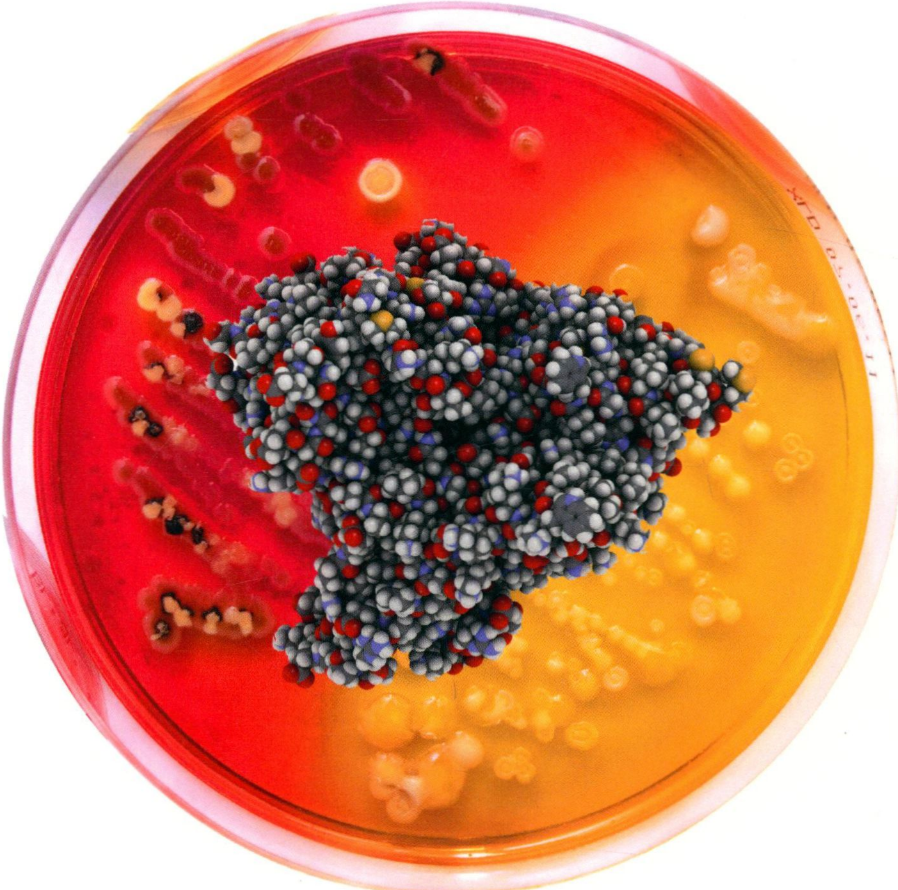
ENZYMES IN HUMAN AND ANIMAL NUTRITION

Principles and Perspectives



Edited by

Carlos Simões Nunes
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Edited by
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Enzymes in Human and Animal Nutrition: Principles and Perspectives is a detailed reference on enzymes covering information on all relevant aspects fundamental for final use of enzymes in human and animal nutrition. Topics explored include depolymerization of enzymes, both from a food and feed perspective, different enzymes and some direct-fed microbials, and the important technological issues related to enzyme use and production. Readers will develop new insights for applications of enzymes in foods and feeds with the wealth of knowledge brought forth by the authors of the various chapters.

Key Features

- Examines the role of enzymes in nutrition and in the production of food and animal feed so that food industry and academic researchers can understand applications of enzymes in the health of humans and animals
- Provides a thorough overview of selection, engineering, and expression of microbial enzymes and looks at extremophile organisms as a potential new source of enzymes
- Includes discussion of analytics, economics, and intellectual property to increase applicability of the rest of the book outside of the laboratory

About the Editors

Dr. Carlos Simões Nunes is a researcher and teacher with appointments at Estação Zootécnica Nacional in Fonte Boa, Portugal, and at INRA in France. He holds a PhD in Nutrition, a PhD in Veterinary Science, and a Doctorate in Veterinary Medicine. He heads the Veterinary Department of Luena, Angola and manages cattle farms, slaughter houses, and a fish industry in Angola as well as Invited professor in several European Universities. He has worked extensively in industry as group leader at Hoffman-la-Roche and DSM and is an invited professor at Nancy University in France and at Vila Real at Universidade de Trás-os-Montes e Alto Douro in Portugal. He has published 189 peer papers and is the author or the coauthor of 19 patent applications and/or patents.

Dr. Vikas Kumar is an assistant professor at University of Idaho. His research revolves around fish nutrition, physiology and nutrigenomics, with aims of developing nutritionally-balanced, environmentally sound, and cost-efficient aqua and animal feeds. For his outstanding contribution to research and teaching in the global fisheries sector, he was awarded the "Dr. T.V.R. Pillay and Dr. M.V. Gupta Best Overseas Fisheries Scientist Award" by the Professional Fisheries Graduate Forum (PFGF) in India. He is currently serving as an editorial board member of Scientific Reports by the Nature Publishing Group and as leading Associate Editor for the *Journal of the World Aquaculture Society*. He has authored more than 70 papers in peer-reviewed journals, 8 book chapters, and 25 magazine articles. Along with extensive research work, his duties also include development of new undergraduate and graduate courses in aquatic animal nutrition, feed technologies, nutrigenomics and metabolism.

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Preface

In modern-day nutrition research, there is a distinct division between the nutrition of humans and animals. Even within animal nutrition, there are clear demarcations between those involved in nutrition research for pigs, poultry, ruminants, fish, and companion animals (dogs, cats, and horses). This discrimination between species may seem logical from a practical point of view as there are distinct physiological and biochemical differences between humans and terrestrial animals, as well as between animals. However, there are far more aspects of nutrition which all species have in common. In academia, research, and even industry, animal and human nutrition appear as two separate worlds, each with its own scientific approaches and foci. Human and animal nutrition departments exist side by side in many organizations (e.g., universities, companies), more often than not, living within their own world with cooperation between these departments being, unfortunately, rather the exception than the norm. Over recent years, the concept of OneHealth has been (re)introduced. Within this concept, a multiple-discipline approach is taken to provide the best health for people, animals, and our environment.

A recent example of a OneNutrition approach can be found in the area of protein quality evaluation of human foods. Whereas in the past the protein digestibility corrected amino acid score (PDCAAS) system was used for the evaluation of protein quality of human foods, the recent FAO approach has been to capitalize on the decades of methodology development in pig nutrition (ileal digestibility) and adopt the digestible indispensable amino acids score (DIAAS) system. Where the previous PDCAAS values were hindered by (often major) inaccuracies from a correction by rat fecal nitrogen digestibility values, the new DIAAS system utilizes state-of-the-art standardized ileal digestibility values of individual amino acids of an animal species more similar in digestive physiology to humans. Although ultimately such measurements should be conducted on the species of interest, in this case humans, until more accurate methodologies are developed, a OneNutrition approach will provide more accurate data.

The OneNutrition concept also provides an excellent approach in our understanding of the nutrition of individual species. Differences (and similarities) in anatomy and digestive physiology as well as specializations (or adaptations) of species in their metabolism of nutrients as a result of diet-induced evolutionary adaptations, can provide insights into species-specific nutrition. For example, the spatial localization of alanine:glyoxylate aminotransferase 1 (AGT1), responsible for the removal of glyoxylate which is involved in hyperoxaluria, seems to be species dependent. In carnivores and insectivores, AGT1 is mainly present in mitochondria of liver cells, while in humans, Old World monkeys (macaques, baboons), rabbits, and guinea-pigs, AGT1 is almost exclusively located in the peroxisome. The mitochondrial localization of AGT1 is seen in carnivorous and insectivorous species of different genera (mammals, birds, reptiles), indicating that AGT1 localization in the mitochondrion might be required when consuming high-protein, low-carbohydrate diets. In rodents (rats, mice, hamsters) and marmosets (New World monkey), AGT1 is distributed approximately equally between both organelles. These species differences in intracellular localization of hepatic AGT1 provide clear indications of dietary selection pressure during evolution and hence guidance to the nutrition of individual species.

An area in which there appears to be little overlap between human and animal nutrition, research is in the application of enzymes. The use of enzymes in human food production dates back to 6000 BC or earlier, first as a product of microbial fermentation, for example in the production of beer, wine, cheese, and yogurt. For decades, industrially produced, more or less pure, enzymes have been used in the production of bread and lactose-free milk, to mention a few, to improve quality (bread) or make nutritious milk accessible to lactose-intolerant people. Most enzymes used in human nutrition are, however, used during food production/processing. Very few enzymes are used as such, meaning they are consumed (as a small pill or a few drops) to act within the gastrointestinal tract where they should degrade specific unwanted factors. Current examples are enzymes to degrade lactose and gluten for lactose- and gluten-intolerant people, respectively.

In contrast, apart from enzymes produced during fermentation processes as in the production of silage, enzyme application in animal feeds is only a relatively recent phenomenon. In the 1980s, the first enzyme products gaining commercial importance entered the feed market. These first enzymes mainly degraded fiber; xylanase and β-glucanase. In 1991, the first commercial viable phytase entered the feed market, which rapidly changed the entire landscape. Nowadays, most poultry and pigs feeds contain specific enzyme products. They are active in the stomach and intestines and degrade antinutritional factors or improve the nutritional value of the feed for the animal.

Obviously, the OneNutrition approach can also be applied to enzymes. Unwanted (antinutritional) factors in animal feeds are very likely also unwanted in the diet of humans, and vice versa. What are such common unwanted factors? Which compounds can be broken down to improve the nutritional value for animals and could also improve the nutritional value for humans? This could be either by degradation of unwanted factors (similar to lactose and gluten), or by increasing the digestibility and availability of (relatively) scarce nutrients. Examples are increasing the availability of some amino acids for athletes, sick, or elderly people, and of specific minerals in populations of people with deficiencies. Improving the nutritional value of foods in general is a clear objective for feeding undernourished people. And, vice versa, can enzymes that are presently used in food production be applied to improve the nutritional value of animal feeds?

This book reintroduces the once (pre-1970) common approach to nutrition, that of OneNutrition by posing these questions and thoughts. The editors, Prof. Dr. Carlos Simões Nunes and Assistant Professor Dr. Vikas Kumar, realized that enzymes are important in both animal and human nutrition and that by bringing our current knowledge in these, hitherto, two separate areas into one book, the reader is able to develop new insights for applications of enzymes in foods and feeds. The authors of the various chapters have a wealth of knowledge in various aspects of enzymes for feed and food. After chapters dealing with more general aspects of enzymes, phytase is discussed in detail. The direct application of phytase has been extensively investigated in pigs and poultry, but has also been investigated for use in humans. Although for humans the improved digestibility of phosphorus may be less important than for animals, its effect on microminerals such as iron and zinc may be of great importance for many people.

In 10 chapters, depolimarizating enzymes are discussed, both from a food and feed perspective. The multitude of applications of such enzymes is amazing, and we are only at the beginning of our understanding of how these can be used more effectively. Increased understanding of vegetable cell wall composition and morphology, and of their effect within the gastrointestinal tract will ultimately result in the development of more specific enzymes to break down or modify these complex structures. In combination with currently used enzymes such as xylanases and cellulases, they will not only enable the more complete use of potential food/feed energy, but also induce specific effects on the gut wall and the gut microflora, resulting in improved gut health (for both humans and animals). Lately, the importance of the microbiota in human diseases such as obesity has been described. The production of prebiotics by a specific (combination of) enzyme(s) may promote a favorable microbial balance, and thus promote health.

In the third part of the book a number of different enzymes and some direct microbials are discussed. I was especially triggered by the chapter on chitinases. Given the future predicted protein shortage and our focus on the use of insects in feed and food, this enzyme may prove to be of great importance. Protein digestibility of many insects is limited because of the presence of chitin, but may be greatly improved by effective application of chitinases.

The final area of focus of the book deals with important technological issues related to enzyme use and production: formulation and analysis, the continued discussion of regulatory aspects, and the overall questions regarding economy. The final chapter of the book contains a great review regarding the potential of enzymes for both humans and animals, discusses general perspectives, and provides conclusions.

The editors should be complemented on bringing together many experts in the field of enzyme use in feeds and foods and achieving the OneNutrition approach in the use of enzymes in nutrition. We have to look into each other's kitchens and silos more often! This book allows the reader to look into those silos and kitchens, and be able to develop new insights and understanding of the application of enzymes in food and feed.

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First of all, we would like to thank Elsevier for accepting our proposal for this book more than 2 years ago. The challenge has been handled professionally and also in a friendly manner, particularly by Jaclyn Truesdell and Megan Ball.

We believe in the interest of the present book, taking into account that humans and animals are targets for dietary supplements of microbial enzymes. There are plenty of published works on industrial applications and, more rarely, on direct human or animal applications. Thus far, extensive reviews on both aspects have not been frequently available. Also, subjects such as the basis and the state-of-the-art regarding the use of enzymes in therapy, decontamination, and remediation, as well as intellectual property, represent quite a new development in the present work.

Obviously we would like to express our deep recognition of the difficult, but fantastic work performed by all of the co-authors. One of us, Carlos Simões Nunes, has cooperated, for a longtime, with many of them in a very stimulating, constructive, and productive manner; they have become, after the start of shared work more than 30 years ago, very good friends. On this matter, we would like to mention the following in a very similar rank: Kjell Malmlöf, Glenn Monastersky, Petra Phillips, Guido Rychen, and Kurt Vogel.

We miss the contribution of two very important colleagues, for reasons that are independent of their personal choice. Both are highly personally respected colleagues and furthermore friends, with a stimulating capacity for discussions. They share a broad knowledge and experience of nutritional physiology and food and feed additives. We have always had, and continue have interesting exchanges with them, particularly on several perspectives of the development of new enzymes and the prospect of enzyme applications.

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Introduction

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INTRODUCTION

Enzymes are pivotal for the mechanisms which maintain all forms of life. To name just a few processes, photosynthesis, respiration, and homeostasis could not occur with necessary speed, without enzymes. Enzymes are proteins, and their importance in nature is indicated in human medicine by the fact that the mutation of one single base pair, leading to the disruption of the expression of just one protein-enzyme, can result in disabling metabolic disorders, or even death of the neonate. The power of these natural, chemical catalysts can be harnessed for industrial purposes; in the context of this chapter, most specifically for improving the speed and outcome of hydrolytic, digestive processes.

The definition of catalysis is to enable a reaction to proceed at an increased speed than it would otherwise. All processes catalyzed by enzymes would occur in their absence but at a much reduced rate. These activities may be synthetic, hydrolytic, or transformative, but at such a slow rate that it would not be of any value for the process under catalysis, whether it be to sustain life or for industrial conversion purposes. From the beginning of the reaction to the end, there is energy released which drives the reaction forwards, but in order to initiate the reaction a certain amount of energy has to be provided, the activation energy, in order to move the substrate from its current and probably stable state to a transition state. At this point, the forward reaction produces the product and the reverse takes it back to the substrate. The rate at which a reaction would proceed in the absence of the enzyme is dependent on the energy released in the conversion of the substrate to the product, and the activation energy needed to reach the transition state. Some reactions are incredibly slow due to a very high activation energy, which can also be related to how stable the substrate is, and a marginal release in energy in the whole reaction. In such cases, enzymes can speed up the reaction by an almost unimaginable rate. For example, the decarboxylation of orotidine-5′-phosphate to uridine monophosphate (a step in the pyrimidine synthetic pathway) would normally take millions of years, but in the presence of orotidine-5′-phosphate decarboxylase this takes place in milliseconds. This is the most extreme example of what enzymes are capable of, as it accelerates the uncatalyzed process by a factor of 10^{17}.

To put this into perspective, this is greater than the number of seconds that the universe has been in existence. Clearly most enzymes are not responsible for such extreme degrees of acceleration of a reaction, and indeed such feats would be problematic for most synthetic pathways where more than one enzyme is involved.

All enzymes in nature do not work in isolation, but as part of a co-ordinated process, or pathway, such that the product of one enzyme may become the substrate of another. Evolution has resulted in pathways that employ multiple enzymes in the transformation, synthesis, or hydrolysis of compounds into the desired outcome, and the successful integration of many if not all of the individual pathways involved in the process of life means that each process needs to be aware of the overall status and needs of the cell and indeed the whole being, whether it is a microbe or mammal. Thus each enzymatic pathway, which may involve tens of individual enzymes, has to be controlled in its overall rate and be able to change its speed if circumstances change and alter the requirements for its product. As a result, the enzymes which have evolved in nature are adapted to catalyze a reaction under the specific circumstances/conditions under which the organism lives, and as stated above, the rate at which it catalyzes the reaction will depend on the needs of the organism. Consequently, maximum speed of the reaction may not be the specific priority of a given enzyme if its involvement in a pathway is not a critical step. Most if not all enzymes are up- or downregulated by compounds which may or may not be related to the reaction it catalyzes in order to enable co-ordination of the whole pathway into all other pathways in the cell. This means that such enzymes may be optimally adapted to a specific set of conditions—temperature, pH, or ionic strength, for example, which is optimal for the organism. Such conditions may be significantly divergent from those in which industry currently employs enzymes. The first and most obvious difference is that most enzymes employed in industry are used in single-step processes, and as a result there is no need for integration of the enzyme into up- or downstream enzymatic processes. Secondly, the conditions under which industrial enzymes are employed are often hugely divergent from those from whence the enzyme originated, thus there is often significant room for improvement in their catalytic properties. Much of the development of enzymes in the feed or food industry has in fact focused on adapting them to function optimally under the conditions of the industrial process.

Enzymes can be categorized into six classes, as defined by the International Union of Biochemistry and Molecular Biology (Table 1). This is useful for nomenclature but also to describe the types of reactions catalyzed by enzymes.

As modern day industry evolved, it was noted that specific reactions may be better suited to include, or indeed be based upon, an enzymological rather than physicochemical process, and as a result the search for candidates began. In the beginning, microbial or organ-based extraction methods of the enzyme of interest were entirely dependent on the enzyme of interest being present in sufficient quantities to be of economic interest. Evolutionary pressures and selection techniques used in microbial fermentation processes were rudimentary, but nevertheless progress was made in evolving candidate enzyme characteristics to that they suited the industrial process needs more so than those of the organism.