AMINONews®

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Analysis

Immunity and inflammatory system: Basics

Key information

- Important initial barriers to infection are physical (e.g. the skin), enhanced by substances secreted by the body, such as saliva and tears, that contain molecules that can neutralize bacteria. The internal mucosal tissues (e.g. lungs and airways, gut, and reproductive tract) are coated with mucus that is able to trap potential infectants. In the airways, mobile ciliate hairs work together to transport contaminants away from vulnerable areas. Likewise, in the intestines, mucus and gut motility work together to transport potentially infectious organisms or materials away from susceptible tissues.

Life Cycle Thinking in Animal Production

Evonik´s sustainability journey to reducing crude protein in pig and broiler production

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Editorial

Dear Reader,

Animal production is facing new challenges in many parts of the world. This new issue of AMINONews® is addressing two of them: How to limit the use of antibiotics in feed and how to reduce the environmental footprint of animal production.

A good understanding of the immune system is a prerequisite to any decision when it comes to the change of management practices. Dr. Behnam Saremi has summarized some current knowledge with a special emphasis on the inflammatory response. The second article is the first part of a series of articles focusing on sustainability in animal production. In this article Dr. Michael Binder describes how to evaluate the ecological performance of animal production and how amino acid utilization can positively influence this performance.

Happy reading!

Vincent Hess

Research Highlights: Page 22–23

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- Immune system is classified to humoral and cell-mediated immunity, based on whether or not these responses are specific to a particular pathogen or mediated by immune cells designed to cope with general infections. It is also classified to innate and adaptive immunity. Innate immunity is important because it is non-specific and reacts immediately. Adaptive immunity takes longer to respond; however, it generates a memory of the required response to the initial infection. This will create a faster and more powerful response to a similar infection in the future.
- Inflammation is an adaptive response that is triggered by stimuli, such as infection and tissue injury. Classical inflammation is defined by the signs of redness, swelling, heat, and pain.
- Common methods of assessing systemic immune function include the measurement of: serum antibody titers, proportions of different blood cell subsets, serum concentrations of cytokines and other immune mediators and the weights of lymphoid organs, as well as morbidity and recovery from infectious disease.

Introduction

The study of animal immunology is of ever increasing importance to the feed additive industry. Nutrients themselves, such as amino acids, may play a significant role in the immune function of an animal. Additionally, alternative feed additives with direct effects on animal health such as probiotics, prebiotics, organic acids, and immune stimulants are continually being introduced to the market. Therefore, a clear understanding of the immune system will become essential to elucidating and differentiating the potential benefits of the available products in the feed additive industry. This article aims to provide a general introduction to what the immune system is, how it can be classified and the various functions therein. Additionally, the concept of inflammation will be discussed as this is a relevant parameter in gastrointestinal health of livestock species.

Immunology has its origins in the study of how the body protects itself against disease causing organisms known as pathogens. Pathogenic microorganisms can be bacteria, viruses, protozoa, fungi, and parasitic organisms, such as worms.

An important initial resistance to infection is physical barriers, for example the skin. These physical barriers are enhanced by secreted substances, for example in saliva and tears, that contain molecules with the ability to neutralize bacterial pathogens. The internal mucosal tissues (e. g. lungs and airways, and the gut) are aptly named as they are coated with mucus that is able to trap potential pathogens, toxins or debris. In the airways, mobile hair-like structures known as cilia work together to transport contaminants away from vulnerable areas. Tissues such as the skin, mucosal surfaces and airways also contain populations of immune cells that can respond to pathogens that breach these physical defenses.

The immune system can be classified into humoral and cell-mediated immunity. Humoral immunity is the aspect of immunity that is mediated by macromolecules found in extracellular fluids such as secreted antibodies and certain antimicrobial peptides. Cell-mediated immunity is an immune response that does not involve antibodies, but rather involves the activation of phagocytes, T and B cells.

While the humoral and cell-mediated classifications are still referenced a more modern and common classification divides the immune system into two branches; innate and adaptive. The innate immune system utilizes certain 'hard-wired' strategies to provide a rapid, general, response when alerted by certain typical signals of infection (essentially forming a first-line of defense). The adaptive immune system is able to develop highly specific responses (and a persistent 'immune memory') to target infection with extraordinary accuracy (Calder, 1995). Both systems work in close cooperation and, to an important extent, the adaptive immune system relies upon the innate immune system to alert it to potential targets, and shape its response to them. These two systems are highly inter-related through cytokines and signaling molecules (Li *et al.*, 2007).

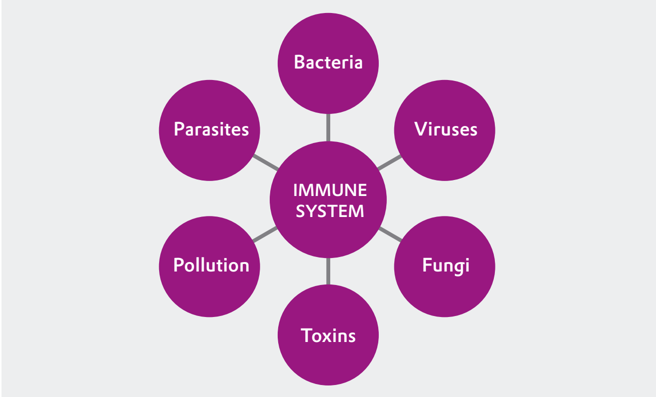
Pathogens

Pathogens are defined as any organism that can cause disease within a host. Pathogens are quite diverse and include bacteria, viruses, fungi, and parasites (Figure 1). It is therefore, unsurprising that pathogens also have diverse effects on the immune system. Some pathogens induce a strong activation of immune cells, such as many bacterial diseases. Conversely, pathogens such as parasitic worms hinder the activation of cells so that they can hide from the immune system and live in their host for years or even decades. Other pathogens like viruses have a high rate of mutation to create new strains in order to prevent the immune system from easily identifying them and developing a memory response. This is the main reason why humans and animals may be susceptible to getting the common cold every year. The viral pathogen responsible constantly mutates into new strains so that our immune system is surprised each year despite having already encountered similar pathogens in the past.

Immune tissues

The bone marrow, bursa of Fabricius and thymus are known as primary lymphoid tissues. All immune cells originate from a common stem cell type in the bone marrow and the bone marrow is also where a cell type known as B lymphocytes matures in mammalian species. In avian species this occurs in the specialized lymph organ known as the bursa of Fabricius, which is where B cells derive their name. T lymphocytes are named as such as unlike B lymphocytes these cells undergo maturation in an organ known as the thymus. Secondary lymphoid tissues, namely the lymph nodes, spleen and mucosa-associated lymphoid tissues (MALT) are important sites for generating adaptive immune responses and contain the lymphocytes (key adaptive immune cells). The lymphatic system is a system of vessels draining fluid (derived from blood plasma) from body tissues. Lymph nodes, that house lymphocytes, are positioned along draining lymph vessels, and monitor the lymph for signs of infection. The spleen essentially serves as a 'lymph node' for the blood (Mak and Saunders, 2006). MALT tissues are very important in mucosal immune responses as they are most exposed to various pathogens, and reflect the particular importance of the gut and airways in immune defense.

Figure 1 A list of pathogens as common immune system stimulators.



Immune cells

The immune cells of the blood, known as leukocytes can be categorized into two groups. The first group is referred to as granulocytes for the presence of granules in their cytoplasm or polymorphonuclear leukocytes, referring to the various shapes of their multi-lobed nuclei. The other group of leukocytes are referred to as agranulocoytes or mononuclear leukocytes as comparatively they have a lack of cytoplasmic granules and a uniform non-lobed nucleus (Janeway *et al.*, 2001).

Granulocytes consist of three different cell types; neutrophils, eosinophils and basophils. However, in common parlance, the term polymorphonuclear leukocyte often refers specifically to neutrophil granulocytes as they are the most abundant of the three. Neutrophils are involved in engulfing and neutralizing pathogenic organisms as well as inducing inflammatory responses to pathogens. The second most abundant granulocytes are the eosinophils that are involved in allergic inflammation, play a role in the response to parasitic worm infections and are important to the recruitment and survival or different agranulocytes. Basophils are the least abundant of the granulocytes and like eosinophils are also mostly involved in allergies and the response to parasite infections.

Another granular type of immune cell is the mast cell. Mast cells are very similar in both appearance and function to the basophil, but while basophils circulate in the blood mast cells reside in the tissues (e.g. in mucosal tissues). Mast cells can play a role in both the innate and adaptive immune systems. They contain many granules rich in histamine and heparin and are therefore best known for their role in allergy and anaphylaxis. However, mast cells play an important protective role as well, being intimately involved in wound healing and early inflammatory defense against pathogens (Stone *et al.*, 2010).

The two types of agranulocytes in the blood circulation are lymphocytes and monocytes. Monocytes are immature, undifferentiated cells that circulate in the blood and are drawn into the tissues in response to different attractant molecules (chemokines or cytokines) during infection. In the tissues these cells then differentiate into macrophages that can activate lymphocytes, engulf and neutralize different pathogens and induce inflammation (Mak and Saunders, 2006). Other subsets of macrophages are not attracted from the circulatory system, but reside in the tissues (for instance the immune tissues of the gut) are aptly named resident macrophages. These resident macrophages play a role in maintaining homeostasis in times without stress, but also sample the surrounding areas for pathogens and offer a first line of defense when they sense danger.

Dendritic cells, like monocytes are agranular and play some similar roles. However, while in some species particular subsets of dendritic cells circulate in the blood, they are generally more prominent in various immune tissues. Dendritic cells are members of the innate immune system that are particularly efficient at stimulating lymphocytes, like T cells, to respond to antigen (Mak and Saunders, 2006;Banchereau and Steinman, 1998). Dendritic cells develop in the bone marrow and migrate to the tissues in an immature form. Immature dendritic cells efficiently take up antigens from the environment, but they are poor activators of T cells until they differentiate into specific subsets and are mature (Caetano Reis e Sousa 2006).

The other type of agranulocytes is lymphocytes. While lymphocytes are a type of mononuclear leukocyte that circulates in the blood, they are much more common in the lymphatic system and tissues. Lymphocytes include B cells, natural killer cells (NK cells) and T cells. B cells are so named as they mature in the bone marrow in mammals or in an immune organ known as the bursa of Fabricus in birds. B cells are responsible for making antibodies that bind to pathogens to enable their destruction. They are important in the development of immune memory to different pathogens and are therefore necessary in building a protective antibody response after vaccination.

Natural killer cells are a type of cytotoxic lymphocyte critical to the innate immune system. The role NK cells play is to kill cells of the body that are infected by intracellular pathogens, like viruses (Janeway *et al.*, 2001). Like B cells, NK cells possess a unique 'memory' system which allows them to remember past invaders and prevent disease when a similar invader is encountered again (Janeway *et al.*, 2001).

T cells are a very diverse type of lymphocyte with many different subsets involved in many different functions. As was previously mentioned T cells are derived from immature cells in the bone marrow, but mature in the thymus where T cells with reactivity to host molecules undergo a programmed cell death to prevent them from attacking tissues of the host organism and causing autoimmune disease.

Cytotoxic T cells are often referred to as CD8+ T cells as they express a marker known as CD8 on their surface. Analogous to the function of NK cells in the innate system, cytotoxic T cells in the vertebrate adaptive immune response are also able to respond to intracellular pathogens, like viruses, that invade host cells. However, in comparison to NK cells that provide a rapid response to infected cells, acting at around 3 days after infection, cytotoxic T cells take longer to be initiated in the response.

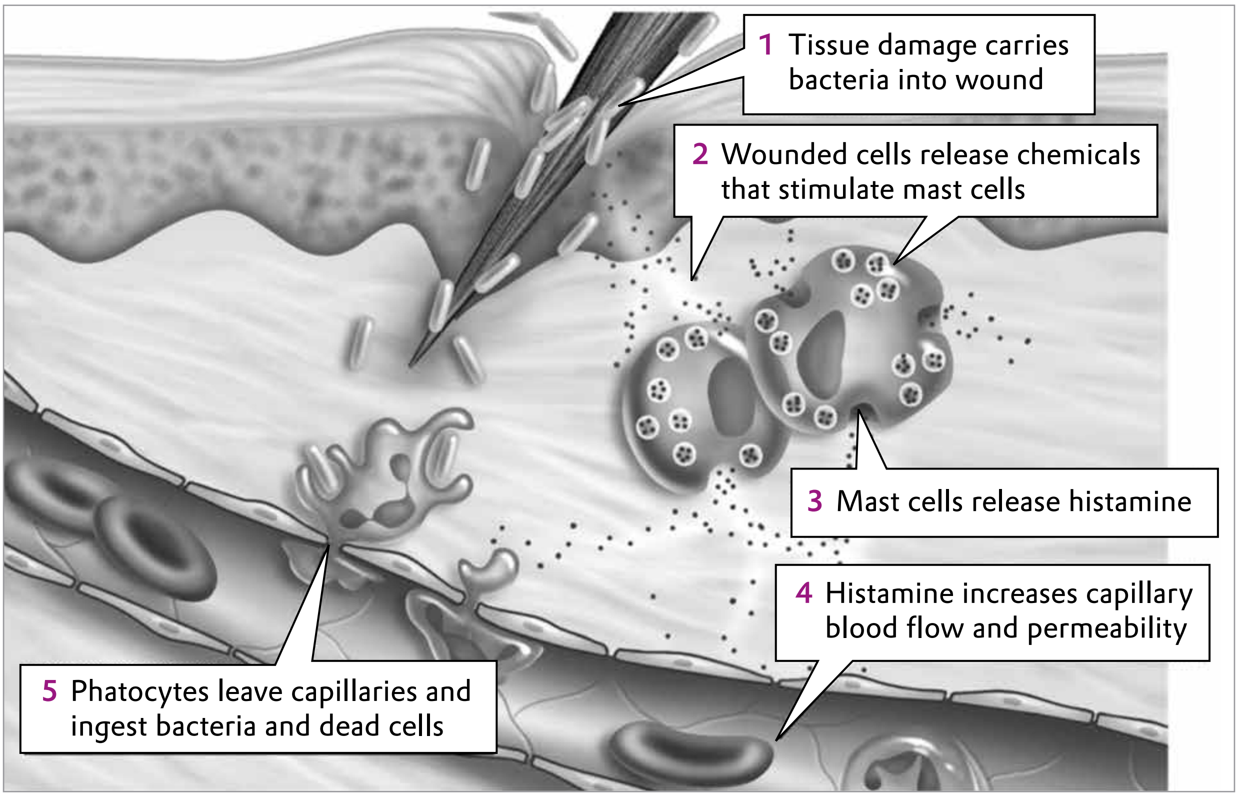
T helper cells (Th cells) are also often called CD4+ T cells referring to the presence of a marker known as CD4 that they express on their surface. These T cells co-ordinate the immune response between the innate and adaptive immune responses. They are also important in initiating antibody production by B cells and supporting B cells with memory function.

Regulatory T cells (Treg cells), formerly known as suppressor T cells, also express the CD4+ marker. They are crucial for the maintenance of immunological tolerance. Their major role is to shut down T cell-mediated immunity toward the end of an immune reaction and to suppress any auto-reactive T cells that escaped the selection process in the thymus to prevent an immunological response to self-antigens.

Inflammation

Underlying many physiological and pathological processes, inflammation is an adaptive response that is triggered by adverse stimuli and conditions, such as infection and tissue injury (Medzhitov, 2008). Classical inflammation is defined by the signs of redness, swelling, heat, and pain. In response to inflammatory stimuli, the body often increases the release or expression of inflammatory mediators that are critical for both vascular changes and leukocyte infiltration (Newton and Dixit, 2012). The primary immune cells involved are part of the innate immune systems first line of defense. These immune cells and the mediator molecules they release form complex regulatory networks that result in increased blood flow to the infected tissue, and the infiltration and activation of immune cells from both the innate and adaptive systems (Figure 2). These cellular interactions can stimulate systemic responses, including increased body temperature, increased heart rate, and decreased appetite (Dantzer and Kelley, 2007).

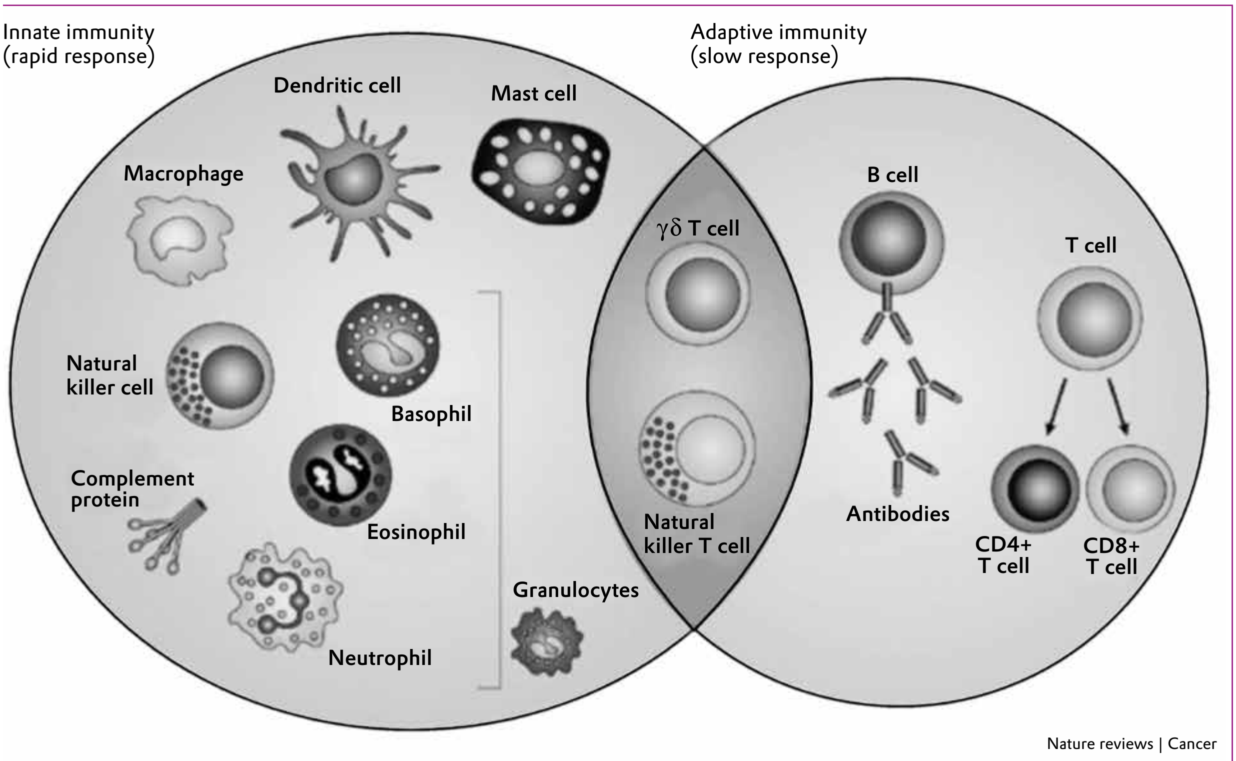
Figure 2 Schematic presentation of acute phase process (Audesirk *et al.* 2008).



Innate immune system

The innate immune system is so named as it developed relatively early during evolution and therefore at least some form of this immune system is present in all animal species. It offers the only line of defense in evolutionarily simple species like shrimp, and a first line of defense in more evolutionarily advanced organisms like vertebrate fish, poultry, and mammals such as swine, cattle and humans. The cells of the innate immune system are not specific to particular pathogens. They, therefore, offer a rapid and widespread response to invading microbes. However, this non-specificity has a major disadvantage as it means that the innate system largely lacks the ability to produce a memory to infections. When infection cannot be fully cleared by the innate immunity over a short period, the adaptive immune system (in relevant species) is activated to destroy infectious agents (Li *et al.*, 2007). The innate immune system consists of physical barriers (e.g. skin, the endothelial cell layer in the respiratory tract, and the gastrointestinal tract) as well as various mononuclear agranulocytes (e.g. monocytes/macrophages, dendritic cells, NK cells), polymorphonuclear granulocytes (e.g. neutrophils, eosinophils and basophils) and mast cells (Figure 3). Many other factors such as platelets which are important for clotting and antibacterial enzymes in secretions like tear lysozymes are also involved in the innate system (Li *et al.*, 2007).

Figure 3 Schematic presentation of innate and adaptive immune cells (Dranoff, 2004).



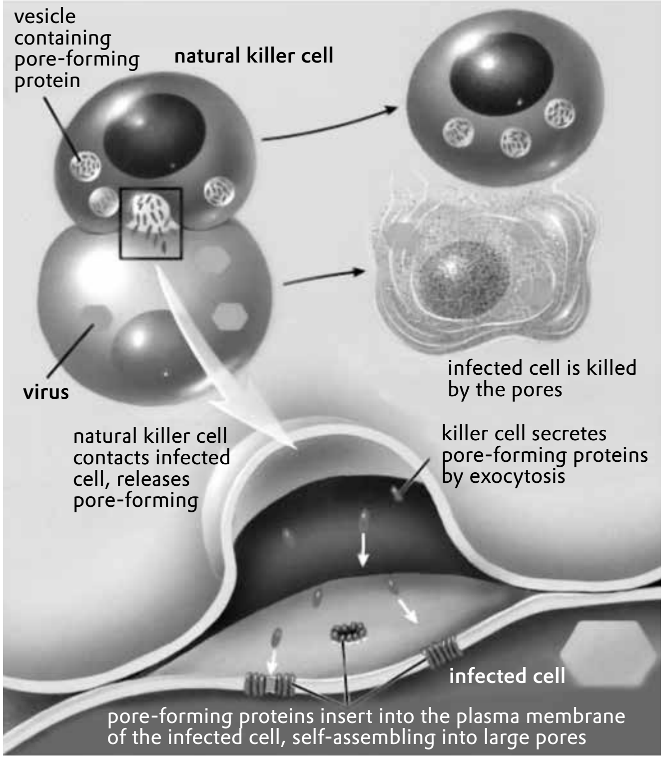
The innate immune system as a first line of defense is capable of disabling many pathogens. For example, macrophages and neutrophils are known for engulfing and killing a variety of different pathogens. However, while macrophages may kill many cells, neutrophils die upon consumption. Likewise, when natural killer cells contact infected cells, they release pore-forming proteins which are preserved in vesicles within their cytoplasm. These pore-forming proteins then disable the cell wall causing the infected cell and hopefully the pathogen to die (Figure 4).

Adaptive (acquired) immune system

As might have been guessed from the description of innate immunity, the innate system is only the first line of defense in evolutionarily advanced animal species like vertebrate fish, poultry, swine, cattle and humans; because these organisms have evolutionarily adapted to have additional defense systems. This acquired system becomes effective only within days after the initial stimulation. This adaptive immune system, while not as fast acting to an initial pathogen infection as the innate system, is able to form a very specific memory response to pathogens. This allows for an even stronger, more rapid and specific, response upon re-infection with the same pathogen, that the innate system alone would not be able to provide. Vaccines make use of the adaptive system by exposing the body to pieces of pathogens or pathogens that have either been killed or inactivated so that a memory response can be formed to these dangerous infectious agents without the need for a real infection. This allows the body to respond quickly and efficiently to these specific pathogens if they ever do encounter them.

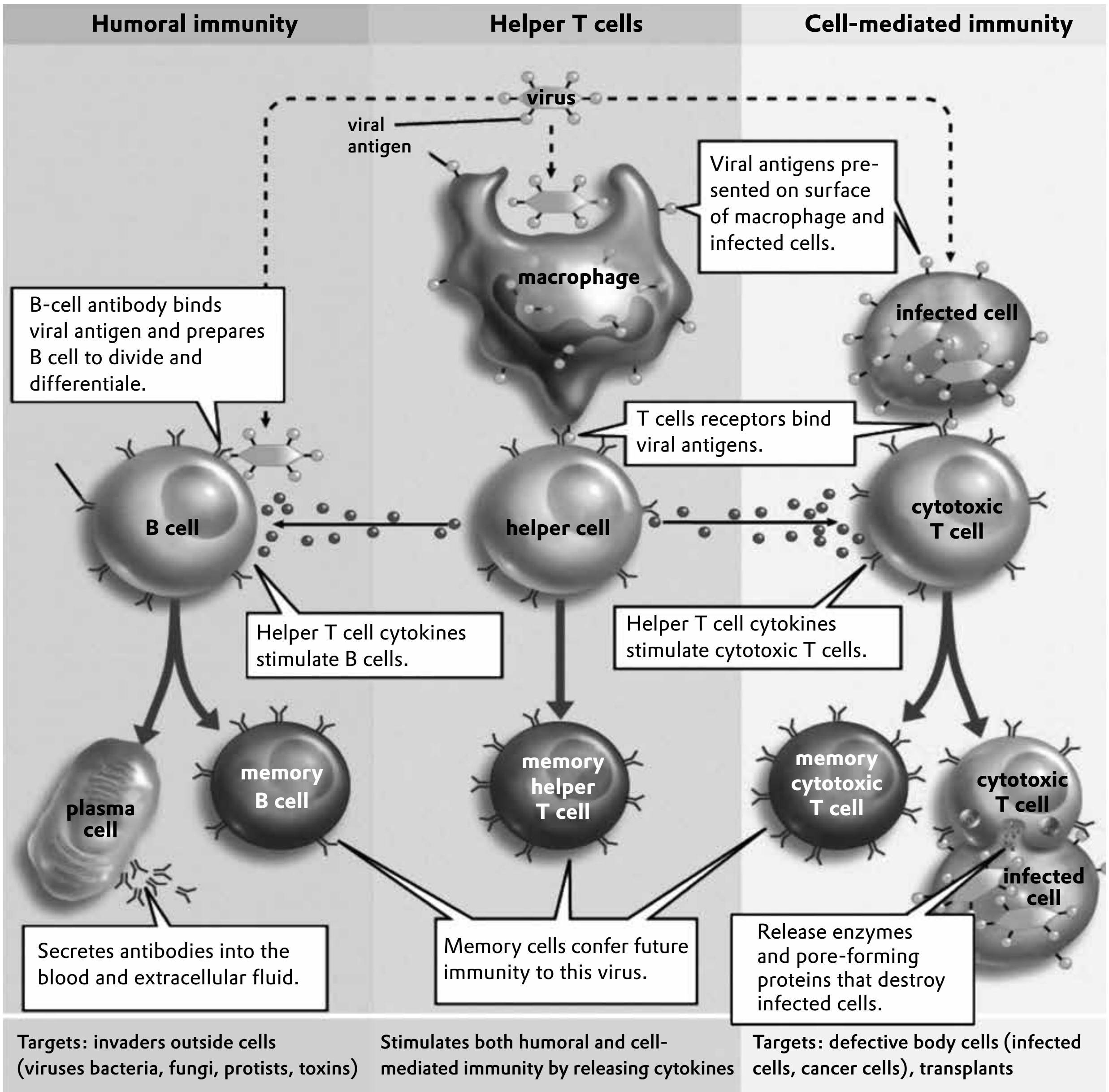
The adaptive (acquired) immune system (Figure 3) mainly consists of T lymphocytes and B lymphocytes (Calder and Yaqoob, 1999). The high specificity of the acquired immune response is because each lymphocyte carries surface receptors for a single pathogen specific molecule, known as an antigen. B lymphocytes are unique in their ability to produce and release specific antibodies in the humoral immunity. The antibodies can immobilize and neutralize microorganisms (including viruses) or toxins by binding to them. Conversely specific antibodies can activate protein systems in the blood that signal innate cells to engulf the antibody bound pathogen and destroy it.

Figure 4 Natural killer cells attack infected body cells using proteins and enzymes to lyse cells (Audesirk *et al.* 2008).



T lymphocytes are also involved in the adaptive immune response to pathogens. Like B cells, each T cell has receptors for a single specific antigen (pathogen molecule). However, unlike B cells, T cells do not produce antibodies. T cells can support B cells by activating them to produce antibodies or instructing them what kind of antibody to produce. Additionally T cells can coordinate specific types of responses best suited to parasite, bacteria, or viral infections by producing chemical messengers known as cytokines. While antibodies are highly effective against extracellular pathogens like many bacterial infections, T cells mechanisms are more effective when host cells are infected with intracellular pathogens (e.g. viruses and certain bacteria). These infections can be cleared by cytotoxic T lymphocytes that recognize fragments of pathogens that the host cell presents on its surface as a kind of SOS (Li *et al.*, 2007). T cells, like B cells, are also important in generating a memory after each case of pathogenic exposure (Figure 5). This will create a faster and more powerful response to a similar infection in future.

Figure 5 Creation of memory after immune response to pathogens (Audesirk *et al.* 2008).



Innate and adaptive (acquired) immune system regulation

The innate immune system is an ancient and diverse collection of defenses, including the recognition of pathogens through the use of germline-encoded pathogen receptors. The adaptive immune system, encompassing T and B cell responses, is a more recent development that utilizes somatically recombined antigen receptor genes to recognize virtually any antigen. The adaptive immune system has the advantage of flexibility and immunological memory but it is completely dependent upon elements of the innate immune system for the initiation and direction of responses (Clark and Kupper, 2005). Appropriate innate and acquired immune system interactions lead to highly efficient recognition and clearance of pathogens, but maladaptive interactions between these two systems can result in harmful immunological responses including allergy, autoimmunity, and allograft rejection (Wu, 1995; Field, 2005).

Innate and acquired immune systems are regulated by a highly interactive network of chemical communications, which includes the synthesis of the antigen-presenting machinery, immunoglobulins and cytokines (Calder, 2006).

Assessments of immune function

It is likely that nutrients influence several or all aspects of the immune system. Additionally, the alternative feed additives, like probiotics, have known effects on the immune system. Thus, there are multiple, complex methods for assessing immune function in individuals, depending on experimental conditions, the availability of analytical facilities and the investigator's interest (Calder and Yaqoob, 1999; Field, 1996).

The classic functional measurements in live animals (referred to as *in vivo*) include:

(1) The amount of time and type of response induced after the skin is exposed to an antigen (pathogen particle) – This gives the researcher indications as to what arm (innate or adaptive) of the immune system is stimulated by the antigen.

(2) Serum antibody titers – This allows the observer to measure the amount of antibodies produced in response to primary or secondary immunizations or infections.

(3) Blood levels of different lymphocyte subsets as well as serum concentrations of cytokines and other immune mediators – The quantity and proportions of different cells and their mediators can give the observer an indication as to the type of immune response that is being mounted and therefore the type of infection or stimulation that has occurred.

(4) The weights of immune organs – As immune cells proliferate the organs that house them often increase in size to accommodate this. Therefore the weight of immune organs gives an indication as to the severity of the infection and/or response. Additionally, it can help the observer to determine if the immune response is only local at a specific site of infection (e.g. the intestine, and gut draining lymph nodes) or if it is systemic throughout the entire body (e.g. affecting the spleen or liver).

(5) Morbidity and recovery of an animal from an infectious disease.

Assays of immune function in laboratory cell experiments (referred to as *in vitro*) often consist of:

(1) The metabolism of immunocytes – This is a clear indicator of activation of a specific immune cell after stimulation.

(2) The proliferation and activation of lymphocytes in response to different stimuli can help to determine what kind of immune cells respond and how strong the response is to the stimuli.

(3) Cell morphology and apoptosis - The shape and viability of different cell types can be investigated as an indicator of their respective health, maturation and function.

(4) The ability of monocytes and macrophages to phagocytose (engulf) and destroy particles or microbes.

(5) The production of antibodies, cytokines or cytotoxic substances by different immune cells in response to specific stimuli.

Conclusion

Herein, we provided basic knowledge about the immune system and its elements (innate and adaptive immune system) and a list of common cells and their functions. Indeed, the interrelationship between the innate and adaptive immune system and the assessment of the immune system is complex. However, a general description of the various parameters of the immune system is provided in an attempt to introduce a field that is becoming increasingly more relevant to the feed additive industry.

Acronyms

MALT	Mucosa-associated lymphoid tissues
NK cells	Natural killer cells
Th cells	T helper cells
Treg cells	Regulatory T cells

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Life Cycle Thinking in Animal Production

Evonik´s sustainability journey to reducing crude protein in pig and broiler production

Abstract

Consequent crude protein reduction in pig and poultry feed leads to improved sustainability performance by adding increased amounts of supplemental amino acids. Life Cycle Assessments (LCAs) are now becoming the new world standard supported by Non-Government Organizations (NGOs) and the industry for sustainability assessment of livestock production. These observations are reflected again by the latest Evonik LCA study within the series of reports since 2002.

Introduction

Feed additives such like amino acids are effective measures to lower the amount of resources – land, feed, water and energy – leading to the production of healthy, nutritious food for a growing world population. This technique further saves thousands of acres of forest and pasture that would otherwise have to be transformed into cropland. Sustainable food production, guided by the responsibility to increase resource productivity while reducing environmental impact, is seen as the innovative solution for the future. One element of that solution is the further consequent reduction of the crude protein in animal diets using supplemental feed amino acids (FAO, 2015[1]; Makkar Harinder and Ankers, 2014[2]).

Agricultural production of meat and milk products for human nutrition is increasingly blamed as one of the main sources of detrimental greenhouse gases. Due to this, increasingly more in-depth discussions are taking place with regards to changes in the consumer´s behavior leading to less consumption of products of animal origin. At the same time the world population is steadily growing, and this combined with a steady increase of wealth in numerous emerging markets, is resulting in the growing demand of meat and milk products. Evonik started more than 10 years ago to investigate the use of amino acids in animal production through the standardized method of life cycle assessment following the ISO14044:2006 standard. Currently Evonik is able to present the fourth life cycle assessment study (February 2015) assessing and reporting on the continuous sustainability improvement of Evonik's amino acids and production processes.

This new study also highlights the benefits of the next step in protein reduction by introducing the new feed amino acid ValAMINO®, which has been included in the assessment of the pig and poultry production.

[1] FAO (2015): Animal Performance of feed supply chains, Guidelines for assessment.
[2] Makkar Harinder, P. S., P. Ankers (2014): Towards sustainable animal diets: A survey-based study Livestock Production Systems Branch, Animal Production and Health Division, Food and Agriculture Organization of the United Nation (FAO).

The concept of the present approach on evaluating the ecological performance of the use of feed amino acids in animal nutrition is also now the current technology at an international level and will be used as an industry standard in future[3]. Both go along with the international initiatives designed for improving sustainable performance of animal feeding and livestock performance such as the Global Agenda of Action (GAA) of the Food and Agriculture Organization of the United Nations (FAO) or the Product Environmental Footprint Category Rules (PEFCR) Initiative of the European Commission.

Material and methods

LCA

The study intends to be a comparative LCA from cradle to grave respectively farm gate in line with the requirements defined under ISO 14040 ff. As the study was published, it was accompanied by an independent critical review.

The study is based on specific product category rules (PCRs) set for assessment of feed additives with respect to specialty feed ingredients in livestock production (IFIF FEFANA, 2014, in progress). The scientific basis for these PCRs is a LCA study report conducted by the industry and a scientific council accompanied with a critical review panel in 2014 (IFIF-FEFANA, 2014)[3].

Additionally these PCRs are part of other standards for the assessment of environmental impacts of livestock production respectively feed production. A draft was issued in 2014 for public consultation, the final version will be published later 2015[4].

Products

Methionine, lysine, threonine, tryptophan and valine are the five first limiting essential amino acids in animal production. Methionine as the first limiting amino acid in typical compound feed for poultry has a particular importance. Lysine is the first limiting amino acid in pig nutrition and plays a particularly important role here. Threonine and also valine are further limiting amino acids for both species. Further, tryptophan plays another important role in pig nutrition. It is of utmost importance that the respective daily amino acid requirement for each species is fully covered in order to guarantee a healthy and well balanced nutrition. Otherwise a distinct drop in performance and a detrimental effect on the animal's health will occur.

Feed formulation and functional unit

To compare several feeds as the different options when following the LCA methodology, the overall principle of the so-called functional equivalence must be given. This means that all options under investigation must provide the same nutritional minimum value to meet the animals' demand for a certain performance level and health. The different elements of the functional equivalence are the same minimum amount of essential amino acids, same minimum amount of other nutrients such as vitamins, trace elements, minerals etc. and also the same minimum amount of metabolizable energy.

The individual steps in the definition of the specific functional unit (FU) for the supplementation of amino acids for animal nutrition are described in the following figures and explanations (Evonik, 2015[5]). For simplification purposes, the examples are focusing on a broiler feed only supplemented with MetAMINO® and a small number of basic feed ingredients.

Accordingly, the simplified explanations are also valid for any other amino acid premix and an increased number of feed ingredients per diet. Detailed information on the different feeds for pigs and broilers are given in the overview tables of the Annex.

The supplementation of feed amino acids helps to close the gap in the nutritional balance. Thus, the deficient situation given is the overall basic scenario to support the animal with the least amount of the recommended metabolizable energy and other nutrients. The missing amount of methionine can be now compensated by either adding another diet supplemented with MetAMINO® or through another methionine rich feed based on other ingredients such like

[3] IFIF-FEFANA (2014): Report LCA on the role of Specialty Feed Ingredients on Livestock Production´s Environmental Sustainability.
[4] FAO (2015): Environmental performance of animal feeds supply chains, Guidelines for quantification.
[5] Evonik (2015): Comparative life cycle assessment of MetAMINO®, Biolys®, ThreAMINO®, TrypAMINO® and ValAMINO® in broiler and pig production.

soybean meal (SBM) or rapeseed meal as the main component. Since the oilseeds provide also additional nutritional value beside the methionine content, MetAMINO® is also supplemented together with an additional amount of wheat to balance the nutritional content compared to the oilseed option (Figure 1). This is again to meet the functional equivalence as already explained earlier in that chapter.

The differences between Feed 1-A, Feed 2-A and Feed 3-A, respectively, is the net amount of additional feed necessary to compensate the methionine deficiency (Figure 2). Since the basic scenario was counted in all three options, it will not be longer displayed in the further considerations in Figure 3. This is the basis to define the FU of 1.0 kg of methionine or 1.0 kg of amino acid premix, respectively. To achieve that, the amount of wheat from the supplemented solution (Feed 1) is counted negative in the Feeds 2 and 3 (Figure 3).

Following this concept, 4 scenarios (options) have been set up to build the FU for the different comparisons (Table 1). The compositions of the different feeds are given in the Tables 1 to 4 in the Annex.

Figure 1 The basic feed formulations for the breakdown of the FU for LCA comparison. The basic diet "A" provides the minimum amounts of nutrients and metabolizable energy, but a lack of methionine. Feeds 1 to 3 provide the additional amounts of methionine from different sources.

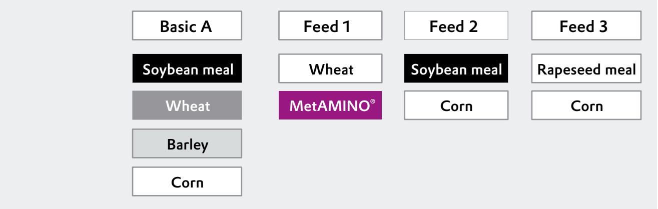


Figure 2 The first step to calculate the FU. The difference is made with the basic feed formulation "A" with the different options from feeds 1 to 3 to display the net difference of the different approaches.

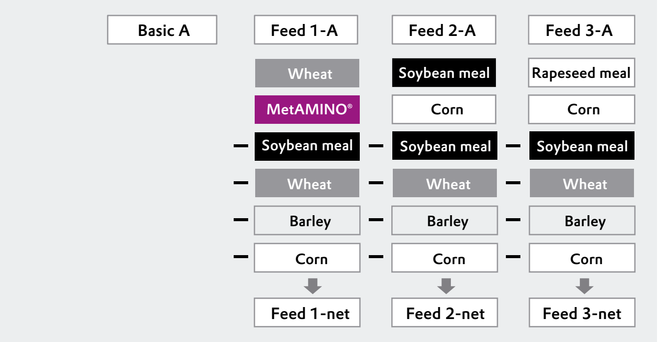


Figure 3 The final FU in terms of the LCA methodology is achieved through the difference of the amount of wheat from the other diets. Thus, the real impacts of 1.0 kg of MetAMINO® can be compared with the adequate amount of methionine covered through other sources.

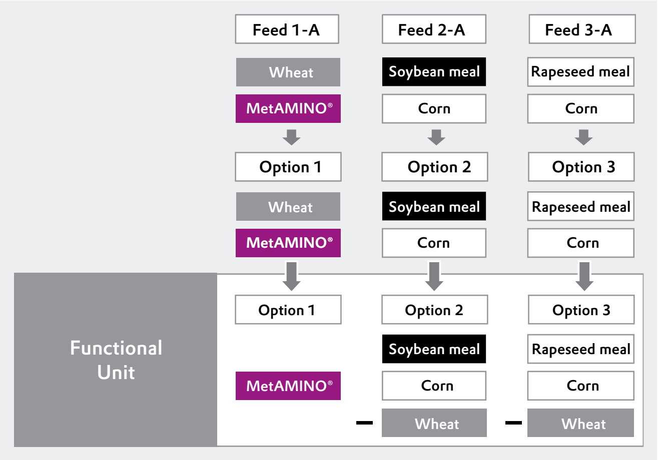


Table 1 Alternative options for broiler and pig feeding. All 4 options for broilers and, respectively, 3 options for pigs, reflect the nutritional minimum requirements for feeding (nutrients, metabolic energy). The amino acids are provided either through supplemental amino acids or extended amounts of vegetable sources such like soybean meal or rapeseed meal.

Pigs	Description
Option 1	Supplementation with the 5 amino acids MetAMINO®, Biolys®, ThreAMINO®, TrypAMINO® and ValAMINO® with a what/barley basal diet
Option 2	Compound feed based on SBM without amino acid supplementation
Option 3	Compound feed based on rapeseed meal without amino acid supplementation
Broilers	**Description**
Option 1	Supplementation with the 4 amino acids MetAMINO®, Biolys®, ThreAMINO® and ValAMINO® with a corn basal diet
Option 2	Compound feed based on rapeseed meal without amino acid supplementation
Option 3	Compound feed based on SBM without amino acid supplementation
Option 4	Compound feed based on SBM only with MetAMINO® supplementation

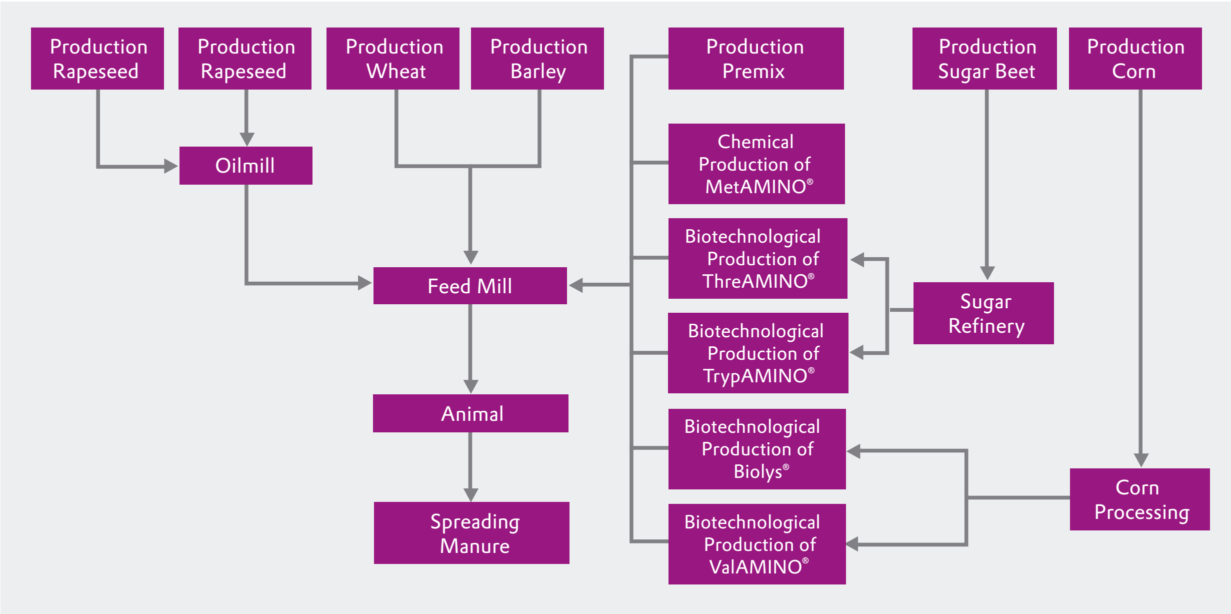
tilizer for agricultural production, the harvesting and processing of the agricultural raw materials as well as the transportation of all feed ingredients, raw materials and intermediates, through to including all emissions related to the feeding and distribution of manure. Figure 4 provides an insight into all the levels of the LCA.

Impact categories

The evaluation of impact as per the family of ISO-Norms 14040-44 is the combination of the parameters of the life cycle inventory with those environmental categories for which they make a contribution to today's knowledge.

Focusing the parameters of the life cycle inventory to a few relevant environmental categories helps in concentrating the multitude of the single parameters of the life cycle inventory. Thus, in line with the previously conducted studies, the current report focuses again on a few, but important, environmental categories for the specific application of amino acids in animal production and performance:

Figure 4 System boundaries (cradle to grave respectively farm gate) for the options analyzed in broiler and pig feeding.



System boundaries

All system boundaries for the different scenarios equivalent to the 3, respectively 4, compound feed options follow the principle „from cradle to grave“, i.e. they start from providing the raw materials used for production of the supplemental amino acids, the cultivation of the feed raw materials, the manufacturing of the mineral fer-

The environmental impact categories global warming potential (GWP), acidification potential (AP) and europhication potential (EP) have been evaluated using the CML-methodology [CML 2001] with updated characterization factors of April 2013. The results are summarized and discussed in the following paragraphs. Consideration of global warming potential excluding biogenic carbon has been added compared to earlier studies to stay in line with international and national standards such as PAS 2050[7].

Results

Global warming potential

The option 1 in the scenario with the pig feeding clearly shows with only 4 kg CO_2e/FU that the supplemental amino acid option is the more environmental friendly solution compared to the other options with 79 kg CO_2e/FU, respectively 66 kg CO_2e/FU (see Figure 5-1).

The GWP including biogenic carbon of option 1 with the supplemented essential amino acids (EAA) in broiler feed was very low (3 kg CO_2e/FU) as compared to the reference scenarios option 2, 3 and 4 (Figure 5-2). Option 2 with rapeseed as a local oil seed alternative shows a value of 53 kg CO_2e/FU which is much lower than the result for option 3 with 107 kg CO_2e/FU. This large deviation is caused by greenhouse gas emissions from land use change (LUC) occurring at soybean cultivation in Brazil and Argentina. Option 4 with 56 kg CO_2e/FU can be compared to option 3 as both diets are based on (SBM). The lower GWP of option 4 shows the effect of adding only MetAMINO® as first limiting amino acid to a SBM based broiler diet compared to option 3.

Table 2 Relevant environmental impact categories considered in the study.

Category		Dimension
Global warming potential, including biogenic carbon	(GWP100)	[kg CO_2- equiv.]
Global warming potential, excluding biogenic carbon *	(GWP100)	[kg CO_2- equiv.]
Acidification potential	(AP)	[kg SO_2- equiv.]
Eutrophication potential	(EP)	[kg Phosphate-equiv.]
Primary energy demand *	(PED)	[MJ]
Consumption of resources *		[kg Crude oil-equiv.]

* Results not shown in the present publication, but assessed and documented in the full study[6].

Eutrophication potential

The AP and the EP are dominated by the nitrogen based emissions of the agricultural plant and animal production. Accordingly, the picture for EP is similar to the category of AP. The current study looks at EP in total and does not split into terrestrial and aquatic EP. Option 1 (Figure 6-1) with the amino acids supplementation in pig production only contributes very low levels of 0.02 kg PO_4e/FU. Option 2, the standard mix (0.33 kg PO_4e/FU) and option 3, the rapeseed/soya alternative (0.34 kg PO_4e/FU) had an EP approximately 17 fold higher than option 1. This is well in line with the results of the earlier studies (IFEU, 2004; Evonik, 2010).

Figure 5-1 Global warming potential GWP100 [CML 2001] of pig production.

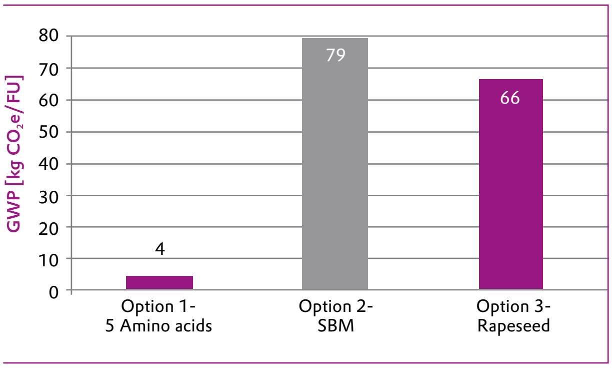
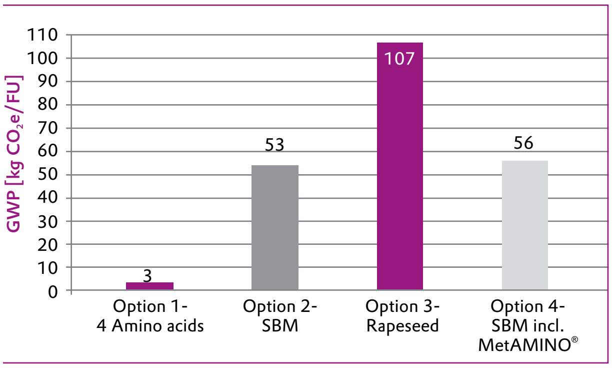


Figure 5-2 Global warming potential GWP100 [CML 2001] of broiler production.



[6] Evonik (2015): Comparative life cycle assessment of MetAMINO®, Biolys®, ThreAMINO®, TrypAMINO® and ValAMINO® in broiler and pig production.
[7] PAS 2050 (2011): Specification for the assessment of the life cycle greenhouse gas emissions of goods and services, page 9.

Similar to the impact categories addressed before option 1 with the supplemented amino acids, the broiler scenario again shows a distinct environmental advantage with only 0.02 kg PO_4e/FU (Figure 6-2). The options with the amino acids from oilseed sources are substantially worse with values of 0.78 kg PO_4e/FU for option 2 and 0.59 kg PO_4e/FU for option 3 (Figure 6-1). The supplementation with methionine only (option 4) shows advantages regarding EP of 0.32 kg PO_4e/FU. The reasons are again higher nitrogen emission from animal production where increased levels of oilseeds were used to satisfy the amino acid demand (Figure 6-2). Also the emissions from agricultural production and fertilizer use in plant cultivation contribute significantly to the EP, especially for production of wheat and soybean meal and oil. Regarding to the FU there is again a credit for EP from corn in option 2, 3 and 4.

Figure 6-1 Eutrophication potential EP [CML 2001] of pig production.

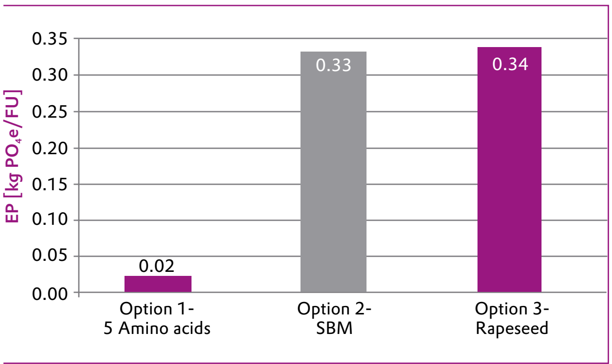
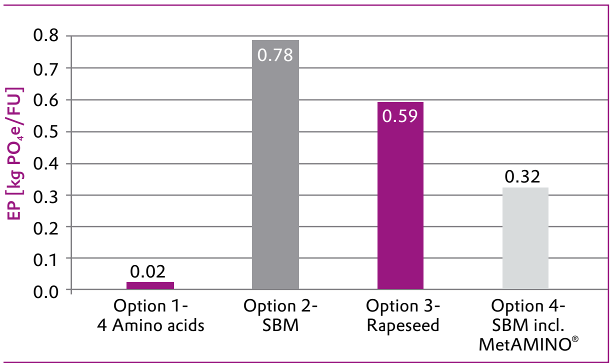


Figure 6-2 Eutrophication potential EP [CML 2001] of broiler production.



Acidification potential

For the AP, the pig production again displays a comparable trend as seen for EP, but depending on the different macro ingredients, the level of impact is slightly different (Figure 7-1).

As compared with the options 2 and with increased oilseed use, option 1 with the supplemented amino acids again has a very low value with 0.06 kg SO_2e/FU AP, while option "rapeseed" has 1.14 kg SO_2e/FU and "SBM" 1.24 kg SO_2e/FU (Figure 7-1).

As compared with the options 2, 3 and 4 in broiler production with increased oilseed use, option 1 with the supplemented amino acids again has a very low value with 0.06 kg SO_2e/FU AP, while option "rapeseed" shows 2.12 kg SO_2e/FU and "SBM" 1.65 kg SO_2e/FU (Figure 7-2). Option 4 shows a significantly lower AP of 0.90 kg SO_2e/FU caused by including MetAMINO®.

The influence of higher nitrogen emissions of the options soybean meal "SBM" and "rapeseed" are even more striking for AP compared to EP. While the scenario of supplementation with the amino acid mix contributes only marginally (0.06 kg SO_2e/FU) to the AP, the options 2 and 3 with oilseeds as alternatives have a 19 to 20 fold higher AP in pigs and in broilers even more.

Impact of land use change on GWP

In line with the earlier study from 2010 the aspect of LUC was evaluated again. In the meantime, this topic has gained increasing popularity and importance in the discussion on renewable raw materials for biofuels and bio based products during the last years.

The base scenario, assumed as a reference situation for soya production in South America, included a certain extent of LUC and the value was set at 52.9 %. In an additional sensitivity analysis a varying percentage of soya grown in the respective regions was studied (from 0 to 70.4 %)[8]. Indirect LUC was not considered as the methodology and the mode of calculation is still the subject of scientific discussions.

The LUC primarily affects emissions relevant for the climate factors which then has an impact on GWP (see Figure 8 for broiler production). The major effect is caused by the degradation of biomass stored in the soil releasing the CO_2 fixed in the soil.

[8] Evonik (2015): Comparative life cycle assessment of MetAMINO®, Biolys®, ThreAMINO®, TrypAMINO® and ValAMINO® in broiler and pig production.

Figure 7-1 Eutrophication potential AP [CML 2001] of pig production.

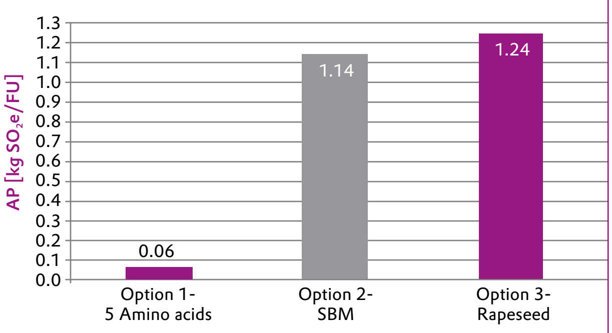
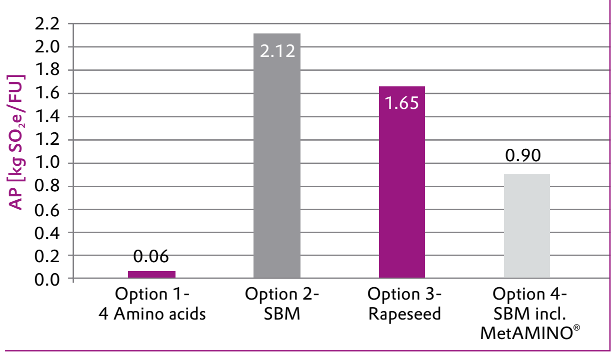


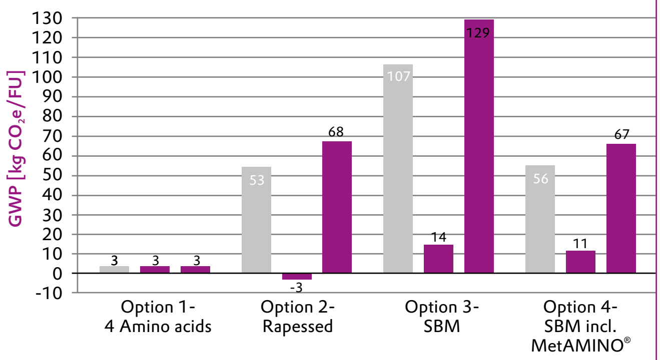
Figure 7-2 Eutrophication potential AP [CML 2001] of broiler production.



The range assumed that soya does not have an impact on the scenario "amino acids" as no SBM is included in the FU for this option. The GWP for option 1 remains unchanged accordingly at a level of 3 kg CO_2e/FU. Assuming no direct LUC emissions at all in option 2, the GWP reduces by approximately 56 kg CO_2e/FU to a level of – 3 kg CO_2e/FU while increasing the area of LUC brings GWP to a level as high as 68 kg CO_2e/FU. The corresponding values for option 3 vary between 14 kg and 129 kg CO_2e/FU, and for option 4 between 11 kg and 67 kg CO_2e/FU respectively.

Assuming no LUC for soybean cultivation can refer to a better performance in GWP for option 2. This seems to be reasonable as biotechnological production of amino acids is an energy intensive progress compared to crop production. Including biogenic carbon, the background dataset for rapeseed meal shows a lower carbon footprint than for SBM even without any emissions from LUC. Thus, using rapeseed instead of SBM leads to a reduced overall GWP result.

Figure 8 GWP [CML 2001] from broiler production – sensitivity analysis for "land use change soya"



Conclusions

The evaluation based on the updated process data and compound feed mixes shows that the precise adjustment of the amino acid content in the compound feed for broiler and pig production by supplementing amino acids provide distinct environmental advantage over the reference scenarios. These results are in line with the earlier results from the studies several years ago (IFEU, 2002[9]; IFEU, 2004[10]; Evonik, 2010[11]). As further demonstrated, the inclusion of ValAMINO® as the next limiting amino acid in pig and broiler production as a supplement to the final compound feed is the next innovative step for further improvement of the environmental performance of livestock production.

As already proven in the different previous studies, the most significant improvements can be achieved for the impact categories EP and AP. Supplemental amino acids play the major role in

[9] IFEU (2002): Ökobilanz für DL-Methionin in der Geflügelmast, Endbericht, Seiten 99-122.
[10] IFEU (2004): Ökobilanz für den Einsatz von DL-Methionin, L-Lysin und L-Threonin in der Geflügel- und Schweinemast, Endbericht, Juli 2004, Seiten 141-190.
[11] Evonik (2010): Comparative life cycle analysis of DL-Methionine, L-Lysine, L-Threonine and L Tryptophan in broiler and pig production July 2010.

the nitrogen metabolism, thus, the reduction of the nitrogen flow is the main effect and in consequence excretion of nitrogen based emission to water and soil are significantly impacted. Impacts respectively reduction potentials on GWP are related to process improvements or the handling of fewer raw materials either in the amino acid production or the feed milling producing the compound feed. As already pointed out in the previous studies, the interpretation of the environmental impact categories again showed that some assumptions made for the agricultural production may have a significant impact on the results. For instance, the GWP of the reference scenarios depends to a significant extent from the LUC for soya production. The data sets for soya including LUC assume a „depreciation" over a period of 20 years for the LUC emissions which are caused by converting the original vegetation into culture land. This way of handling the LUC emissions was chosen due to the current methodological discussions on LUC in LCA (Finkbeiner, 2014a[12]; Finkbeiner, 2014b[13]). The extent of nitrogen emissions from animal production, storage and spreading manure is another assumption that has a large influence on the LCA for the categories EP and AP. Due to the large differences between the agricultural systems for broiler and pig production the results vary dramatically.

As another effect animals need less feed for an equal performance, which also reduces the impact on GWP due to the reduced feed volumes, and thus, fewer raw materials needed for feed formulation and also reduced transportation activities, especially for imported oilseeds from overseas.

The additional supplementation of the new product ValAMINO® as the next limiting amino acid in animal nutrition clearly continues that effect, which could be properly demonstrated in the present study.

Without the use of supplemental amino acids, an effective and more sustainable animal protein production is simply not feasible. These findings reflect also the current publications on LCA reports within the animal feed and protein production (Mosnier *et al.*, 2011[14]; Garcia-Launay *et al.*, 2014[15]; Gallo *et al.*, 2014[16]; Makkar Harinder and Ankers 2014[17] and Kebreab Ermias, 2015[18], in progress).

Thus, the comprehensive results of Evonik´s series of LCA studies and the above mentioned scientific studies build a sound basis for the monitoring of the ecological benefits for the environment through advanced feeding technologies.

However, to monitor and improve daily feed formulations in feed milling and farm applications, ready to use tools would be helpful to analyze the ecological footprint of each individual diet. Such a tool for implementing theoretical standards into daily business can be seen in AMINOFootprint® 2.0 from Evonik. This tool is now the second generation of a web-based application for desk computers, laptops and tablets. It focuses on calculating the impact of feed ingredient production and transportation as well as crude protein reduction in pig and poultry diets. The updated version now also considers the animal performance contributing to the ecological score linked to the amount of compound feed consumed respectively life weight produced. As another new option, the tool now allows to simulate different phases of production for pigs and broilers, which are typically different in feed composition and nutritional value, and thus also in their ecological impact. A detailed description of AMINOFootprint® 2.0 will follow soon.

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[12] Finkbeiner, M. (2014a): The International Standards as the Constitution of Life Cycle Assessment: The ISO 14040 Series and its Offspring, in: LCA Compendium - The Complete World of Life Cycle Assessment –Volume 1: Background and Future Prospects in Life Cycle Assessment, W. Klöpffer (ed.), Springer: Dodrecht, The Netherlands, pp. 85-106, ISBN 978-94-017-8696-6.

[13] Finkbeiner, M (2014b): Indirect land use change: Help beyond the hype?. Biomass and Bioenergy, 62, 218-221.

[14] E. Mosnier, H. M. G. van der Werf, J. Boissy and J.-Y. Dourmad (2011): Evaluation of the environmental implications of the incorporation of feed-use amino acids in the manufacturing of pig and broiler feeds using Life Cycle Assessment.

[15] F. Garcia-Launay, H. M. G.van der Werf, T. T. H. Nguyen, L. LeTutour, J. Y.Dourmad (2014): Evaluation of the environmental implications of the incorporation of feed use amino acids in pig production using Life Cycle Assessment.

[16] L. Gallo, G. Dalla Montà, L. Carraro, A. Cecchinato, P. Carnier, S. Schiavon (2014): Growth performance of heavy pigs fed restrictively diets with decreasing crude protein and indispensable amino acids content.

[17] Makkar Harinder, P. S., Philippe Ankers (2014): Towards sustainable animal diets: A survey-based study Livestock Production Systems Branch, Animal Production and Health Division, Food and Agriculture Organization of the United Nation (FAO).

[18] Kebreab Ermias, Alexander Liedke, Dario Caro, Sabine Deimling, Michael Binder, and Matthias Finkbeiner: Environmental impact of specialty feed ingredients use in swine and poultry production: A life cycle assessment Mitigating environmental impact in European and American animal production.

Abbreviations

AP	Acidification Potential
EAA	Essential Amino Acids
EP	Eutrophication Potential
FAO	Food and Agriculture Organization
FU	Functional Unit
GAA	Global Agenda of Action
GWP	Global Warming Potential
LCAs	Life Cycle Assessments
LUC	Land Use Change
NGOs	Non-Governmental Organizations
PCRs	Product Category Rules
PEFCR	Product Environmental Footprint Category Rules
SBM	Soybean Meal

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Appendix

Diets

Table 1 Diet options of broiler production.

Feed raw materials, kg	Option 1 "4 amino acids"	Option 2 "Rapeseed"	Option 3 "SBM"	Option 4 "SBM incl. MetAMINO®"
Wheat		41.89	30.57	26.69
SBM	29.10	29.26	38.01	32.89
Soya oil	5.15	10.99	8.24	7.01
Extracted rapeseed meal		15.00		
Corn	61.97		20.00	30.00
Dicalciumphosphate	1.64	1.34	1.55	1.57
$CaCO_3$	0.75	0.62	0.71	0.74
Premix Blank Poultry	0.50	0.50	0.50	0.50
Salt	0.31	0.28	0.29	0.29
Sodium carbonate	0.10	0.12	0.12	0.12
Amino acids				
MetAMINO® (99.0 %)	0.24			0.19
Biolys (54.6 % L-Lysine)	0.18			
ThreAMINO® (98.5 %)	0.04			
ValAMINO® (98.0%)	0.02			

Table 2 Diet options grower phase 1 of pig production.

Feed raw materials, kg	Option 1 "5 amino acids"	Option 2 "SBM"	Option 3 "Rapeseed"
Wheat	45.05	41.01	25.22
Barley	30.75	11.95	24.03
SBM (48 % CP)	10.83	26.18	24.25
Extracted rapeseed meal			4.53
Corn-DDGS	2.92	15.00	15.00
Soya oil	4.27	3.63	
Rapeseed oil			4.53
Vitamin Mineral Premix	0.50	0.50	0.50
Dicalciumphosphate	0.58	0.36	0.41
$CaCO_3$	3.85	1.24	1.15
Salt	0.19	0.12	0.12
Amino acids			
MetAMINO® (99.0 %)	0.13		
Biolys® (54.6 % L-Lysine)	0.71		
ThreAMINO® (98.5 %)	0.17		
TrypAMINO® (98.0 %)	0.01		
ValAMINO® (98.0 %)	0.03		

Table 3 Diet options grower phase 2 of pig production.

Feed raw materials, kg	Option 1 "5 amino acids"	Option 2 "SBM"	Option 3 "Rapeseed"
Wheat	45.05	43.49	14.60
Barley	39.22	15.02	40.00
SBM (48 % CP)	8.20	22.07	20.38
Extracted rapeseed meal			3.99
Corn-DDGS		15.00	15.00
Soya oil	1.51	2.34	
Rapeseed oil			3.98
Vitamin Mineral Premix	0.50	0.50	0.50
Dicalciumphosphate	0.50	0.25	0.35
$CaCO_3$	3.89	1.20	1.08
Salt	0.21	0.13	0.11
Amino acids			
MetAMINO® (99.0 %)	0.11		
Biolys® (54.6 % L-Lysine)	0.65		
ThreAMINO® (98.5 %)	0.15		
TrypAMINO® (98.0 %)	0.01		
ValAMINO® (98.0 %)	0.02		

Table 4 Diet options finisher phase of pig production.

Feed raw materials, kg	Option 1 "5 amino acids"	Option 2 "SBM"	Option 3 "Rapeseed"
Wheat	45.05	31.50	31.50
Barley	40.00	40.00	40.00
SBM (48 % CP)	1.80	18.04	18.04
Extracted rapeseed meal			
Corn-DDGS	6.39	7.46	7.46
Soya oil	3.33	1.16	
Rapeseed oil			1.16
Vitamin Mineral Premix	0.50	0.50	0.50
Dicalciumphosphate	0.26	0.19	0.19
$CaCO_3$	1.28	0.99	0.99
Salt	0.43	0.17	0.17
Amino acids			
MetAMINO® (99.0 %)	0.08		
Biolys® (54.6 % L-Lysine)	0.71		
ThreAMINO® (98.5 %)	0.17		
TrypAMINO® (98.0 %)	0.01		
ValAMINO® (98.0 %)	0.001		

Fish

Dietary methionine level affects growth performance and hepatic gene expression of GH–IGF system and protein turnover regulators in rainbow trout (*Oncorhynchus mykiss*) fed plant protein-based diets

Marine Rolland, Johanne Dalsgaard, Jørgen Holm, Pedro Gómez-Requeni and Peter V. Skov

The effects of dietary level of methionine were investigated in juvenile rainbow trout (*Oncorhynchus mykiss*) fed five plant-based diets containing increasing content of crystalline methionine (Met), in a six week growth trial. Changes in the hepatic expression of genes related to i) the somatotropic axis: including the growth hormone receptor I (GHR-I), insulin-like growth hormones I and II (IGF-I and IGF-II, respectively), and insulin-like growth hormone binding protein-1b (IGFBP-1b); and ii) protein turnover: including the target of rapamycin protein (TOR), proteasome 20 delta (Prot 20D), cathepsin L, calpains 1 and 2 (Capn 1 and Capn 2, respectively), and calpastatin long and short isoforms (CAST-L and CAST-S, respectively) were measured for each dietary treatment. The transcript levels of GHR-I and IGF-I increased linearly with the increase of dietary Met content ($P < 0.01$), reflecting overall growth performances. The apparent capacity for hepatic protein degradation (derived from the gene expression of TOR, Prot 20D, Capn 1, Capn 2, CAST-L and CAST-S) decreased with increasing dietary Met level in a relatively linear manner. Our results suggest that Met availability affects, directly or indirectly, the expression of genes involved in the GH/IGF axis response and protein turnover, which are centrally involved in the regulation of growth.

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Part B 181; 2015: 33–41

Ducks

Effect of dietary methionine content on growth performance, carcass traits, and feather growth of Pekin duck from 15 to 35 days of age

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A study was conducted to establish the response of Pekin ducks to dietary Met from 15 to 35 d age. Experimental diets were formulated to contain 0.35, 0.45, 0.55, 0.65, and 0.75% Met (0.30, 0.39, 0.45, 0.56, and 0.68% on an analyzed basis, respectively) and 0.3% cysteine (0.25, 0.27, 0.26, 0.26, and 0.28% on an analyzed basis, respectively). Each diet was fed to 10 pens of 55 ducks/pen. Carcass yields and feather growth were determined at 28 and 35 d. Results showed that feeding 0.30% Met (0.55% Met+Cys) significantly impaired ADG, feed-to-gain (F:G) ratio, breast meat yield, and feather growth in comparison to the other dietary treatments ($P < 0.05$). BW, ADG, F:G, carcass and breast meat weight and yield, breast skin and subcutaneous fat weight and yield, the fourth primary wing feather length, and feather coverage showed significant quadratic broken-line or quadratic polynomial response to increasing dietary Met ($P < 0.05$). From 15 to 28 d age, the optimal Met requirement for the BW, breast meat yield, and the fourth primary wing feather length were 0.510, 0.445, and 0.404 %, respectively, based on quadratic broken-line model, and correspondingly were 0.606, 0.576, and 0.559 % by quadratic regression. For ducks from 15 to 35 d age, the optimal Met requirement for BW, breast meat yield, and feather coverage were 0.468, 0.408, and 0.484 %, respectively, by quadratic broken-line model, and 0.605, 0.564, and 0.612 %, by quadratic regression, respectively.

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Poultry

Oxidative damage to poultry: from farm to fork

M. Estévez

Poultry and poultry meat are particularly susceptible to oxidative reactions. Oxidation processes have been for decades the focus of animal and meat scientists owing to the negative impact of these reactions on animal growth, performance, and food quality. Lipid oxidation has been recognized a major threat to the quality of processed poultry products. The recent discoveries on the occurrence of protein oxidation in muscle foods have increased the scientific and technological interest in a topic that broadens the horizons of food biochemistry into innovative fields. Furthermore, in recent years we have witnessed a growing interest in consumers on the impact of diet and oxidation on health and aging. Hence, the general description of oxidative reactions as harmful phenomena goes beyond the actual impact on animal production and food quality and reaches the potential influence of oxidized foods on consumer health. Likewise, the current antioxidant strategies aim for the protection of the living tissues, the food systems, and a potential health benefit in the consumer upon ingestion. Along these lines, the application of phytochemicals and other microelements (Se, Cu) with antioxidant potential in the feeds or directly in the meat product are strategies of substantial significance. The present paper reviews in a concise manner the most relevant and novel aspects of the mechanisms and consequences of oxidative reactions in poultry and poultry meat, and describes current antioxidant strategies against these undesirable reactions.

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Poultry

The mRNA expression of amino acid transporters, aminopeptidase N, and the di- and tri-peptide transporter PepT1 in the embryo of the domesticated chicken (*Gallus gallus*) shows developmental regulation

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The mRNA expression profile for 10 amino acid transporters, the di-and tri- peptide transporter (PepT1), and aminopeptidase N (APN) during chick embryogenesis was determined. Fertilized eggs were sampled at d 9, 11, 15, 17, 19, and 20 of incubation. Three to 4 embryos were sampled at each time period. At d 9 and 11, the entire intestine was collected due to its undifferentiated appearance. The ceca, duodenum, midgut, and liver were sampled at d 15, 17, 19, and 20. Gene expression was measured using absolute quantitation quantitative reverse-transcription PCR. In the liver, all genes except for PepT1 were expressed at most time points. At d 9, only the expression of Na+-independent cationic amino acid transporter 1, Na+-independent cationic amino acid transporter 2, and excitatory amino acid transporter 3 was detectable in the intestine, but by d 11, all genes associated with transporters of the basolateral surface were expressed, and at higher levels than genes associated with brush border transporters. By d 15, all of the genes tested were expressed in the duodenum, midgut, and ceca at high levels that remained relatively constant until d 20. Statistical analysis shows that at d 15, 17, 19, and 20 there is a significant interaction between the 2 main effects (days of incubation and region of the gut); therefore, it is likely that gene expression in different regions of the gut is dependent on the age of the embryo. At d 9 and 11, the gut may not function in amino acid uptake from the lumen and possibly relies on other structures such as the yolk sac. As the gut matures and protein becomes available in the lumen, amino acid transporters become highly expressed in all parts of the intestine. The data suggest that by d 15 of embryo development the gut may be capable of amino acid absorption.

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