolate/min, at 37°C.

Protein content (mg/g pancreas) was determined according to Hartree (1972), using bovine soroalbumin as a standard.

RESULTS AND DISCUSSION

There was no significant interaction among the factors (energy, thermal stress and age) on the activity of the pancreas enzymes amylase, lipase and trypsin and the intestinal enzyme phosphatase alkaline. As shown in Table 2, pancreas amylase activity was affected ($p<0.05$) by energy level of the diet and by thermal stress. Stressed birds had a higher enzymatic activity than control birds.

Details about chemical and physiological mechanisms involved in the regulation of pancreatic amylase level under normal or thermal stress conditions are still unknown. Thus, Slaughter *et al.* (2001) studied amylase activity in order to determine the kinetics and catalytic efficiency in relation to starch from different feedstuffs, and to establish how the enzymatic levels in gastrointestinal tract are related with glucose and insulin levels in blood. According to these authors, high levels of amylase in the gastrointestinal tract indicate that the enzymatic hydrolysis of starch could be the limiting factor in carbohydrate digestion, mainly due to factors related to its physical and chemical properties. On the other hand, Osman & Tanios (1983) reported that thermal stress causes a consistent increase in amylase activity in broilers, which could result in a significant increase in starch hydrolysis and, consequently, in the supply of glucose for internal oxidation and posterior metabolic heat production.

Thus, high pancreatic amylase activity indicates that pancreas plays an important role in the regulation of the levels of intestinal amylase and glucose supplied to achieve energetic requirements under heat stress conditions. Furthermore, higher pancreatic amylase activity in response to an increase in environmental temperature suggests that the levels of pancreatic amylase could be regulated by a complex interaction among paracrine and endocrine pancreatic secretions and neuroendocrine stimuli that could explain the abdominal fat deposition in animals exposed to chronic heat stress.

Pinchasov *et al*. (1990) studied digestive enzymes in birds aged 14 to 83 days and fed *ad libitum* or intermittently. Pancreas amylase levels were reported to increase with age. These authors did not find an age effect on lipase and trypsin activities when these enzymes were expressed in units/g pancreas. Nevertheless, when the results were expressed in units/g body weight, a difference was seen between 20 and 33 days of age in the birds fed intermittently.

According to Table 2, different treatments (diet energy level, heat stress and age) did not result in different trypsin activities. Similar results were observed by Pinchasov *et al*. (1990) for trypsin activity in poultry from 14 to 83 days of age.

In relation to lipase activity, the results suggested that energy levels of 2,900 and 3,200kcal ME/kg were not enough to detect effects of added fat in diet. It has been suggested that the amount and type of triglycerides in the diet regulate pancreatic lipase activity but the mechanism is still unclear (Ricketts & Brannon, 1994). Other studies have shown that different fat levels added to the diet rapidly resulted in adaptive changes that reached a plateau in five days (Deschodt-Lanckman *et al*., 1971).

Alkaline phosphatase activity was affected by diet

Table 2 – Effect of thermal stress and dietary energy level on intestinal alkaline phosphatase (AP), pancreatic lipase (L), trypsin (T) and amylase (A) extracted from poultry.

Sources of variation	Energy (kcal ME/kg)		P value	Stress		P value	Age (days)		P value
	2,900	3,200		Yes	No		23	46	
AP(U/mg protein)[1]	477.90	391.15	*	419.03	450.02	NS	508.91	360.14	**
L(U/mg protein)	14.56	12.48	NS	13.53	13.51	NS	16.85	10.19	**
T(U/mg protein)	35.83	34.71	NS	37.90	32.63	NS	38.54	31.99	NS
A(U/mg protein)	217.65	146.16	**	200.38	163.43	*	184.73	179.08	NS

1- Statistical difference between means in the lines for each treatment
NS – non significant, $p > 0.05$;
* $p < 0.05$
**$p < 0.01$

energy and animal age; it was higher ($p<0.05$) in animals fed the diet containing 2,900kcal ME/kg and at 23 days of age. There was no effect of environmental temperature ($p>0.05$) on alkaline phosphatase activity. Considering that alkaline phosphatase activity could be modified by several factors (Komada *et al*., 1989), the results obtained in the current work suggest that alkaline phosphatase activity extracted from broiler gut is influenced by dietary energy level. In rats, contrasting results were found; alkaline phosphatase activity increased immediately after the rats were fed a meal rich in fat (Alpers *et al*., 1995). Thus, it seems reasonable to accept that these differences were originated by species characteristics, tissues and/or different methods used to prepare the enzymatic substrate, besides other unknown factors.

CONCLUSIONS

Pancreas amylase activity was affected by dietary energy level and thermal stress conditioning. There was no difference among treatments on pancreatic trypsin and lipase activity. Dietary energy level and animal age affected intestinal phosphatase alkaline activity and there was no effect of thermal stress on this enzyme.

The findings of this study suggest that the activity of pancreas amylase, lipase and trypsin, in animals under thermal stress, could be regulated by different mechanisms. However, it is still unclear if these changes are essential for poultry to better use carbohydrates, lipids and proteins under thermal stress conditions.

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