

Research Article

DISTRIBUTION OF HYDROLYTIC ENZYMES ACTIVITY IN THE DIGESTIVE TRACT OF GRASSCUTTER (*Thryonomys swinderianus*)

SORO Soronipoho, KARAMOKO Yahaya*, TRAORE Beh and FANTODJI Agathe

Natural Sciences Research and training Unit, Laboratoire de Biologie et cytologie Animales,
Nangui Abrogoua University, 02 BP 801 Abidjan 02, Republic Côte d'Ivoire

Article History: Received 15th November 2013; Accepted 4th January 2014; Published online 10th January 2014

ABSTRACT

Hydrolytic enzymes activities in digestive tract of grasscutter were measured on 40 adult grasscutters. The selected animals were apparently healthy and adulte. Each portion of digestive tract and the contents was recovered in a jar which contained physiological water. Amylolytic, cellulolytic and invertasic activities were measured at pH 2.6, pH 3, pH 4, pH 5, pH 5.6, pH 7, pH 8 and pH 9 in each portion of digestive tract. Only the proteolytic activity was measured at 1.7, 7, 9 and 10. Enzymatic activities mixture showed that amylolytic, cellulolytic, invertasic and proteolytic activities changed according to pH and portions of digestive tract. The amylolytic activity is dispersed in all digestive contents, glands and digestive muscle excepted the oesophagus. Cellulolytic activity is detected in the digestive contents, precisely in the caecum. An important proteolytic activity was detected in the pancreas and no xylanasic activity was detected in tissues and digestive contents.

Keys words: Grasscutters, digestive tract, enzymatic activity, digestive content.

INTRODUCTION

Grasscutter meat is the most preferred and expensive meat in West Africa, including Nigeria, Togo, Benin, Ghana and Cote d' Ivoire. It contributes to both local and export earnings of most West African countries and is therefore hunted aggressively. Thus, the excessive and uncontrolled decimation of this animal for consumption poses a threat to the ultimate survival of the species. Grasscutter domestication has not been successful so far, despite the consented efforts. The lack of information on the biology, veterinary care and nutritional would be the principal causes. The weak nutritional value of natural forage served to grasscutter and their wasting give generally bad zootechnics performances. Feeding remains an important pillar in animals breeding because it gives to animals a good health, a good growing and a good reproduction (Zougou-Tovignon 2005).

It is assumed that hydrolytic activity and digestive volume correlate positively with the digestion efficiency (Marounek *et al.*, 1994). Generally distribution and intensity of intestinal enzyme activity along the gut varies with feeding

habits and intestinal morphology (Hofer and Schiemer, 1981; Kuz'mina and Smirnova, 1992; Sabapathy and Teo, 1993). Then, nutriment availability in all body depend on hydrolytic enzymes, the season and the microbial flour (Juana and Alejandra, 2008).

To elaborate a composed aliment which can permit to have good performances, the digestive physiology must be known. Thus, this study chose to determine the hydrolytic enzyme in the grasscutter gut.

MATERIALS AND METHODS

1. Materials and chemical

Twenty (20) grasscutter ranging in age from ten (10) month high at the experimental farm of the University Nangui Abrogoua (Ex Abobo-Adjamé) were used. They were fed daily at will with green fodder (*Panicum maximum*, *Pueraria phaseoloides*, *Pennisetum purpureum*), the stems of Manhiot esculenta and a dietary supplement consisting of grains of corn, dried leaflets of *Leucaena leucocephala*, dried tuber of cassava grain, powder of shells of snails or calcined bone and salt. The substratum (starch, carboxy

*Corresponding author e-mail: y.karamoko@gmail.com

méthylcellulose, xylane and saccharose) used come from Sigma Aldrich when the casein was a Merck product. The others products and reactive used where an analytic quality.

2. Methods

2.1. Isolation and preparation of the crude enzyme extract: Animals were stunned and dissected immediately according to the classic dissection of mammals. The digestive tube was identify, unrolled and carved in portions corresponding to oesophagus, stomach, duodenum, jejunum, ileum, caecum, colonist and rectum. No digesta was found in the oesophagus. All the digesta were mixed and crushed with NaCl 0.9 % (p/v) then kept at -2 °C. The crude enzyme extract was obtained after homogenization, sonication during 10 minutes at 4 °C.

2.2. Enzymatic activities dosage: The dosage of polysaccharidasic and invertasic activities performed in standard conditions. The buffer

solutions used were citrate phosphate (100 mM, pH 2.6 and 3), acetate (100 mM, pH 4; 5 and 5.6) and tris-HCl (pH 7; 8 and 9). Xylane and carboxymethylcellulose were incubated for 2 hours and starch was incubated for 30 mn. Proteins were dosed according to the method of Lowry *et al.* (1951). The coloration intensity was determined with a spectrometer (SHIMADZU UV-120-02) at 540 nm. Then the optic density was converted using standard curved obtained in the same conditions.

RESULTS

1. Invertasic activity

Invertasic activity (saccharidasic activity) was observed in the duodenum, the jejunum and the ileum tissues. This activity was higher in the jejunum at pH 5 and 5.6 (Figure 1). Concerning the digesta, invertasic activity was found in the entire portion excepting the stomach. The optimum hydrolytic activity pH was 5 and 5.6 (Figure 2).

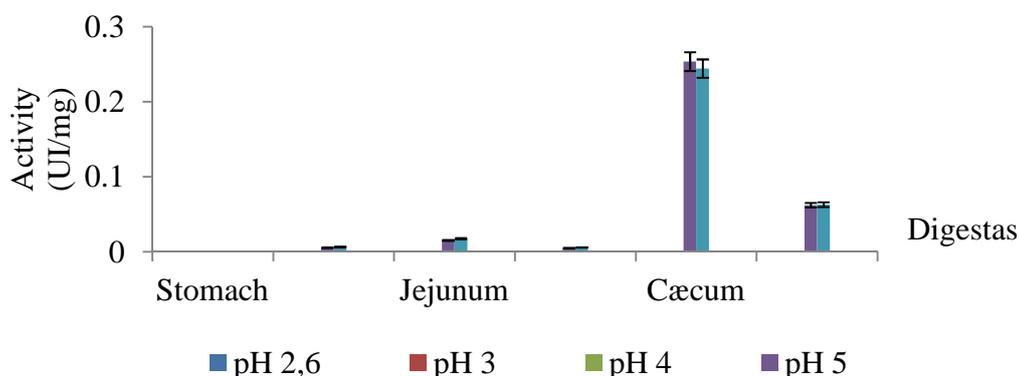


Figure 1. Distribution of invertasic activity in the tissues of digestive tuber and the annexes glands of grasscutter.

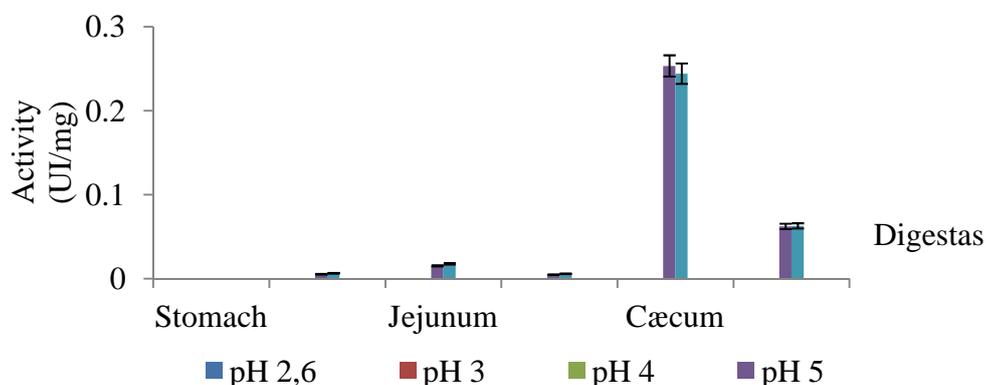


Figure 2. Distribution of invertasic activity in the digestas of digestive tuber of grasscutter.

2. Polysaccharidasic activity

2.1. Amylasic activity

The amylasic activity was widely distributed in the pancreas, the salivary gland and the digestive tuber, excepting the oesophagus. It was optimal

in the colonist at pH 5.6 and 7, in the salivary gland at pH 5.6 (Figure 3). This activity is present in the entire portion excepting the stomach and the duodenum (Figure 4). It was higher in jejunum and ileum digesta with the maximum activity at pH 5.

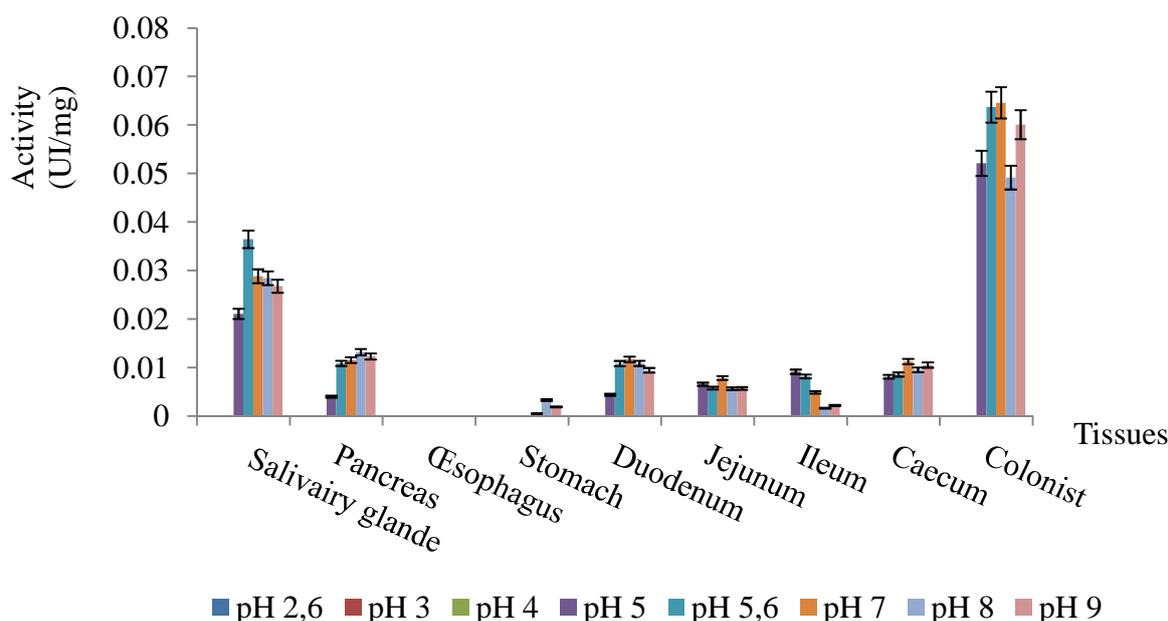


Figure 3. Distribution of amylasic activity in the tissues of digestive tuber and annexes glands of grasscutter.

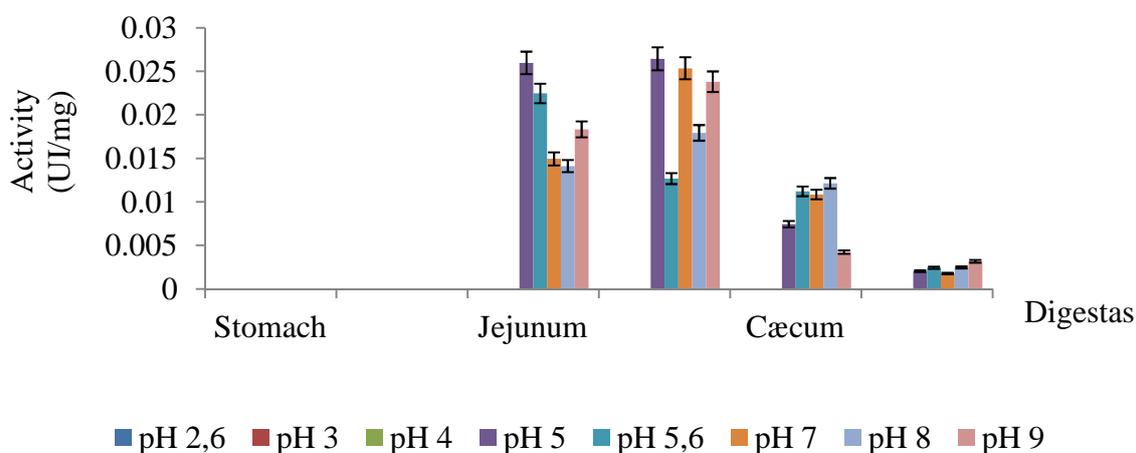


Figure 4. Distribution of amylasic activity in the digestas of grasscutter.

2.2. Cellulasic and xylanasic activities

The cellulasic activity wasn't detected in gland tissues. It was only present in caecum and

colonist digestas (Figure 5). Xylanasic activity wasn't detected.

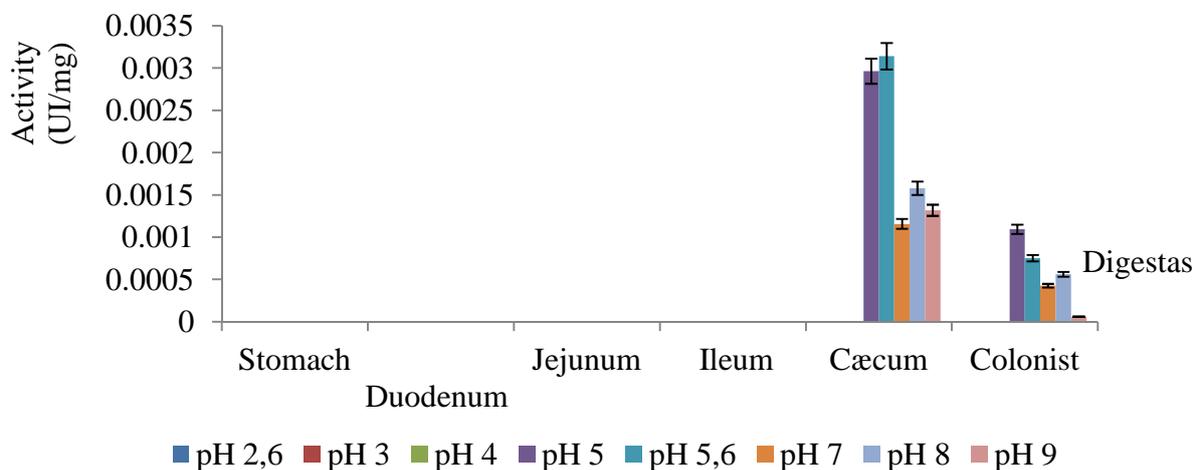


Figure 5. Distribution of cellulasic activity in the digestas of grasscutter.

2.3. Protéasic activity

Excepting the oesophagus, this activity was observed in the rest of digestive tuber, in the

glands and the digestas (Figure 6). The higher activity was obtained with the Tris-HCl buffer in the pancreas. No proteasic activity was underlined in the digstas (Figure 7).

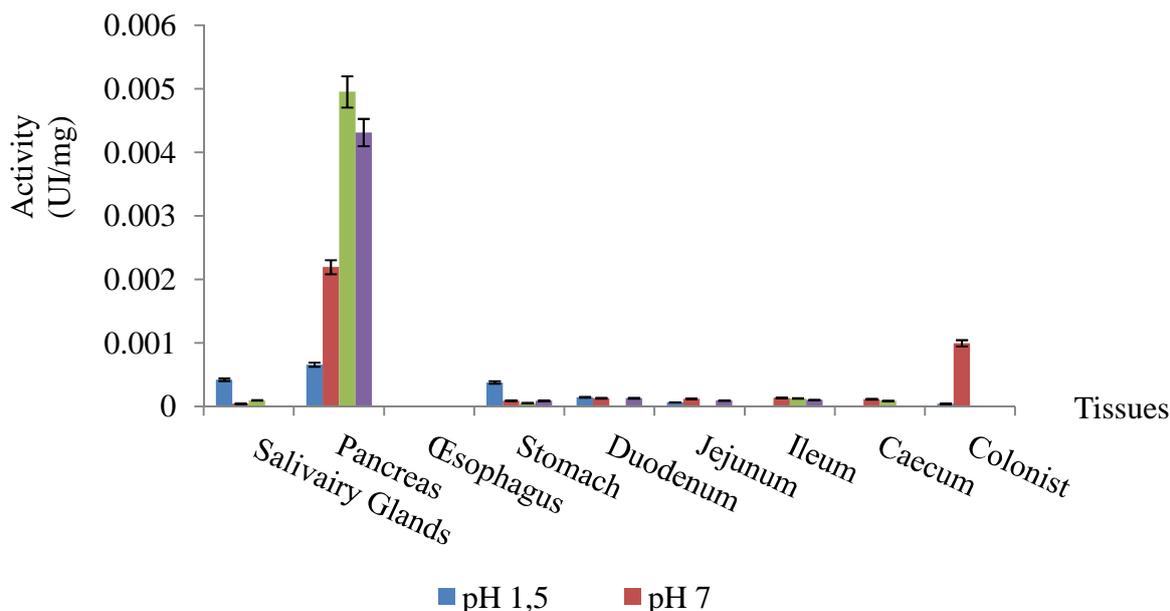


Figure 6. Distribution proteasic activity in the tissues of digestive tuber and annexes glands of grasscutter.

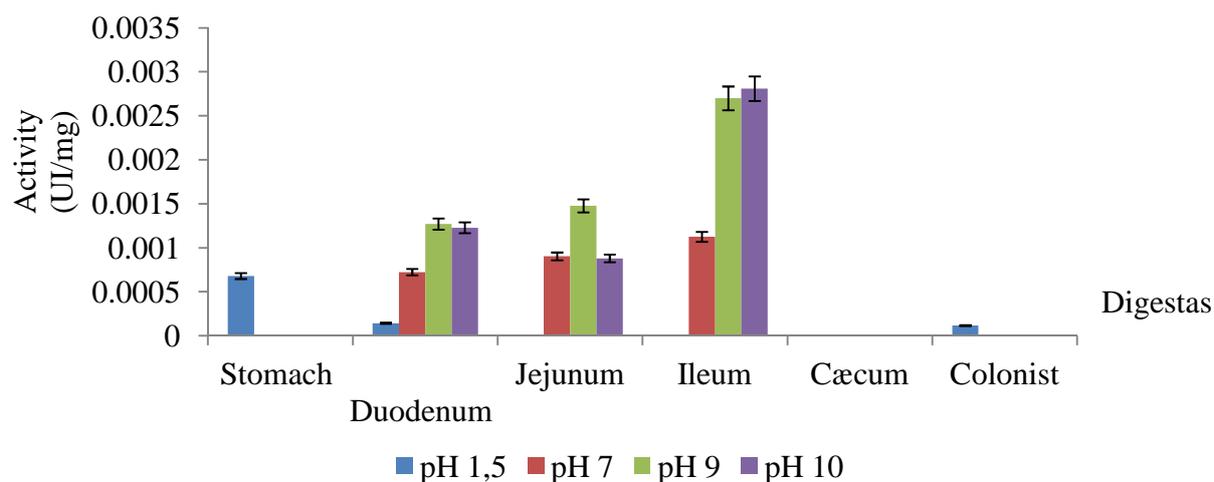


Figure 7. Distribution of protease activity in the digestas of grasscutter.

DISCUSSION

The enzymatic activities, especially polysaccharidase, invertase and protease activities in the tissues of the digestive tubers and the glands showed an irregular distribution. This irregularity was so observed with the digestas. The invertase activity was only found in the tissues of small gut and in the digestas of caecum and the colonist. Buts *et al.* (1986), and Adrian and Phillip (2005) reported the presence of this enzyme in the rat's small gut. The presence of invertase activity only in the tissues of small gut is in accord with the results of Champ (1985). This one denoted that invertase is produced in special manner by the cells of this portion. The presence of such an activity in the digestas suggests that the microbial flora takes part in the digestion of saccharose. Fevrier *et al.* (1973) come to the same conclusion after their work on the pigs. They showed that the disaccharides which are not hydrolysed by the enzyme in the small gut were hydrolysed by the microbial flora and absorbed in the caecum or the colonist. Silva *et al.* (2004) sustain that hydrolysis of saccharose comes from glucosidase and fructosidase which are the principal enzymes of degradation of this sugar. Kubota *et al.* (2004) assert that these enzymes are secreted by the intestine cell. The existence of the saccharase in the grasscutter can be proved by his manner which consists to appreciate and consume food according to the total sugar content. The starch was hydrolysed in all the digestive portions excepting the oesophagus. This situation suggests that the digestion of amylaceous composite of the

grasscutter begins from the buccal cavity and continues along of digestive tubers. The α -amylase activity was determined by Lebas *et al.* (1971) and Debray *et al.* (2003) in the rabbit. According to Kidder and Manners (1980), the presence of this activity in the digestas of small intestine and the large intestine comes from the pancreas. Even if, α -amylase activity is important in the tissues of colonist, the main of starch hydrolysis was noted in the jejunum and the ileum, where the activity is present in the digestas. Champ (1985) reported such an observation. He asserted that the starch digestion is done in the upper part of digestive tubers. Cellulase and xylanase activity was not found in the digestive tubers and glands of the grasscutter. The cellulose is a major component of herbivorous, notably grasscutter aliment. Nevertheless, the absence of cellulase and xylanase activities in mentioned digestive apparatus portions was underlined by Schrage and Yéwadan (1995). Pratima *et al.* (2012) sustain that the gut flora is implicated in cellulose digestion. In order to hydrolyse fiber, the bacteria produce fibrolitic enzymes which carve cellulose and hemicellulose molecules (François, 2007). That justifies the presence of cellulase activity in caecum and ileum digestas. Cellulase activity doesn't come from salivary gland and pancreas secretion, but from microorganism which live in those portions. Some studies on herbivorous showed clearly that the presence of bacteria in different portion of digestive tubers facilitates cellulose degradation (Kolb, 1975). Suh-Ching *et al.* (2005) also asserts that certain fibers are not hydrolysed by

the digestive enzymes; they reach the colon unabsorbed and are utilized selectively as a substrate for the growth of bacteria. Saccharidasic, amylasic and cellulosic activities are showed into the range of 5 to 5.6 pH. Cathy (2006) reports that the stomach pH of rabbits falls into the range of 5.0–6.5. The proteasic activity is distributed as well in the tissues and the digestas. According to Papoutsoglou and Lyndon (2006), this activity corresponds to pepsin activity. Thus, this activity is present in the pancreas and the salivary gland because those organs excrete and discharge their enzymatics contents along the digestive tuber (Vernay and Kaynaud, 1975; Candau *et al.*, 1986). The pepsin activity is also found by Kageyama (1988) with the pig and by Dziekonska-Rynko *et al.* (2004) in guinea pig.

CONCLUSION

Observation shows that amylolytic, cellulolytic, invertasic and proteolytic activities varied according to pH and portions of digestive tract. The amylolytic activity is dispersed in all digestive contents, glands and digestive muscles excepted the oesophagus. Cellulolytic activity was detected in the digestive contents, particularly in the caecum. A significant proteolytic activity was detectable in the pancreas and no xylanasic activity was detected in tissues and digestive contents.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this article.

ACKNOWLEDGEMENTS

This study was conducted within the framework of the consortium Afrique One “Ecosystem and Population Health: Expanding Frontiers in Health”. Afrique One is funded by the Wellcome Trust (WT087535MA).

REFERENCES

- Adrian, R.W. and Philip, S.O., 2005. Decrease sucrase and lactase activity in iron deficiency is accompanied by reduced gene expression and upregulation of the transcriptional repressor PDX-1. *Am. J. Physiol. Gastrointest. Liver Physiol.*, 289: 1108-1114.
- Buts, J.P., Bernasconi, P., Van Craynest, M. P., Maldague, P. and De Meyer, R., 1986. Response of human and rat small intestinal mucosa to oral administration of *Saccharomyces boulardii*. *Pediatr. Res.*, 20 (2) :1-9.
- Candau, M., Auvergne, A., Comes, F., Bouillier-Oudot, M., 1986. Influence de la forme de présentation et de la finesse de mouture de l'aliment sur les performances zootechniques et la fonction caecal chez le lapin en croissance. *Annales de zootechnie*, 35, 373-386.
- Cathy, J.D.A., 2006. Anatomy and Physiology of the Rabbit and Rodent Gastrointestinal System; *Assoc. Avian Vet.* pp. 9-17.
- Champ, M., 1985. Digestion des glucides chez les monogastriques. *Reprod. Nutr. Dévelop.*, 25(4 B), 819-842.
- Debray, L., Huerou-Luron, I. L., Gidenne, T., Fortun-Lamothe, L., 2003. Digestive tract development in rabbit according to the dietary energetic source: correlation between whole tract digestion, pancreatic and intestinal enzymatic activities. *Comp. Biochem. Physiol. A*, 135, 443-445.
- Dziekonska-Rynka, J., Rokicki, J. and Jablonowski, Z., 2004. Effects of 3rd stage *Anisakis simplex* larvae on digestive tract protease activity of guinea pigs 24 and 48 hours after infection. *Helminthologia*, 41(1): 21-24.
- Février, C., Collet, J. and Bourdon, D., 1973. Utilisation de divers types de lactosérum dans les régimes de sevrage des porcelets et durant la période de croissance finition. *Journées Rech. Porcine en France, Paris, LN.R.A., ITP*, pp.79-86.
- François, G., 2007. Les effets d'une supplémentation en enzymes fibrolytiques exogènes dans l'alimentation des vaches laitières. Faculté des sciences de l'agriculture et de l'alimentation ; Université Laval ; *Séminaire en Sciences Snimales SAN-12474 du 18 avril 2007*, p. 25.
- Hofer, R. and Schiemer, F., 1981. Proteolytic activity in the digestive tract of several species of fish with different feeding habits. *Oecologia*, 48: 342-345.
- Juana, D.V.C. and Alejandra L.M.A., 2008. Digestive strategies in the South American subterranean rodent *Ctenomys talarum*.

- Comp. Biochem. Physiol. A Mol. Integr. Physiol., 150(4), 387-394.
- Kageyama, T., 1988. Analysis of the activation of pepsinogen in the presence of protein substrates and estimation of the intrinsic proteolytic activity of pepsinogen. *Eur. J. Biochem.*, 176: 543-549.
- Kidder, D.E., Manners, M.J., 1980. The level and distribution of carbohydrases in the small intestine mucosa of pigs from 3 week of age to maturity. *Br. J. Nutr.*, 43: 141-153.
- Kolb, E., 1975. La digestion dans le gros intestin chez le cheval, les ruminants et le porc. In: *Physiologie des animaux domestiques*, Vogt Frères, Paris : 301-305.
- Kubota, M., Tsuji, M., Nishimoto, M., Wongchawalit, J., Okuyama, M., Mori H, Matsui, H., Surarit, R., Svasti, J., Kimura, A. and Chiba, S., 2004. Localization of alpha-glucosidases I, II, and III in organs of European honeybees, *Apis mellifera* L. and the origin of alpha-glucosidase in honey. *Biosci. Biotechnol. Biochem.*, 68: 2346-2352.
- Kuz'mina, V.V. and Smirnova, Y.G., 1992. Distribution of alkaline phosphatase activity along the length of the intestine of freshwater teleosts. *J. Ichthyol.* 32: 1-9.
- Lebas, F., Corring, T., Courtot, D., 1971. Equipement enzymatique du pancréas exocrine chez le lapin, mise en place et évolution de la naissance au sevrage. Relation avec la composition du régime alimentaire. *Ann. Biol. anim. Bioch. Biophys.*, 11(3): 399-413.
- Marounek, M., Vovk, S.J., 1995. Distribution of activity of hydrolytic enzymes in the digestive tract of rabbits. *Br. J. Nutr.*, 73, 463-469.
- Papoutsoglou, S. E. and Lyndon, A. R., 2006. Digestive enzymes of *Anarhichas minor* and the effect of diet composition on their performance. *J. Fish Biol.*, 69: 446-460.
- Pratima, G., Kalpana, S., Avinash, S. and 2012. Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. *Int. J. Microbiol.*, doi:10.1155/2012/578925.
- Sabapathy, U. and Teo, L.H., 1993. A quantitative study of some digestive enzymes in the rabbitfish, *Siganus canaliculatus* and the sea bass, *Lates calcarifer*. *J. Fish Biol.*, 42: 595-602.
- Schrage, R. and Yewadan, L.T., 1995. Abrégé d'élevage des aulacodes. *GTZ, N° 251*, p. 103.
- Silva, C.P., Carneiro, C.N., Isejima, E. M. and Samuels, R. I., 2004. Sucrose hydrolases from the midgut of the sugarcane stalk borer *Diatraea saccharalis*. *J. Insect Physiol.* 50: 1093-1101.
- Suh-Ching, Y., Ju-Yen, C., Huey-Fang, S., Ting-Ying, C., Su Chen, T. and Jiun-Rong, C., 2005. Effect of synbiotics on intestinal microflora and digestive enzyme activities in rats. *World J. Gastroenterol.*, 11(47): 7413-7417.
- Vernay, M., Kaynaud, P., 1975. Repartition des gras volatils dans le tube digestif du lapin domestique. I, lapins nourris en luzerne et avoine. *Annales des Recherches vétérinaires*, 6 : 357-368.
- Zougou-Tovignon, G.C., 2005. Influence des parties végétatives de manioc (*Manihot esculenta*) sur les performances zootechniques des aulacodes (*Thryonomys swinderianus*, Temminck, 1827) d'élevage. Mémoire en gestion des ressources animales et végétales en milieux tropicaux, Université de Liège, 79.