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PANCREATIC TRYPSIN ENZYMATIC ACTIVITY IN BROILERS WHICH INTAKE ROYAL PALM NUT MEAL

ACTIVIDAD ENZIMÁTICA TRIPSINA PANCREÁTICA EN POLLOS DE CEBA QUE CONSUMEN HARINA DE PALMICHE

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To study the pancreatic trypsin enzymatic activity in broilers which intake royal palm nut meal, a total of 40 animals (HE_{21}) distributed in a completely random design were used. Four treatments were designed: control and inclusion of 5, 10 and 15 % royal palm nut meal. The animals were weighed and sacrificed at 42 days and the pancreas were extracted, they were weighed and expressed relative to live weight and the trypsin enzymatic activity was determined, which increased with the inclusion of 5 and 10 % compared to the control and 15 % (5744.50 and 5131.50 vs 2415.67 and 1540.67 mg of released tyrosine. min^{-1}. mg^{-1} of protein, respectively). It is concluded that the inclusion of royal palm nut meal up to 10 % in broilers diets increase the pancreatic trypsin enzymatic activity to digest proteins in the gastrointestinal tract.

Key words: *birds, digestive enzyme, royal palm fruit*

Para estudiar la actividad enzimática de la tripsina pancreática en pollos de ceba que consumen harina de palmiche, se utilizaron 40 animales (HE_{21}), distribuidos en un diseño completamente aleatorizado. Se diseñaron cuatro tratamientos: control e inclusión de 5, 10 y 15 % de harina de palmiche. Los animales se sacrificaron a los 42 días y se extrajeron los páncreas, se pesaron y expresaron como relativos al peso vivo y se determinó la actividad enzimática de la tripsina, que aumentó con la inclusión de 5 y 10 % respecto al control y 15 % (5744.50 y 5131.50 vs 2415.67 y 1540.67 mg de tirosina liberada. min^{-1}. mg^{-1} de proteína, respectivamente). Se concluye que la inclusión de hasta 10 % de harina de palmiche en la dieta de pollos de ceba aumenta la actividad de la enzima tripsina pancreática para digerir las proteínas en el tracto gastrointestinal.

Palabras clave: *aves, enzima digestiva, fruto de la palma real*

One of the central organs of the gastrointestinal tract is the pancreas. It plays an active role in regulating the digestive system by secreting enzymes that hydrolyze proteins, lipids, and carbohydrates from food (Kuzmina *et al.* 2024). Regarding poultry farming, researchers has been reported in the scientific literature that shows the adaptation of the digestive glands of animals to the chemical composition of foods (Vertiprakhov *et al.* 2023).

In studies performed by Martínez-Pérez *et al.* (2021), where royal palm nut meal was included in the broilers diet, it was shown that the protein fraction of this fruit is considered relatively low (8.77 %) and that the levels of ether extract are high (16.06 %). Vertiprakhov *et al.* (2020), when analyzing different sources of vegetable oils in laying hens, observed that the exocrine function of the pancreas is modified depending on the source under study. The objective of this study was to study the pancreatic trypsin enzymatic activity in broilers which intake royal palm nut meal.

A total of 40 HE_{21} hybrid male broilers with an average initial live weight of 141±5 g were used. They were housed in metal cages from 8 to 42 days of age. The experimental diets consisted of: control (corn-soybean cake) and the inclusion of 5, 10 and 15 % of royal palm nut meal, so that they remained isoproteic and isoenergetic. The formulation was made for the start, growth and finishes stages, reported by Vives *et al.* (2020). Throughout the experiment, broilers had free access to water and food.

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At 42 d, the broilers were weighed and slaughtered, exactly two hours and thirty minutes after food ingestion. Traditional procedures were used: desensitization by stunning with electric shock before the exsanguination method by jugular puncture. Subsequently, the abdominal cavity was opened and the pancreases were removed. They were weighed on a technical scale (SARTORIUS, Germany) and expressed relative to live weight (g.kg^{-1} of LW). Then, they were immersed in 4 mL.g^{-1} of physiological saline solution, between 0 and 4 ºC, for transfert to the laboratory. The organs of three animals per treatment, which constituted a sample, were manually homogenized and centrifuged at 1500 r.p.m for 10 min at 4 ºC in a Thermoscientific IEC CL31R (China) refrigerated centrifuge. The pellet was discarded and the supernatant was stored in small aliquots at -80 °C until further analysis.

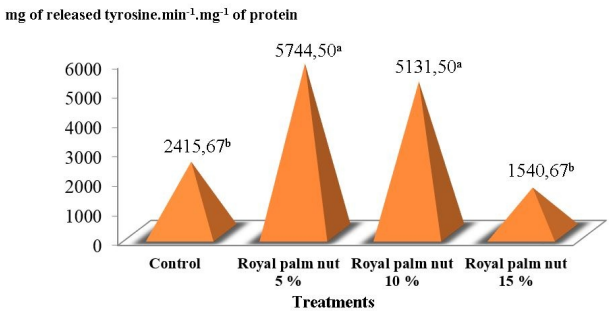
Protein concentration was determined by the Bradford (1976) method on a UV-vis spectrophotometer (Rigol Ultra-3400 series, China). Concentrations were calculated by interpolating absorbance values of samples in the standard curve of bovine serum albumin (BSA) (0.01-5 mg/mL). This was normalized for all homogenates under study at 3 mg/mL, for which the corresponding dilutions were made in each case. All analyses were performed in triplicate.

Trypsin activity was determined in pancreatic homogenate, according to the technique described by León *et al.* (2007). The same (0.3 mg of protein) was incubated for 15 min at 41 °C with 200 µL of the 1 % casein substrate in 100 mM pH 7.4 sodium phosphate buffer. The reaction was stopped with the addition of 1 mL of 5 % w/v trichloroacetic acid and the mixture was filtered through Whatman 42 paper. 250 µL of the filtrate was reacted with 1,250 µL of 200 mM Na_2CO_3 and 250 µL of the Folin-Ciocalteu reagent, diluted 1:5 in distilled water. After incubation for 20 min at 41 °C, the absorbance was measured at a wavelength of 625 nm. The values were compared with those of a standard curve prepared from a tyrosine solution (1 mg/mL). The specific activity of trypsin was expressed as mg of tyrosine released.min^{-1}.mg^{-1} of protein.

A completely randomized design was used, with four treatments. For the final and relative live weights of the pancreas, a total of 10 replicates from one animal were used and for the pancreatic trypsin enzymatic activity, three replicates, which corresponded to a sample of three pancreases each. Differences between means were determined according to Duncan (1955) for $p<0.05$ where necessary. The statistical package Infostat (Di Rienzo *et al.* 2012) was used.

There were not differences in the final live weights and relative weights of the pancreas among the four treatments (table 1). Similar performance was recorded by Vives *et al.* (2021), when studying the accessory gland in broilers. These authors also suggested that, although macroscopically no morphometric changes were observed, there was an increase in its specific functions due to the presence of royal palm nut meal in the ration, which favors the digestion process.

The treatments with the inclusion of 5 and 10 % of royal palm nut meal did not differ from each other, and showed higher enzymatic activity of pancreatic trypsin compared to the rest (figure 1). The increase represents a physiological benefit for the animal, since according to Vertiprakhov *et al.* (2023) this enzyme breaks protein bonds through hydrolysis to form smaller peptides or amino acids that are more easily digestible and bioavailable. In this way, the digestion of these biomolecules increases, which perform essential biological functions in the body: structural, enzymatic, hormonal, regulatory, homeostatic, among others.



a,b Values with different letters show significant differences ($p<0.05$)

Figure 1. Pancreatic trypsin enzymatic activity in broilers which intake different levels of royal palm nut meal in the diet. SE±0.8758, p=0.0014.

The 15 % reduction in enzymatic activity compared to the other treatments that include royal palm nut meal can be related to the high correlation showed by Martínez-Pérez *et al.* (2021) between the fat and fiber contents (86 %) provided by royal palm nut with this level in the diet. According to Liu *et al.* (2017) lipids have a greater impact on food intake than starch and protein, and the concentrations of the latter

Table 1. Final live weights and relative weights of the pancreas of broilers, which intake royal palm nut meal in the ration

Weights	Inclusion of royal palm nut meal , %				SE±	p-value
	Control	5	10	15		
Final live (kg)	2.24	2.36	2.34	2.15	0.15	0.7506
Pancreas (g.kg^{-1} LW)	2.93	2.58	2.50	2.45	0.21	0.3910

are diluted by the inclusion of additional lipids in the ether extract of isoenergetic diets. Vertiprakhov *et al.* (2020) stated that at high physiological concentrations of fat, a selective increase in lipase is observed with an overall decrease in the rest of the enzymatic secretion. This result is consistent with that expressed by Vives *et al.* (2020), who observed higher enzymatic activity of pancreatic lipase for 15 % inclusion of royal palm nut meal in the ration of broilers.

Inhibitory effects on exocrine pancreatic secretion by nutrients, bile salts, some gastrointestinal hormones and trypsin inhibitors have been described (Kuzmina *et al.* 2024). According to Singh and Kim (2021), fiber increases the excretion of bile salts, which are responsible for the emulsification of lipids so that their digestion can occur. Therefore, this performance could also be associated with inhibitory effects by the fibrous fraction of royal palm nut. It could be that 15 % of royal palm nut meal in the diet increased the concentrations of trypsin inhibitors, which would reduce trypsin activity. This hypothesis needs to be confirmed with further studies.

It is concluded that the inclusion of up to 10 % of royal palm nut meal in the diet of broilers increases the activity of the pancreatic trypsin enzyme in the gastrointestinal tract.

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