REVIEW: II - DIET AND IMMUNITY : CURRENT STATE OF KNOWLEDGE AND RESEARCH PROSPECTS FOR THE RABBIT

FORTUN-LAMOTHE L., DROUET-VIARD F.[1]

Station de Recherches Cunicoles, INRA, BP 27, 31326 CASTANET TOLOSAN. France

[1]Station Bio-Agresseurs Santé Environnement, INRA, 37380 NOUZILLY. France

ABSTRACT: Feeding plays an important role on the immune system of the animals. Nutriments contained in food can have a direct role on immunity while being used as substrates for the cellular proliferation and the synthesis of the effector and informant molecules. They can also have an indirect role: the nutritional status acts on immunity throughout the neuroendocrine system and some nutrients (unsaturated fatty acids and vitamins A, D and E) have an immunomodulatory action. In addition to the nutriments that it contains, the food can affect the sensitivity of the animals to the infections by modifying the intestinal flora, the attachment capacity of the micro-organisms and the intestinal epithelium integrity. In addition, some molecules not having a major nutritional function, such as carotenoids, can modulate the immune answer. Lastly, the food can be a vector for molecules or organisms that have a role on immune system (glucans, probiotics, mycotoxins). If the feeding can affect the immune system, reciprocally, a stimulation immune system (in case of infectious disease for example) modifies the nutritional needs of the sick animal. The whole of the data concerning the interactions between feeding and immunity obtained in other species make it possible to propose interesting perspectives of research in rabbit. Indeed, the control of digestive pathologies is one of the major difficulties of the rabbit breeding and appropriate feeding could contribute to improve immune capacity of the animals.

Key words: rabbit, immunity, nutrition, diet, health.

RÉSUMÉ: Revue: II - Alimentation et immunité: état des connaissances et perspectives de recherches pour le lapin.
L'alimentation joue une rôle important sur le système immunitaire des animaux. Les nutriments contenus dans l'aliment peuvent avoir un rôle direct sur l'immunité en servant de substrats pour la prolifération cellulaire et la synthèse des molécules effectrices et informatrices. Ils peuvent également avoir un rôle indirect : le statut nutritionnel agit sur l'immunité par l'intermédiaire du système neuroendocrinien tandis que certains nutriments (acides gras polyinsaturés et vitamines A, D et E) ont une action immunomodulatrice. L'aliment peut également affecter la sensibilité des animaux aux infections en modifiant la flore intestinale, la capacité d'attachement des microorganismes aux entérocytes et l'intégrité de l'épithélium intestinal. Par ailleurs, certaines molécules n'ayant pas de rôle nutritionnel majeur, tels que les caroténoïdes, peuvent moduler la réponse immunitaire. Enfin, l'aliment peut servir de vecteur à des molécules ou à des organismes qui ont un rôle sur le fonctionnement du système immunitaire (glucanes, probiotiques, mycotoxines). Si l'alimentation peut affecter le système immunitaire, réciproquement, une stimulation mettant en jeu la réponse immune (maladie infectieuse par exemple) modifie les besoins nutritionnels de l'animal malade. L'ensemble des données concernant les interactions entre alimentation et immunité obtenues sur les autres espèces permettent de proposer des pistes de travail intéressantes chez le lapin. En effet, la maîtrise des pathologies est une des difficultés majeures de l'élevage cunicole et une alimentation adéquate pourrait contribuer à renforcer les défenses immunitaires des animaux.

**Mots clés:** immunité, lapin, santé, nutrition, alimentation.

INTRODUCTION

The prevention and control of diseases, and especially digestive diseases, is one of the main difficulties of rabbit breeding. Hence, in French rabbit farms which use cyclic production, we can deplore a mortality rate of about 24% between birth and sale (RENACEB results, 1999; GUERDER, 2001). This mortality is the cause of large financial loss to the breeder. The losses associated with chronic diseases, such as prolongation of the fattening period or declines in food conversion rate, are more insidious and much more difficult to quantify but nevertheless real. Because of this high disease risk, the use of medicaments is a widespread practice, not necessarily effective and always expensive. At the same time there is greater and greater pressure from the European Union to reduce the use of antibiotics in animal production. In this context, one of the possible preventative approaches is to improve the non-specific immunity resistance of rabbits. For this purpose the installation of microflora barriers or even modification of the diet are alternative solutions, which may be envisaged to reinforce the animals' immunological defences. Indeed, it has been known for a long time that, through its role in modifying the immune response, the diet can alter the susceptibility of individuals to infectious diseases.

Our knowledge of the nutritional requirements of bred animals allows us to make tables of feeding

Needs for production
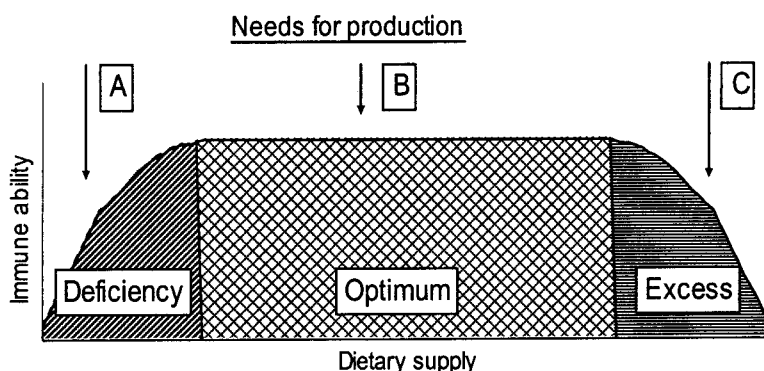
Figure 1: Relations between the nutrional needs for production and immune system functionning. Example: for one nutrient, the nutrionnal need for growth (indicated with an arrow) could be lower (A), equal (B) or higher (C) than those for an optimal functionning of immune system (from KLASING and LESHCHINSKY, 2000).

recommendations which define the minimum rate of incorporation of each nutrient in relation to the physiological stage of the animal for most species receiving rational husbandry (INRA, 1989). These requirements are generally determined from studies called "in range" which, for the nutrient studied, enable the content needed for maximum growth or reproduction, in the absence of clinical signs of deficiency or excess, to be determined. In these studies, the influence of the factor studied on the resistance of the animal to infections is rarely taken into consideration. The content of a specific nutrient which maximises growth or reproduction does not necessarily induce the greatest defence capacity in the animals (see figure 1). It is therefore of interest to take a fresh look at the nutritional requirements of the animals for good immune resistance. The aim of this article is to present the interactions between the different nutrients and the immune system and we will present the effect of a challenge to the immune system (an infectious disease, for example) on the modification of nutritional requirements of the sick animal. The studies carried out on the rabbit in the field of interactions between nutrition and immunity are unfortunately extremely rare. For this reason we will report mainly on work carried out on other species. We will try to find out which of these interactions might be worth studying in the rabbit.

Remarks: 1/ in this article the term immunity is taken in its wide sense, and includes natural non-specific resistance to infections; 2/ we have deliberated omitted to recall basic knowledge on the immune system functions and we advise

the reader to refer to DROUET-VIARD and FORTUN-LAMOTHE (2002) for the information on this topic.

MECHANISMS OF NUTRITIONAL EFFECTS ON IMMUNITY

Several mechanism are distinguishable whereby nutrients may affect immunity (KLASING and LESHCHINSKY, 2000 ; see table 1).

A direct role in the supply of substrates
The animal has to find in its food, even during foetal life, sufficient amounts of substrates 1/ at the time of establishment of the immune system (before and just after birth), 2/ in the immuno-surveillance phase in the mature immune animal and 3/ at the time of the immune response to a challenge by a pathogenic agent, allergen etc.

In the rabbit, the immune system is established during foetal life and continues to mature during the first weeks of life. It has been shown for other species that nutritional deficiencies or excesses during this period (notably in linoleic acid, vitamin A, iron, selenium and several of the B group vitamins) can affect the resistance capacity of animals. One should recall that the nutritional status of the foetus (and thus of the newborn) and the unweaned young animal, particularly as regards reserves of vitamins and trace elements, are dependent on that of its mother. Consequently, the determination of nutritional requirements for optimum development of the immune system should be made with regard to the feeding of the female during gestation and lactation.

If one adds together all the structural components of the lymphatic organs, the immune

Table 1: Mechanisms of nutritional modulation of immune response (from KLASING, 1998).

Mechanism	Implicated nutrients
Supply of substrates	All the nutrients
Immunomodulating action, modification of intra and inter-cellular communication	Vitamins A, D, E, polyunsaturated fatty acids
Change of hormonal milieu	Energy, proteins
Reduction of pathology associated with immune response	Vitamin E, selenium
Nutritional immunity	Iron, biotin, zinc (?)

26

system does not represent more than 3-4% of the total body weight, making it a "pseudo-organ" of low weight compared with the intestines, the muscles, or the nervous system (KLASING, 1998). Thus, in the chicken, the mass of the leucocytes and immunoglobulins produced each day (about 800mg/kg live weight) represents less than 1% of the weight gain of a 2-week old animal. Furthermore, when there is a possibility of competition (i.e. if the food supply is less than the total requirements), it has been shown that the immune system takes a high priority compared with other organs. It is known, for example, that lymphocytes use the same glucose transporter as the neurones and erythrocytes, which is insensitive to insulin or to IGF-I, thus permitting transport even in the fasting animal. The presence of this type of transporter indicates that the lymphocytes take priority over other cells such as those of muscles or the liver for the utilisation of glucose. The same phenomenon is observed for amino acids. These two factors, a small total requirement and a high priority, mean that the satisfaction of the nutritional substrate requirements of

the mature animal in the immuno-surveillance phase is not generally a major nutritional problem, especially in livestock breeding where general severe deficiencies are rare (KLASING and LESHCHINSKY, 2000).

During an infection episode, the requirements change because many substrates (amino acids, glucose, fatty acids etc) and enzyme co-factors (vitamins, minerals etc) are needed by the sick animal to respond effectively to the attack. These substrates play a role in the clonal proliferation of lymphocytes, the recruitment of new monocytes and heterophils and in the synthesis of effective molecules (immunoglobulins, nitric acid, lysozyme etc) and messenger molecules (cytokines and eicosanisoids). But they allow the animal, in particular, to respond to the increased hepatic utilisation of amino acids. In fact most immune responses begin with an inflammatory response which involves the synthesis of significant quantities of inflammation proteins by the liver. This sudden
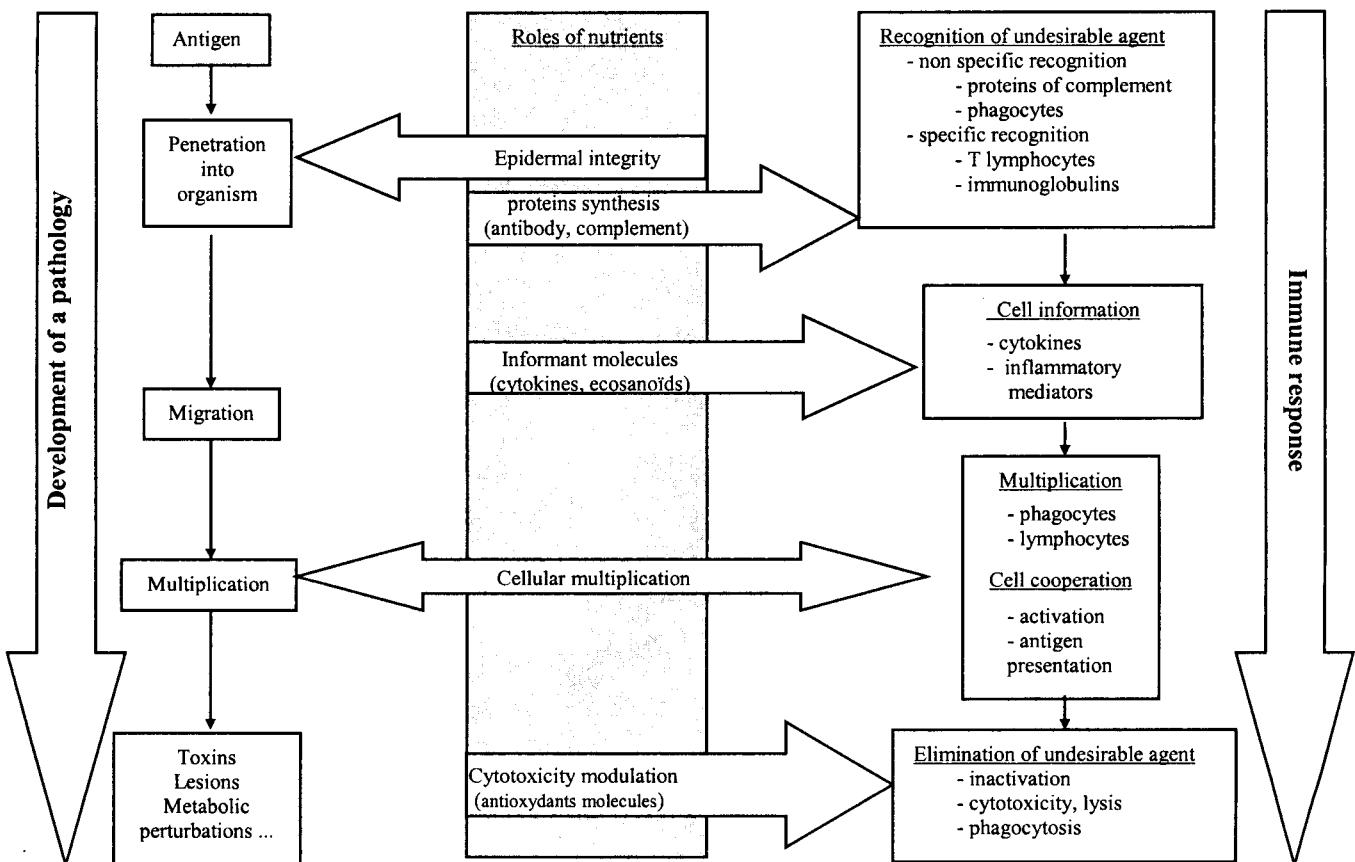
Figure 2: Directs (substrates supply) and indirects roles (modulation of infection or of immune response) of nutrients on immune response. Remark : this figure does not indicate some time axis, and differents steps of pathology developpement or immune response which face together are not necessarily contemporary.

increase in the requirements is partly satisfied by the liberation of nutrients, such as amino acids and micronutrients, from other tissues (muscle and bone catabolism). However the real cost of all these operations is not known for any species.

Immunomodulating action of certain nutrients

The immune response brings into play numerous cell populations, which communicate between themselves by means of a great numbers of molecules, such as cytokines. Certain nutrients, either essential or non-essential (such as polyunsaturated fatty acids, vitamins E, A and D) can affect the immune response by modifying the intra- and extra-cellular communication pathways (see figure 2). For example, the amount and profile of fatty acids in the diet determines the type of fatty acids which are

incorporated into the cell membranes and hence the fluidity of the membranes and the type of eicosanoid secreted as informant molecules (see chapter B-2). Similarly, vitamin E reduces the secretion of prostaglandins and modulates the secretion of cytokines by the stimulated leucocytes, while vitamin A increases the specific response of T lymphocytes.

The activation of cells of the immune system, such as the cytotoxic T cells, NK (natural killer) cells, macrophages and neutrophiles leads to the production of large numbers of destructive molecules, such as reduction products of oxygen, nitric oxide, hydrolases etc., which remain inside the cells or may be secreted. These molecules are cytotoxic and kill pathogenic micro-organisms and the infected cells, but they can also affect the neighbouring host tissues, creating tissue lesions. The oxidation index of polyunsaturated
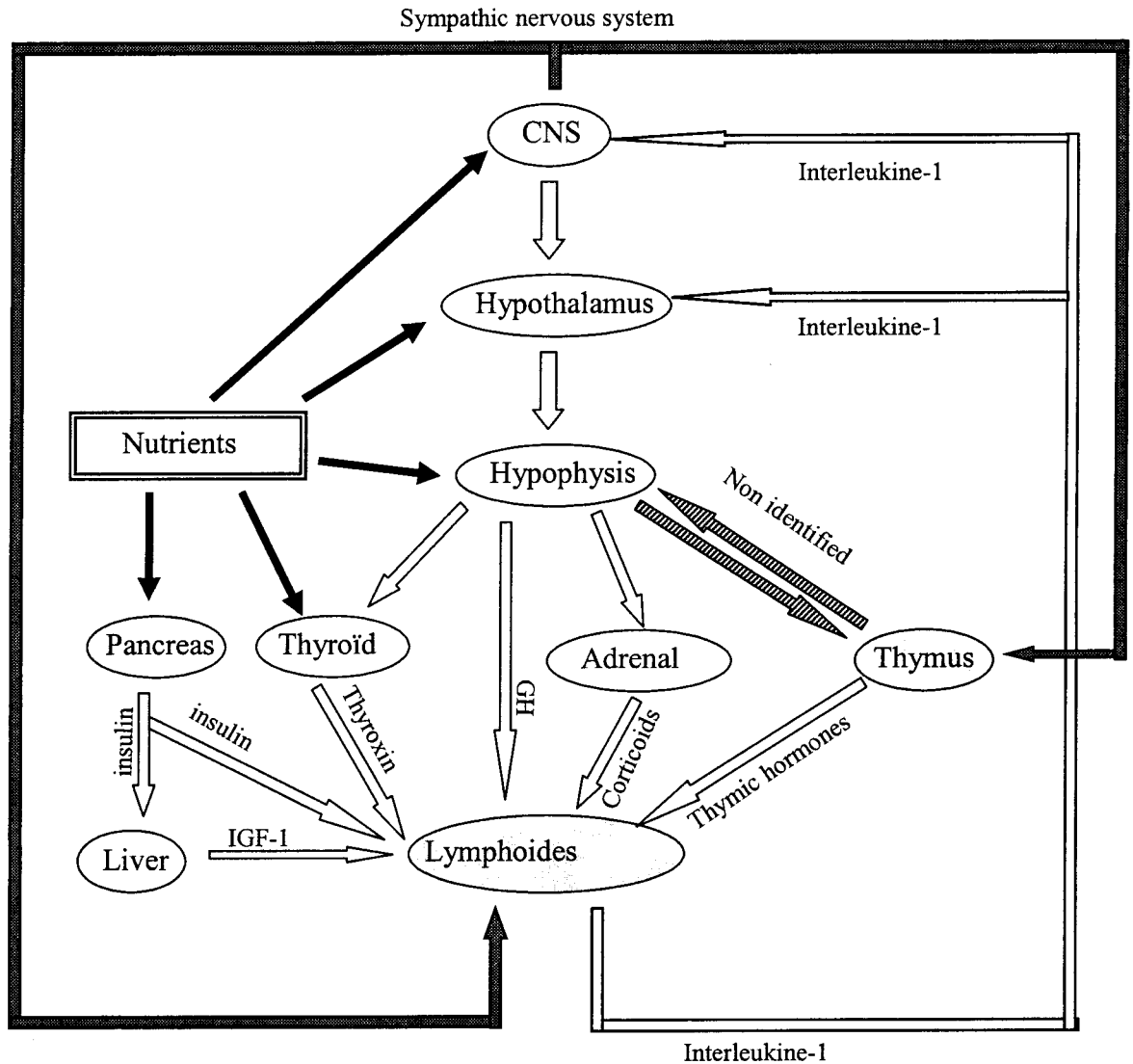
Figure 3 : Indirect modulation of immune response by nutrients, *via* neuroendocrin system. White arrows indicate hormonal interactionsand grey arrows indicate nervous connections (adapted from ROITT et al., 1997).
CNS : central nervous system.

fatty acids (the consequence of the undesirable action of these molecules) also substantially increases during an inflammatory response to an endotoxin. Certain nutrients, notably those which are anti-oxidants (vitamins E and C, β-carotene), may limit these undesirable effects. Inflammatory proteins secreted by the liver can also play a protective role, and an increase in their synthesis may be beneficial.

Several factors such as iron, zinc and biotin are necessary for the multiplication of certain infectious agents. The immune system can regulate the concentration of these elements in the blood and other body fluids. For example, during an inflammatory response, the availability of iron in the plasma is reduced by hepatic uptake for the synthesis of transferrin, while a redistribution of zinc is due to an increase in hepatic synthesis of metallothionin under the influence of interleukin-6. This decline in availability for the pathogenic agent compromises its multiplication in the host tissues.

Indirect modulation of the immune response *via* the neuro-endocrine system

The immune system is not an independent system but is, on the contrary, regulated by numerous hormones most of which are responsive to nutritional factors (see figure 3). For example, the quantity of energy used, or rather the protein/energy ratio influence the somatotropic axis (GH, IGF-1...) and the levels of insulin, glucagon, corticoids, endorphin and enkephalin. In return these molecules modulate the immune system as the immune cells possess receptors for most of these hormones and neurotransmitters and the lymphoid tissues receive sympathetic innervation (neuro-lymphocytic synapse) and are well supplied by the blood vessels which traverse them (ROITT et al., 1997). The adrenal corticosteroids play a major role of negative feedback control on immune responses. The lymphocytes themselves may respond to CRF (cortisone releasing factor) by producing their own ACTH which in turn induces the secretion of corticosteroids.

ROLE OF DIFFERENT NUTRIENTS AND CONSEQUENCES OF A VARIATION IN THE FOOD SUPPLY ON THE IMMUNE RESPONSE

Under-feeding in terms of energy, affects all aspects of the immune response: the anatomy of lymphoid organs (regression of the thymus and lymphatic ganglions, fall in number of circulating leucocytes), cell immunity (proliferation of lymphocytes and level of circulating T lymphocytes)

and humoral immunity (level of circulating IgA), phagocytic functions and the complement system (JOLLY and FERNANDEZ, 2000). The devastating effects of under-feeding and malnutrition on the functions of the immune system have been demonstrated in man, notably in developing countries, but also in numerous other animal species.

Lipids and fatty acids

In the rabbit, as in other animal species, there are two essential fatty acids (EFAs): linoleic acid (C18:2 series ω6) and linolenic acid (C18:3 series ω3). These fatty acids play a structural role in biological membranes and a more specific functional role as they are the precursors of eicosanoids (a family which includes prostaglandins, thromboxanes and leucotriens). These pro-inflammatory molecules induce beneficial immune reactions, playing an immuno-regulatory role, or harmful reactions if they are mobilised too intensely, reducing the production of cytokines.

Very many studies have been made on the influence of the quantity of lipids and the nature of nutritional fatty acids on the immune response (reviews : KINSELLA et al., 1990 ; YAQOO and CALDER, 1993; PECK, 1994; CALDER and YAQOO, 1997; MILES and CALDER, 1998), including some on the rabbit (KELLEY et al., 1988). Although the EFAs play an important role in the modulation of the immune response, an excess or deficiency of them is harmful. Because they are tissue constituents, a deficiency of EFA causes lesions of the skin which no longer acts as a barrier. It also upsets the lymphocytic response, notably by reducing the synthesis of antibodies (WOLTER, 1990). An excess of lipids also reduces the selective barrier function of the endothelium by provoking a breach in the cell membranes (HENNING et al., 2000). Further, excess lipid alters the immune response by reducing the proliferation of lymphocytes, the production of cytokines (IL-2, IL-1, IL-6, TNF), the activity of NK cells and phagocytosis (MILES and CALDER, 1998; PABLO and CIENFUEGOS, 2000). Polyunsaturated fatty acids of the ω6 series have an effect on immune protection by increasing the production of eicosanoids; however, fatty acids of series ω3 modulate the production of these molecules by inhibiting the metabolic path of synthesis from arachidonic acid (C20:4 ω6; CHAPKIN et al., 2000). Zinc, selenium and vitamin E, because of their antioxidant properties, prevent the degradation of polyunsaturated fatty acids and protect cell membranes. The last two also have a moderating effect on the production of prostaglandins and can counterbalance the effects of an excess of polyunsaturated fatty acids.

Proteins and amino acids

The proteinaceous nature of most of the inflammation and immune reaction mediators (cytokines, immunoglobulins, inflammation and complement proteins, DNA synthesis and proteins for lymphocyte multiplication) confers a key role to protein synthesis and to its inclusion in the diet in terms of the amount of protein given and its quality (amino acid composition) such as to favour this process (LE FLOC'H, 2000). The organism always gives priority of use of amino acids to the immune system, and therefore a very severe overall protein deficiency is needed to affect immunity. Nevertheless, even with a protein supply which is quantitatively adequate for growth and reproduction, an imbalance of certain amino acids can result in a decline in the immune response. As a general rule, it seems that amino acid deficiency therefore affects the humoral response more than the cell-mediated response. As early as 1973, JOSE and GOOD recognised that leucine, tryptophane, sulphur-containing amino acids and valine were very important in the regulation of the immune response in mice. These results have since been confirmed in other species (WOLTER, 1990; GRIMBLE and GRIMBLE, 1998). More recently, the immuno-modulatory role of arginine, glutamine and taurine have been demonstrated and discussed (EVOY *et al.*, 1998; REDMOND *et al.*, 1998; WILMORE and SHABERT, 1998). However, the use in pig and poultry farming of an amino acid supplement (methionine, for example) has given contradictory results (VAN HEUGTEN *et al.*, 1994). Lastly, one should note that an excess of certain amino acids is just as harmful to the immune response (KUHLMANN *et al.*, 1988).

Glucides and cell wall constituents

There are no existing data to attribute a specific role, direct or indirect, to glucides in the functioning of the immune system beyond their contribution to satisfying the total energy requirements of the individual. In the rabbit, the protective role of fibre, particularly if indigestible or barely digestible, on the occurrence of non-specific enteritis has already been widely discussed (GIDENNE, 2000). However, the involvement of the immune system in these effects has not been demonstrated and fibre seems to have more of a role in the balance of the caecal ecosystem and the transit time of digestion.

Minerals and vitamins

Several minerals are recognised as having great

Table 2: Consequences of a deficiency in zinc, copper, magnesium, iron or selenium on immune response (from SCRIMSHAW and SANGIOVANNI, 1997).

	Zn	Cu	Mg	Fe	Se
Humoral-mediated response, B lymphocytes	X	X	X	X	
Cell-mediated response, T lymphocytes		X	X	X	X
Immunoglobulins	X	X	X	X	
Thymus (structure or function)	X	X		X	X
Phagocyte function	X	X	X	X	X
Killer cells	X		X	X	
Cytokines or de lymphokines production	X	X	X	X	X

importance in immunity: zinc, copper, selenium magnesium and iron (CHANDRA, 1999; GALYEAN *et al.*, 1999; FRAKER, 2000; HENNING *et al.*, 2000; KLASING and LESHCHINSKY, 2000). Table 2 summarises all the effects of deficiencies of these minerals on the immune response. Cobalt, molybdenum and chromium have also been studied in ruminants. These minerals are essential to the development and maintenance of immunity, and influence both the lymphocytic response (humoral and cellular), the phagocytic function (SCRIMSHAW and SANGIOVANNI, 1997) and cell communication (cytokines). A 2-3 ppm deficiency of zinc in the diet results in a weight reduction of the lymphoid tissues in the rabbit (MAJUMDER and ALI, 1987).

However, excessive doses of minerals can also affect the immune response (CHANDRA, 1982). For example, although iron is necessary in many of the host's biochemical reactions, micro-organisms also require a lot of iron for their metabolism. In the case of infection, a fall in the concentration of iron, which is responsible for the siderophore properties of conalbumin, transferrin and lactoferrin, appears to be part of the animal's defence mechanism. An excessive dose of iron which exceeds the fixation capacity of the transferrin increases the availability of free iron in the blood and encourages microbial proliferation (WOLTER, 1990).

Nearly all the vitamins are involved in the functions of the immune system cells: phagocytosis, synthesis of molecules regulating the leucocytic function (interleukins), and production of antibodies (immunoglobulins; see table 3). Consequently, a deficiency of certain vitamins reduces the production of antibodies, phagocytosis and the destruction of the infected cells. In many species, the requirement for certain vitamins (for example A, D and E) to maintain optimum immune function is much higher than for

Table 3: Major function of vitamins in immunity system (from KOLB, 1997).

Vitamins [1]	A	B1	B2	B5	B6	B7	B9	B12	C	D	E
Function, effects											
Regulation of transcription	X									X	
Activation and/or response of T cells	X										
Stimulation antibody production	X	X	X	X	X	X	X	X	X		
Antioxidant, cytoprotector											X
Reduction of prostaglandins liberation											X
Stimulation of phagocytosis									X	X	
Immunomodulating										X	
Proliferation of immune cells						X					
Stimulation of specific immunity										X	
Production of nucleic acids							X	X			

Vitamins. A : Retinol, b-carotene ; B1 : Thiamin ; B2 : Riboflavin ; B5 : Pantothenic acid ; B6 : Pyridoxin ; B7 = H: Biotin ; B9 : Folic acid ; B12 : Cianocobalanin ; C : Ascorbic acid.

adequate growth (WOLTER, 1990; KLASING and LESHCHINSKY, 2000). Thus, in the chicken, the vitamin A requirement to optimise the immune response is up to five times higher than that needed to maximise growth (SKLAN et al., 1994; Friedman and SKLAN, 1997; LESSARD et al., 1997). This is why the levels of incorporation of vitamins and minerals in feedstuffs are generally much higher than those recommended purely for growth and reproduction (SANTOMA, 1999; FRAGA and VILLAMIDE, 2000). In his review of the literature, LEBAS (2000) found that work on the vitamin requirements of the rabbit is sparse, and no studies are available on the influence of the level of incorporation of vitamins on the immune system in this species.

The role of vitamins in immunity has been the subject of a review (KOLB, 1997), and the reader is advised to refer to this for more detail. To sum up, it can be stated that:

- Vitamin A is involved in cellular transcription and a deficiency of this vitamin decreases the proliferation of immune cells. It participates in non-specific immunity (because of its anti-oxidant properties it favours the mobility of polynuclear), assures the maintenance of specific immunity (being needed to maintain the cellularity of the lymphoid organs), and increases the serum antibody count.

- Vitamin D acts more on non-specific immunity (monocytes and macrophages), although recent results have shown an effect of this vitamin on the T lymphocytes.

- Due to their antioxidant properties, vitamins E and C protect cell membranes from attack by free radicals and peroxidised lipids and increase the lifespan and the cytotoxicity of immune cells. Vitamin E plays a role in the differentiation of T cells in the thymus. A deficiency of vitamin E reduces synthesis of IL-2 and the number of helper T cells (MORIGUCHI et al., 1994). In many animal species (cattle, sheep, pigs and chickens) dietary supplements of vitamin E and selenium improve the ability to cope with infectious diseases (BOREN and BOND, 1996; LESHCHINSKY and KLASING, 1998); they are recommended in the case of avian salmonella, pig dysentery and genital infections of cows (WOLTER, 1990).

- Vitamin C plays a major role in the activation and maintenance of the phagocytic function and in many species, the plasma vitamin C concentration falls during the course of an infectious disease. This vitamin also participates directly in antibody synthesis by the oxidation of sulfhydryl groups as disulfure bonds which connect light and heavy immunoglobulin chains. It is also used by neutrophiles during their bactericidal activity (KOLB, 1997). In poultry, a vitamin C supplement reduced the lesions associated with an infection by E. coli (GROSS and BAILEY, 1995).

- Vitamins of the B group act as co-factors in numerous metabolic cell reactions, notably directly in protein synthesis and the metabolism of nucleic acids. A deficiency of certain vitamins of this group suppresses the proliferation of B and T lymphocytes and the production of antibodies, and leads to a serious attack on the lymphoid tissue (WOLTER, 1990; KOLB, 1997).

OTHER DIETARY CONSTITUENTS INVOLVED IN THE IMMUNE RESPONSE

Apart from the nutrients it contains, the diet can affect susceptibility to infection by modifying

the structure of the intestinal epithelium. Moreover, certain molecules which have no major nutritional function can modify the immune response. Finally, the diet may serve as a vector for molecules or organisms which affect the functioning of the immune system. These may be added deliberately to the diet as a form of scientific husbandry (as feed additives) or they may occur unintentionally, e.g. mycotoxins etc.

Influence of physical and biochemical characteristics of diet on intestinal microflora

The physical and chemical properties of the diet can affect the intestinal flora, the capacity of micro-organisms to attach to enterocytes and the integrity of the intestinal epithelium (KLASING, 1998). The dietary factors which can influence the microbial ecology are the content and nature of the fibre (via their effect on the viscosity of the bolus) and the presence of certain indigestible fatty materials. Thus, certain unstabilised (rancid) fatty materials cause an increase in the number of E. coli and a fall in the number of lactobacilla (DIBNER et al., 1996). In chickens, a supply of whole wheat grains in addition to pelleted diet reduced the multiplication of Coccidia, a protozoan parasite of the genus Eimeria (CRÉVIEU-GABRIEL, 2000).

Non-nutritional molecules in food

Many herbs and plants have been studied for their anti-inflammatory or immuno-stimulatory activity (review: CRAIG, 1999; CHANG and GERSHWIN, 2000). It has been shown that extracts of certain plants can stimulate the systemic immune response, both specific and non-specific. Nevertheless, in the majority of cases, neither the chemical compounds contained in these extracts, nor those responsible for the immunological effects were clearly identified. Several families of molecules have been suggested as being involved in the immunostimulatory power: flavenoids, carotenoids, terpenoids, polyphenolic compounds and certain alkaloids. Of these compounds, it is the carotenoids which have been the subject of the largest number of studies for their effects on infectious diseases. These molecules, which are widely distributed in the vegetable kingdom, are partly responsible for the colour and flavour of certain raw materials. Their concentration and availability is however dependent on the plant species, the harvesting stage, the method and duration of storage and on the technological processes which may be used for their preparation. They are absorbed at the level of the intestinal barrier. Due to their antioxidant properties, the carotenoids protect cell membranes from attack by free radicals and peroxidised lipids and increase the lifespan of cells,

including immunological cells. Furthermore, numerous studies have shown that certain compounds of this family (such as β-carotene) may have immunostimulatory properties apart from their role as a provitamin. An increase in the number of helper T cells and NK cells is observed after supplementation with β-carotene (KOLB, 1997; SCHMITZ and CHEVAUX, 2000).

Food additives

- Glucans. Glucans, polysaccharides extracted from the cell wall of certain yeasts, stimulate the local immune response (DRITZ et al., 1995). The zootechnical value of the use of these molecules was tested in piglets at weaning time (SANTOMA, 1999). As far as we know, only one work has been done on rabbits using glucans. In an attemp to controle the Epizootic Rabbit Enterocolitis the association of MacroGard (béta glucane) with an antibiotic reduced strongly mortality and morbidity (COUDERT et al., 2000).

- Probiotics. Several studies show that the maturation of the digestive immune system is progressive and that the presence of commensal flora is indispensable to good maturation, especially for the diversification of the antibody repertoire (LANNING et al., 2000). This is why many studies have been made to see whether the ingestion of living micro-organisms in the diet (classified under the generic term of probiotics) might stimulate the immune defences. In a few cases the administration of these micro-organisms, even though they failed to colonise the digestive tract, did stimulate the production of antibodies or increase the number of polynuclear leucocytes and NK and the production of cytokines (BLUM et al., 2000; CHIANG et al., 2000; FANG et al., 2000; PANDA et al., 2000).

In the rabbit, many studies have been published on the influence of probiotics on food digestibility and the performance of females or growing rabbits (El GAAFARY et al., 1992; MAERTENS and DEGROOTE, 1992; VOROS and GAAL, 1992; KAMRA et al., 1996; KERMAUNER and STRUKLEC, 1997; PANDA et al., 2000). However, the effect of probiotics on the immune response has not yet been tested.

Other additives. Other molecules are added to the diet with the aim of stabilising the intestinal or caecal flora and thus reduce the incidence of digestive troubles. Examples are oligosaccharids and organic and inorganic acids. The value of these compounds has been demonstrated in certain species and in certain sanitary conditions (SANTOMA, 1999). Studies have also been made on the rabbit (ZAGHINI et al., 1985; MORISSE et al., 1991; MORISSE et al., 1992; PEETERS

et al., 1992; LEBAS, 1996). However, the involvement of the immune system in these effects has never been shown for any species, and they will not be considered in this paper.

Mycotoxins

Mycotoxins are molecules produced by microscopic fungi. Well-known ones are aflatoxin, rubratoxin, zearalenone, trichothecenes, ochratoxin and citrine. The aflatoxins (mainly products of *Aspergillus flavus* and *Aspergillus parasiticus*) and the trichothecenes (mainly produced by the genus *Fusarium*) are molecules whose immunodepressive role has been demonstrated in numerous species (CHOUDHURY *et al.*, 1998; BAKSHI *et al.*, 2000; Fernandez *et al.*, 2000), including the rabbit (DIMITRI and GABAL, 1996; GHONEIMY *et al.*, 2000). They inhibit lymphocyte proliferation (DIMITRI and GABAL, 1996), reducing the humoral response (CHOUDHURY *et al.*, 1998) and cell mediation (VAN HEUGTEN *et al.*, 1994), and may reduce the efficacy of certain vaccines (GHONEIMY *et al.*, 2000).

THE PARTICULAR CASE OF THE MATERNAL MILK

The maternal milk, especially the colostrum, is a liquid of complex composition which supplies energy and essential nutrients for the development of the new-born animal, and also compounds which protect the young from certain infections.

The anti-infectious properties of milk originate from dissolved or cell-borne factors. The milk contains immunoglobulins in quite large amounts (mainly IgA in the rabbit, but also IgG and IgM), which protect the young from certain infectious diseases (LÖNNERDAL, 2000). The concentration of immunoglobulins in milk does not appear to be greatly affected by the protein supply to the mother during gestation, although the selenium content in the diet of the female should have a positive effect on the concentration and absorption of Ig (DUVAUX-PONTER et GRONGNET, 2000). To exert their protective role, the Ig have to maintain their structural integrity; they are resistant to digestive enzymes, which are active from the moment of birth, thanks to an anti-trypsic factor present in the mammary secretions of many domestic species.

Also, the milk contains numerous leucocytes (3.10^6 cells/ml in man, LÖNNERDAL, 2000). One finds neutrophils (40-60%), macrophages (30-50%) and lymphocytes (5-10%, of which 80% are T cells) in human milk, which are the most often activated. These cells contribute to local protection of the intestine but also to general passive cell immunity as some of them can pass through the intestinal barrier (especially for the colostrum cells).

Apart from the antibodies and immune cells, the milk contains various compounds which have an activity which is anti-inflammatory (cytokines, prostaglandins, cortisol), antimicrobial (lactoferrin, lactoferricine, lysozyme; Hughes, 1999), or bacteriostatic (certain C:8 and C:10 fatty acids, oligosaccharids). Thus the fucosyloligosaccharids in milk reduce the attachment of pathogenic bacteria to the intestinal epithelium by offering these organisms a similar structure to the glycans of the digestive mucous membrane (LÖNNERDAL, 2000).

During gestation in the rabbit, the foetuses receive a generous supply of immunoglobulins via the amniotic fluid, which defends them from birth against environmental agents. After birth, the colostrum and then the milk continues to supply immunoglobulins to the young in significant quantities. In the case of adoption just after birth, the young rabbit will benefit both from the immunity transmitted from its biological mother and that transmitted from its adoptive mother. Up to the age of three weeks the diet of the young rabbit is almost exclusively milk. From 18-20 days the young rabbits begin to eat solid food as well as milk. They benefit from the maternal immunity (which is passive) until weaning which generally take place between 28 and 35 days. Currently there is renewed interest in early weaning (before 26 days of age). In this way the young receive a diet more suited to their digestive capacity than the maternal diet, which is rich in starch, but are partially deprived of the protective immunity conferred by the milk.

CONSEQUENCES OF AN INFLAMMATORY OR INFECTIOUS STATE ON PERFORMANCE, METABOLISM AND NUTRITIONAL REQUIREMENTS

During an inflammatory or infectious state, one usually observes a fall in the zootechnical performance of the animals: a reduction in growth rate sometimes associated with a deterioration in food conversion rate (MADEC *et al.*, 1992). Stimulation of the immune system results in local and systemic responses which lead to death of the undesirable agent and bring about a return to homeostasis after an infectious episode.

There are several mechanisms whereby an

immune response might influence the general physiology of an individual (KLASING and LESHCHINSKY, 2000): 1/ During the course of most immune responses, the monocytes and macrophages liberate free inflammatory cytokines (IL-1 and TNF). To begin with they act locally, but if the immune response is substantial, their concentration also increases in the blood circulation and leads to a generalised effect as the cells of nearly all the tissues possess receptors for these molecules. The mechanisms by which the cytokines modify the metabolism of the sick animal have been described by JOHNSON (1997); 2/ the leucocytes stimulated produce realeasing hormones (ACTH...) which can result in a generalised endocrinal response in the form of secretion of corticosterone; 3/ the spleen and the lymphatic ganglions, which are very innervated, can communicate directly with the central nervous system. These three mechanisms involve cellular, metabolic and behavioural changes, which alter the distribution of nutrients in favour of the immune response to the detriment of other metabolic uses, such as growth and milk production. Thus one observes a decline in physical activity and food ingestion (up to 70%), an increase in body temperature (fever), an increase in muscular proteolysis and the general turnover of body proteins and an increase in neoglucogenesis, but a fall in lipoprotein lipase activity and of adipocyte lipolysis. The circulating levels of corticosteroids, thyroxin, glucagon and insulin are usually increased (KLASING and LESHCHINSKY, 2000). There is also a redirection of the flux of amino acids from peripheral tissues to the liver. The big increase in removal of amino acids from the liver provides the substrates necessary for neoglucogenesis and the synthesis of inflammatory plasma proteins.

All these events may affect the nutritional requirements of the animals. For example the inflammatory and immunity proteins synthesised by the sick animal present a very different profile from the proteins involved in body growth or milk production. This leads to specific amino acid requirements for the immune response. For example in the rat, the requirement for sulphur amino acids, notably cysteine, increases greatly during infection. Conversely, lysine, which is often limiting for growth, seems not an amino acid which is limiting for the immune reaction (LE FLOC'H, 2000). The protein synthesis associated with the immune response greatly increases the liver's copper and zinc requirement, due to synthesis of ceruloplasmin and metallothionin. These micronutrients can become limiting if the body reserves (in the liver and muscles) are insufficient at the moment of infection, especially as intestinal absorption is often reduced during a digestive illness.

Consequently, a better knowledge of specific requirements for the immune response could lead to the formulation of feeds which improve the defence capacity and limit the drop in performance of animals whose immune system is challenged.

WHAT ARE THE PROSPECTS FOR WORK ON THE RABBIT?

This literature review, although far from exhaustive, shows that the amount of research work done on the influence of diet on immune function is colossal. Most of the work has been done on or for man. For rabbit production, the topics of interest, the problems and financial resources are very different from the situation with human medicine. Mainly it is a matter of reducing infectious diseases rather than reducing the problems of auto-immunity, allergy, hypersensitivity or chronic inflammatory diseases. What is more, in intensive livestock farming, one is not managing an individual but a group. All the individual variability, and hence all the individual susceptibilities and potentialities are ignored in the interest of managing the group. Finally, the physiological situation of the animals is usually very different from what one finds in man: the individuals are usually young (at least in the case of growing animals), or reproducing (reproductive females). Despite these differences, parallels can be found and certain studies offer interesting prospects for application to animal production. Moreover, many studies are available on pigs and poultry in experimental conditions and with objectives which are very similar to those needed for rabbit production. This is so, for example, for the adjustment of doses of certain vitamins and minerals (vitamin E and selenium) or unsaturated fatty acids, which have shown beneficial effects against certain infectious diseases.

Bearing in mind all these factors, what prospects are there for work on the rabbit?

Which nutrients require work?

We have seen that nearly all the essential nutrients (plus some others!) have an indirect or even direct action on immune function. Considering the number of nutrients which exist and their interactions and the number of molecules and cell populations involved in immunity, there is an almost unlimited number of possible lines of work. It therefore seems reasonable to make a choice which takes account of what is known for other species and the peculiarities of the rabbit.

- *Energy*. As regards energy supply, a situation of severe under-feeding in terms of energy which could affect the immune system is probably rare in scientific rabbit farming and one may reasonably assume that the growing animal, given a balanced diet *ad lib*, will not be in a position of energy malnutrition which could significantly affect its immune responses. On the other hand, the doe is usually in negative energetic and proteic balance during lactation, especially when this is at its peak (PARIGI-BINI and XICCATO, 1998). This situation could reduce the defence capacity of the female, but also that of the young which she is suckling. In fact the IgA transmitted from the mother to the young in the milk appear to be one of the first factors affected by under-nutrition (McMURRAY *et al.*, 1977). Many studies have tested ways of reducing the energy deficit of rabbits during lactation, including increasing the energy content of the feed, incorporation of fat and altering reproduction frequency or husbandry factors. None has been found to be very satisfactory and this work should be repeated, taking account of the effects on the animals' defences.

- *Amino acids*. In the rabbit, the requirements for different amino acids for growth and reproduction need to be elucidated. It would be advisable to take account of non-specific and specific resistance of rabbits in such studies. In this way knowledge of both these fields would progress simultaneously.

- *Fatty acids*. In the rabbit, the requirements for essential fatty acids for growth and reproduction are easily met if the fat content of the diet is at least 2.5%, thanks to the high essential fatty acid content of the plants used for feeding rabbits (LEBAS, 1989). However in recent years an increase in fat content has been used to increase the energy value of feeds without reducing their fibre content. The results on zootechnical performance are often encouraging so long as the amounts given are moderate. However the results are sometimes contradictory as regards the effects on the health of females, litters or growing young. This can probably be explained by variability in the fatty acid profiles, rates of incorporation of the different feeds studied, and by the microbial status of the experimental animals. Hence it would be interesting to elucidate the relationships between the fat content of the diet and the health of the animals. Particular attention should be given to the sources of the fats used and hence the profile of the fatty acids incorporated. It would be advisable to meanwhile study the effects of the concentrations of certain antioxidants, such as vitamin E, in the diet.

- *Vitamins and minerals*. In the rabbit, the caecal flora synthesises large amounts of water-soluble vitamins which are rendered available by caecotrophy. It is generally agreed that all the vitamin requirements of group B and C are met in this species. However, the course of caecotrophy is frequently disturbed in the sick animal, especially in the case of digestive disorders such as enteritis. In this case a deficiency of vitamins of the B group and of vitamin C could arise, lowering the resistance to attack and delaying recovery. It seems therefore of interest to determine or redetermine the requirements for vitamins of group B and vitamin C in the rabbit, taking heed of the effects on the immune system and assuming that appropriate information on the vitamin status of an individual can be obtained from a simple measurement of the levels in the plasma. Further, vitamin E, selenium and zinc could be given particular attention as a combination of these supplements has already been successfully given to other animal species. Vitamins B, C and E offer attractive possibilities for supplementation as the risk of poisoning from an excess of these vitamins seems to be slight (LEBAS, 2000).

For which diseases?

The digestive tract is the main battlefield between pathogenic organisms and the immune system. This is clear from the huge number of micro-organisms, whether pathogenic or not, localised in the intestinal lumen (15×10^{13}/kg live weight, ROITT et al., 1997), the importance of defence systems in this part of the body (gut-associated lymphoid tissue, GALT) and the high frequency of digestive disorders in the rabbit (coccidiosis, colibacilliosis, clostridiosis, epizootic enterocolitis etc). The intestinal epithelium has to maintain perfect integrity to resist the passage of pathogenic agents into the rest of the body whilst permitting sufficient nutrient absorption.

In view of this, research on the interaction between diet and immunity in the rabbit should be mainly concerned with digestive diseases. The first consideration should be for those individuals most affected by these diseases in commercial practice, namely the young rabbits at around weaning time. In the long term, the role of the diet on the mucous membrane's immune response should be studied and its interaction with the equilibrium of the caecal ecosystem.

CONCLUSION

The amount of research work done on the influence of nutrition on immune function is colossal. In spite of this, it is not yet enough to allow us to propose actual dietary values for stimulating the immune defences of

rabbits. It does however enable us to propose lines of work for this species. Hence, in view of the number of factors at our disposition, it seems to us that work on fats (namely the quantity and nature of fatty acids), vitamin E and selenium and the interactions between these three factors are the ones which, in the current state of our knowledge, offer the most interesting prospects in the short term as far as the rabbit is concerned. The work should concentrate on the rabbit at weaning time as this is the most critical period as regards disease.

REFERENCES

BAKSHI C.S., SIKDAR A., JOHARI T.S., MEENAKSHI M., SINGH R.K., 2000. Effect of graded dietary levels of aflatoxin on humoral immune response in commercial broilers. *Indian J. Comp. Microbiol. Imm. Infec. Dis.*, *21, 163-164.*

BLUM S., DELNESTE Y., DONNET A., SCHIFFRIN E.J., 2000. The influence of probiotic organismes on the immune response. In: *Nutrition and immunology : principles and practices.* M.E. Gerswhin, J.B. German and C.L. Keen Eds. Humana press, Totowa, USA, *Chap 36, 451-455.*

BOREN B., BOND P., 1996. Vitamin E and immunocompetence. *Broiler Ind., 11, 26-33.*

CALDER P.C., YAQOO P., 1997. n-3 polyunsaturated fatty acids and the immune system. *Recent Res. Dev. Lip. Res.,* 1, 31-61.

CHANDRA R.K., 1982. Excessive intake of zinc impairs immune response. *JAMA, 252, 1443-1446.*

CHANDRA R.K., 1999. Nutrition and immunology: from the clinic to cellular biology and back again. *Proc. Nutr. Soc., 58, 681-683.*

CHANG C., GERSHWIN M.E., 2000. The anti-inflammatory effects of chinese herbs, plants, spices. In: *Nutrition and immunology : principles and practices.* M.E. Gerswhin, J.B. German and C.L. Keen Eds. Humana press, Totowa, USA, *Chap 35, 439-450.*

CHAPKIN R.S., MCMURRAY D.N., JOLLY C.A., 2000. Dietary n-3 polyunsaturated fatty acids modulate T-lymphocyte activation : clinical relevance in treating diseases of chronic inflammation. In: *Nutrition and immunology : principles and practices.* M.E. Gerswhin, J.B. German and C.L. Keen Eds. Humana press, Totowa, USA, *Chap 10, 121-134.*

CHIANG B.L., SHEIH Y.H., WANG L.H., LIAO C.K., GILL H.S., 2000. Enhancing immunity by dietary consumption of a probiotic lactic acid bacterium (Bifidobacterium lactis HN019): optimization and definition of cellular immune responses. *Eur. J. Clin. Nut., 54, 849-855.*

CHOUDHURY M.R., SARMA D.K., RAHMAN T., 1998. Immunosuppressive effect of aflatoxin on pigs against swine fever virus vaccination. *Indian J. Comp. Microbiol. Imm. Infec. D. 19, 132-136.*

COUDERT P. , RIDEAUD P. , RABOTEAU D., 2000. Epizootic Rabbit Enterocolitis: spontaneous evolution and attempt to control the disease. World Rabbit Science 8 (Sup 1) : *vol B; 219-224.*

CRAIG W.J., 1999. Health-promoting properties of common herbs. *Am. J. Clin. Nut., 70, 491S-499S.*

CRÉVIEU-GABRIEL I., 2000. Interaction nutrition-santé chez les volailles. Colloque INRA : *Nutrition et santé animale*, Paris,

10 octobre 2000, .

DIBNER J.J.C., ATWELL C.A., KITCHELL M.L., SHERMER W.D., 1996. Feeding of oxidized fat to broiler and swine : effects on enterocyte turnover, hepatocyte proliferation and gut associated lymphoid tissue. *Anim. Feed Sci. Technol.*, **62**, 1-13.

DIMITRI R.A., GABAL M.A., 1996. Immunosuppressant activity of aflatoxin ingestion in rabbits measured by response to Mycobacterium bovis antigen I. Cell mediated immune response measured by skin test reaction. *Vet. Hum. Toxicol., 38, 333-336.*

DRITZ S.S., SHI J., KIELIAN T.L., GOODBAND R.D., NELSSEN J.L., TOKACH M.D., CHENGAPPA M.M., SMITH J.E., BLECHA F., 1995. Influence of dietary beta -glucan on growth performance, nonspecific immunity, and resistance to Streptococcus suis infection in weanling pigs. *J. Anim. Sci., 73, 3341-3350.*

DROUET-VIARD, F., FORTUN-LAMOTHE L. 2002. Review of the organisation and functioning of the immune system: particular features of the rabbit. *World Rabbit Sci.* (submitted).

DUVAUX-PONTER C., GRONGNET J.F., 2000. L'immunité colostrale. Colloque AERA: *Immunité et gestation.* Paris, le 5 décembre 2000.

EL GAAFARY M.N., RASHWAN A.A., EL KERDAWY D.M.A., YAMANI K.A., 1992. Effects of feeding pelleted diet supplemented with probiotic (Lacto-Sacc) on digestibility, growth performance, blood constituents, semen characteristics and reproductive traits of rabbits. *Egypt. J. Rabbit Sci., 2, 95-105.*

EVOY D., LIEBERMAN M.D., FAHEY T.J., DALY J.M., 1998. Immunonutrition: the role of arginine. *Nutrition, 14, 611-617.*

FANG H., ELINA T., HEIKKI A., SEPPO S., 2000. Modulation of humoral immune response through probiotic intake. *FEMS Imm. Med. Microbiol., 29, 47-52.*

FERNANDEZ A., HERNANDEZ M., VERDE M.T., SANZ M., 2000. Effect of aflatoxin on performance, hematology, and clinical immunology in lambs. *Can. J. Vet. Res., 64, 53-58.*

FRAGA M.J., VILLAMIDE M.J., 2000. The composition of vitamin supplements in Spanish pig diets. *Nut. Abstr. Rev. (B), 70, 925-929.*

FRAKER P., 2000. Impact of nutritional status on immune integritry. In: *Nutrition and immunology : principles and practices.* M.E. Gerswhin, J.B. German and C.L. Keen Eds. Humana press, Totowa, USA, *Chap 12, 147-156.*

FRIEDMAN A., SKLAN D., 1997. Effects of retinoids on immune response in birds. *Worlds Poultry Sci., 53, 186-195.*

GALYEAN M.L., PERINO L.J., DUFF G.C., 1999. Interaction of cattle health/immunity and nutrition. *J. Anim. Sci., 77, 1120-1134.*

GHONEIMY W.A., HASSAN H.A., SOLIMAN S.A.A., GERGIS S.M., 2000. Study on the effect of aflatoxicosis on the immune response of rabbit to Pasteurella multocida vaccine. *Assiut Vet. Med. J., 43, 287-303.*

GIDENNE T., 2000. Recent advances in rabbit nutrition : emphasis on fibre requirements. A review. *World Rabbit Sci., 8, 23-32.*

GRIMBLE R.F., GRIMBLE G.K., 1998. Immunonutrition: role of sulfur amino acids, related amino acids, and polyamines. *Nutrition, 14, 605-10.*

GROSS W.B., BAILEY C.A., 1995. Effects of ascorbic acid on stress and disease in chickens. *Avian Dis., 36, 688-692.*

GUERDER F., 2001. Résultats annuels de la GTE RENACEB. *Cuniculture, 28, 125-131.*

HENNING B., TOBOREK M., BOISSONNEAULT G.A., 2000. Lipids, inflammatory cytokines, and endothelial cell injury. In: *Nutrition and immunology : principles and practices.* M.E. Gerswhin, J.B. German and C.L. Keen Eds. Humana press, Totowa, USA, *Chap 17, 203-220.*

HUGHES D.A., 1999. Diet and the maturation of the immune system. *Food Agric. Immunol.*, *11*, 279-285.

INRA, 1989. *Alimentation des animaux monogastriques*. Paris, *282 pp.*

JOHNSON R.W., 1997. Inhibition of growth by pro-inflammatory cytokines : an integrated view. *J. Anim. Sci.*, *75*, 1244-1255.

JOLLY C.A., FERNANDES G., 2000. Protein-energy malnutrition and infectious disease : synergistic inetractions. In: *Nutrition and immunology : principles and practices*. M.E. Gerswhin, J.B. German and C.L. Keen Eds. Humana press, Totowa, USA, *Chap 16*, 195-202.

JOSE D.G., GOOD R.A., 1973. Quantitative effects of nutritional essential amino acid deficiency upon immune responses to tumors in mice. *J. Exp. Med.*, *137*, 1-9.

KAMRA D.N., CHAUDHARY L.C., SINGH R., PATHAK N.N., 1996. Influence of feeding probiotics on growth performance and nutrient digestibility in rabbits. *World Rabbit Sci.*, *4*, 85-88.

KELLEY D.S., NELSON G.J., SERRATO C.M., SCHMIDT P.C., BRANCH L.B., 1988. Effects of type of dietary fat on indices of immune status of rabbits. *J. Nutr.*, *118*, 1376-1384.

KERMAUNER A., STRUKLEC M., 1997. Effect of some probiotics on digestibility of nutrients in rabbits. *Proc. 10th Symposium on housing and diseases of rabbits, furbearing animals and pet animals*, Celle Germany, 14-15 may, 1997.

KINSELLA J.E., LOKESH B., BROUGHTON S., WHELAN J., 1990. Dietray polyunsaturated fatty acids and eicosanoids : potential effects on the modulation of inflammatory and immune cells : an overview. *Nutrition*, *6*, 24-44.

KLASING K.C., 1998. Nutritional modulation of resistance to infectious diseases. *Poultry Sci.*, *77*, 1119-25.

KLASING K.C., LESHCHINSKY T.V., 2000. Interactions between nutrition and immunity : lessons from animal agriculture. In: *Nutrition and immunology : principles and practices*. M.E. Gerswhin, J.B. German and C.L. Keen Eds. Humana press, Totowa, USA, *Chap 30*, 363-374.

KOLB E., 1997. *Vitamins and the immune system*. F. Hoffmann-La Roche Ltd., Bassel, Switzerland, *74 pp.*

KUHLMANN G., ROTH J.A., FLAKOLL P., VANDEHAAR M.J., NISSEN S., 1988. Effects of dietray leucine, ketoisocaproate and isovalerate on antibody production and lymphocytes blastogenesis in growing lambs. *J. Nutr.*, *118*, 1564-1569.

LANNING D., SETHUPATHI P., RHEE K.J., ZHAI S.K., KNIGHT K.L., 2000. Intestinal microflora and diversification of the rabbit antibody repertoire. *J. Immunol.*, *165*, 2012-9.

LE FLOC'H N., 2000. Conséquences d'un état inflammatoire ou infectieux sur le métabolisme et le besoin en acides aminés chez le porc. *INRA, Prod. Anim.*, *13*, 3-10.

LEBAS F., 1989. Besoins nutritionnels des lapines Revue bibliographique et perspectives. *Cuni-Sci*, *5*, 1-27.

LEBAS F., 1996. Effects of fructo-oligo-saccharides origin on rabbit's growth performance in 2 seasons. *Proc. 6th World Rabbit Congress*, Toulouse, France, 9-12 juillet 1996, *1*, 211-216.

LEBAS F., 2000. Vitamins in rabbit nutrition : literature review and recommendations. *World Rabbit Sci.*, *8*, 185-192.

LESCHINSKY T.V., KLASING K.C., 1998. Effect of vitamin E on the immunity of chicken. FASEB meeting, San Francisco.

LESSARD M., HUTCHINGS D., CAVE N.A., 1997. Cell-mediated and humoral immune responses in broiler chickens maintained on diets containing different levels of vitamins A. *Poultry Sci.*, *76*, 1368-1378.

LÖNNERDAL B., 2000. Immunological considerations of breast milk. In: *Nutrition and immunology : principles and practices*. M.E. Gerswhin, J.B. German and C.L. Keen Eds. Humana press, Totowa, USA, *Chap 14*, 171-180.

MADEC F., FOURICHON C., MORVAN P., LABBÉ A., 1992. Economie et santé en production porcine. *INRA Prod. Anim.*, *5*, 149-161.

MAERTENS L., DE GROOTE G., 1992. Effect of a dietary supplementation of live yeast on the zootechnical performances of does and weanling rabbits. *J. Appl. Rabbit Res.*, *15*, 1079-1086.

MAJUMDER M.S.I., ALI A., 1987. Effect of zinc deficiency on peyer's patches of rabbits. *Nutr. Res.*, *7*, 1103-1108.

McMURRAY D.N., REY H., CASAZZA L.J., WATSON R.R., 1977. Effect of moderate malnutrition on concentrations of immunoglobulins and enzymes in tears and saliva of young colombian children. *Am. J. Clin. Nutr.*, *30*, 1944-1948.

MILES E.A., CALDER P.C., 1998. Modulation of immune function by dietary fatty acids. *Proc. Nut. Soc.*, *57*, 277-92.

MORIGUCHI S., OONISHI K., KISHINO Y., 1994. Vitamin E is an important factor in T cell differentiation in thymus of F344 rat. *FASEB J.*, *8*, A 273.

MORISSE J.P., LEGALL G., MAURICE R., COTTE J.P., BOILLETOT E., 1991. Action chez le lapereau d'un mélange de fructo oligo-saccharides sur certains paramètres intestinaux et plasmatiques. *L'éleveur de lapins*, *36*, 35-37.

MORISSE J.P., MAURICE R., BOILLETOT E., COTTE J.P., 1992. Effect of a fructo-oligosaccharides compound in rabbits experimentally infected with E. Coli O 103. *J. Appl. Rabbit Res.*, *15*, 1137-1143.

PABLO M.A.D., CIENFUEGOS G.A.D., 2000. Modulatory effects of dietary lipids on immune system functions. *Immunol. Cell Biol.*, *78*, 31-39.

PANDA A.K., REDDY M.R., RAMARAO S.V., PRAHARAJ N.K., 2000. Effect of dietary supplementation of probiotic on performance and immune response of layers in the decline phase of production. *Indian J. Poultry Sci.*, *35*, 102-104.

PARIGI-BINI R., XICCATO G., 1998. Energy metabolims and requirements. In: *The nutrition of the rabbit*. C. De Blas and J. Wiseman Eds. CABI Publishing, Wallingford, *Chap 7*, 103-132.

PECK M.D., 1994. Interactions of lipids with immune function II: experimental and clinical studies of lipids and immunity. *J. Nutr. Biochem.*, *5*, 514-521.

PEETERS J.E., MAERTENS L., GEEROMS R., 1992. Influence of galacto-oligo-saccharides on zootechnical performance, cecal biochemistry and experimental colibacillosis O103/8+ in weanling rabbits. *J. Appl. Rabbit Res.*, *15*, 1129-1136.

REDMOND H.P., STAPLETON P.P., NEARY P., BOUCHIER-HAYES D., 1998. Immunonutrition: the role of taurine. *Nutrition*, *14*, 599-604.

ROITT I., BROSTOFF J., MALE D., 1997. *Immunologie*. 4th Edition De Boeck & Larcier, Paris, *405 pp.*

SANTOMA G., 1999. Estimuladores de la immunidad. XIV Curso de especializacion de la fundacion Espagnola para el desarollo de la nutricion animal (FEDNA).

SCHMITZ H., CHEVAUX K., 2000. Defining the role of phytochemicals in modulating human immune function. In: *Nutrition and immunology : principles and practices*. M.E. Gerswhin, J.B. German and C.L. Keen Eds. Humana press, Totowa, USA, *Chap 9*, 107-120.

SCRIMSHAW N.S., SANGIOVANNI J.P., 1997. Synergism of nutrition, infection, and immunity: an overview. *Am. J. Clin. Nut.*, *66*, 464S-477S.

SKLAN D., MELAMED D., FRIEDMAN A., 1994. The effects of varying levels of dietray vitamin A on immune response in the chick. *Poulty Sci.*, *73*, 843-847.

VAN HEUGTEN E., SPEARS J.W., COFFEY M.T., KEGKEY E.B., QURESHI M.A., 1994. The effect of methionine and aflatoxin on immune fonction in weanling pigs. *J. Anim. Sci.*, *72*, 658-664.

VOROS G., GAAL C., 1992. Effect of bacillus CIP 5832 and Enrofloxacin on performance and aerob fecal flora of growing rabbits. Déviation of in vitro situation from in vivo one. *J. Appl. Rabbit Res.*, *15*, 1153-1159.

WILMORE D.W., SHABERT J.K., 1998. Role of glutamine in immunologic responses. *Nutrition*, *14*, 618-26.

WOLTER R., 1990. Nutrition et réponse immune. In: *Immunologie animale*. P.P. Pastoret, A. Govaerts and H.

Bazin Eds. Médecine-Science, Flammarion, Paris, *Chap 40, 407-416.*

WOOD J.W., WOODWARD B.D., 1991. Enhancement of primary systemic acuqired immunity by exogenous triiodothyronine in wasted, protein-energy malnourished weanling mice. *J. Nutr., 65, 1534-1539.*

YAQOO P., CALDER C., 1993. The effects of fatty acids on lymphocyte functions. *Internat. J. Biochem., 25, 1705-1714.*

ZAGHINI G., LAMBERTINI L., STEGAGNO G., 1985. Use of organic acids in the feeding of meat rabbits. *Atti Soc. Ital. Sci. Vet., 39, 464-467.*

Feeding programs for young rabbit does based on lucerne: II. Performance of does during first lactation.

Fernández-Carmona, J.[1], Cervera C.[1], Quevedo F.[2], Blas E.[3], Pascual J.J.[1]

[1]Unidad de Alimentación Animal. Departamento de Ciencia Animal. Universidad Politécnica de Valencia. P.O. Box 22012, Valencia 46071, Spain. [2]Departamento de Producción Animal y Ciencia de los Alimentos. Universidad Cardenal Herrera-CEU. Moncada. Valencia 46113, Spain.

Starting from the animals used in the precedent work (Fernández-Carmona et al., 2002), the effect of the described rearing nutritional strategies on the performance of does during their 1st reproductive cycle was evaluated. In trial 1, after parturition all the females (C and F1 groups) had free access to the same commercial diet (C), their litter standardised to 8 pups and inseminated again at 4 days after parturition. In trial 2, from 28th day of gestation all the females (C and F2 groups) had free access to a concentrate diet (E), their litter was standardised to 10 pups and inseminated again at 11 days after parturition. Type of rearing system did not affected on the live weight (LW) gain of lactating does (Table 1), showing in both trials and treatments a LW increase of does during lactation (+224-347 g). Respect to the main objective of rearing systems, dry matter (DM) intake of F does was significantly higher than that observed for C does during lactation in both trials. However, the differences were greater when lucerne-based diets were given earlier (trial 1). As consequence of the higher DM and DE intake, F1 does showed a clearly higher milk yield during all lactation in the 1st trial (173.1 and 193.2 g day^{-1}, for F1 and C groups, respectively; P<0.01). Therefore, litters of F1 group showed significant higher weight gain (+13 g day^{-1} P<0.01), and were weaned with a higher weight (+354 g; P<0.01) than those of the control group. In the 2nd trial, no differences were found on milk yield of does and litter weight gain between groups, that probably was related to the lower DM increase observed on lactating does when fibrous diets were administered from 3 kg LW. However, figures were always higher for does on F2 group (+111 g for litter at weaning).Respect to the effect of rearing system on the following reproductive cycles, although litter size at 2nd parturition was not affected, the parturition interval was 7 days more for F2 group in the second trial. In the 1st trial, animals were controlled for date of parturition, litter size at birth and litter size at weaning for 2 years time, in order to study possible long term effects of the rearing program. No significant differences were detected between C and F1 programs, although the figures for number of weaned pups per doe and years were slightly better for F2 group. In conclusion, and as Nizza et al. (1997) have also observed with low energy diets, the use of rearing systems based in the use of all-lucerne diets seems to increase the DM intake of primiparous does, improving the performance of their litter, especially if they are early administered. However, more information about the possible long term effect of these type of diets on the reproductive performance of does seems to be necessary.

Table 1. Effect of rearing diet on the performance of young rabbit does during first parturition

	Trial 1		Trial 2	
	F1-C	s.e.	F2-C	s.e.
LW gain of does during lactation (g)	+20	36	+18	43
Feed intake: g DM day^{-1}	+27.6**	4.7	+7.0	4.1
g DM kg$^{-0.75}$ day^{-1}	+12.1***	1.4	+4.2*	1.5
Milk yield (g day^{-1})	+20.1**	3.6	+0.4	3.1
Litter weight (g): at 21 days of lactation	+216*	52	+60	46
at weaning	+354**	65	+111	64
Interval between 1st to 2nd partum (days)	-	-	+7.2	3.7
Litter size at 2nd partum: Total	-	-	+0.63	0.55
Alive	-	-	+0.06	0.62
Pups weaned per doe (no. year^{-1})	+4.3+	1.3	-	-

Ref. Nizza A., Di Meo C., Esposito L. 1997. World Rabbit Science, 5: 107-110.
Fernández J., Cervera C., Quevedo F., Blas E., Pascual J.J. 2002 COST Meeting. Varese. Italy.

Feeding programs for young rabbit does based on lucerne: I. Performance of does until first parturition.

Fernández-Carmona, J.[1], Cervera C.[1], Quevedo F.[1], Blas E.[2], Pascual J.J.[1]

[1]Unidad de Alimentación Animal. Departamento de Ciencia Animal. Universidad Politécnica de Valencia. P.O. Box 22012, Valencia 46071, Spain. [2]Departamento de Producción Animal y Ciencia de los Alimentos. Universidad Cardenal Herrera-CEU. Moncada. Valencia 46113, Spain.

The effect on the performance until parturition of does feed with a lucerne based diet (F1 and F2, 96% of lucerne hay with 8.0 and 7.3 MJ/ kg DM for 1st and 2nd trials, respectively) respect to a commercial diet (C, 10.9 MJ/kg DM) during the rear of young rabbit does was evaluated. In the 1st trial, 46 females of 2 kg live weight (LW) (70 days) had free access to one of the diets until 3kg LW, and restricted (150 g/day) from this moment until the parturition day in the case of C group. In the 2nd trial, 60 females of 3 kg LW (90 days) were divided in two groups: 30 does had free access to F diet and other 30 had restricted access (150 g/day) to C diet until 28th day of lactation and then both groups to a concentrate diet (E, 12.6 MJ/kgDM). In both trials, although does given F diets showed a significant higher DM intake during rearing (P<0.001), its DE value was not sufficient to allow energy compensation in different periods of rearing. Therefore, rearing LW gain of does given F diets was lower than for those given C diet, showing a lower LW at the end of rearing (approx. -260 g; P<0.001), and arriving later to an adequate insemination weight (3.5 kg) when F1 diet was given from 2 kg LW. The results were slightly different for both trials. Does receiving F1 diet from 2 kg LW showed the growth delay during 2 to 3 kg LW period, but they seems to show a recovery thereafter. However, does receiving F2 diet from 3kg LW showed the growth delay during 3 to 3.5 kg LW period. During gestation, females given F diets showed a higher DE intake in both trials, and as consequence they arrived to partum with a similar LW than C does. Respect to effect of rearing diet on the parturition parameters, the results were also slightly different for both trials. In the 1st trial, the use of a fibrous diet during rearing time did not affect the litter parameters at birth, showing a similar litter size (8.6 pups) and weight (490 g/pup). However, due to the higher mortality of pups at birth with F2 diet (12.5 vs. 1.0% for F2 and C diets) in the 2nd trial, litter size at birth tended to be lower (-1.4; P<0.1) for does given F2 diet. This result is difficult to be explained, but it could be related to high energy intake observed for F2 does when they received the E diet (rich in fat and starch) during the prepartum period (410, 545, 582 and 911 kJ DE kg$^{-0.75}$d^{-1}, for C, F1, C and F2), that probably could increase the individual weight of foetuses (+5.7 g per pup at birth; P<0.05) and their risk of mortality during parturition. In fact, mortality at birth was significantly higher on C group in the trial 1 (11.6 vs. 2.7%, for C and F1 groups). From these results could be concluded: 1) the use of an 8.0 MJ DE/kg DM diet based on lucerne from 2 kg LW delayed the time to first parturition; 2) the change to a high energy diet 3 or 4 days before parturition could cause problems on litter viability at birth.

Table 1. Effect of rearing diet on the performance of young rabbit does until first parturition

	Trial 1		Trial 2	
	F1-C	s.e.	F2-C	s.e.
LW gain (g/d): Initial to 3 kg LW	-18.94***	1.22	—	—
3 kg LW to first AI	-1.44	0.78	-9.00***	0.92
First AI to partum	+3.57	1.13	+1.15	0.95
Time to first parturition (d)	-7.62*	1.53	+1.64	1.54
DE intake (kJ kg$^{-0.75}$d^{-1}): Initial to 3 kg LW	-105***	11.4	—	—
3 kg LW to 1st AI	+93.0***	9.9	-72.4***	8.92
1st AI to 28th day gestation	+157***	16	+29.3***	9.1
28th day gestation to partum	+135**	28	+328***	32
Litter size at birth (no.): Total	-0.88	0.32	-0.66	0.49
Alive	-0.20	0.42	-1.44^{+}	0.56
Pup weight at birth (g): Total	+0.50	1.55	+5.68*	1.99
Alive	-0.71	2.49	+4.41	1.92

Aunque el diseño de experiencia ni permite estudiar entre sí los resultados obtenidos en los dos ambientes estudiados, es inevitable realizar algún tipo de comparación. Muy brevemente podemos estimar la enorme diferencia entre los índices obtenidos en uno y otro, comentando alguno de ellos en especial.

El peso a la cubrición, al parto o a 7 días post-parto como aconsejan Blasco y Ouhayoun (1996), escogidos como índices representativos del peso de las conejas de ciclo a ciclo, demuestra que aparentemente el peso adulto no pudo alcanzarse en la cámara.
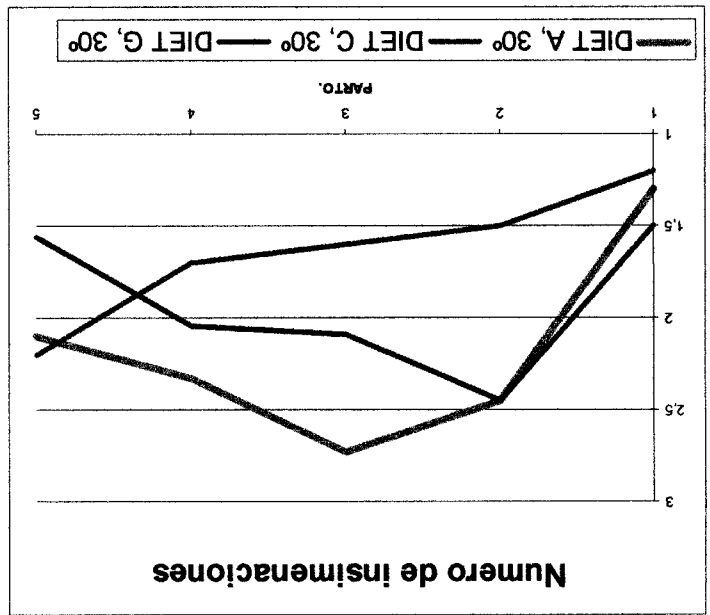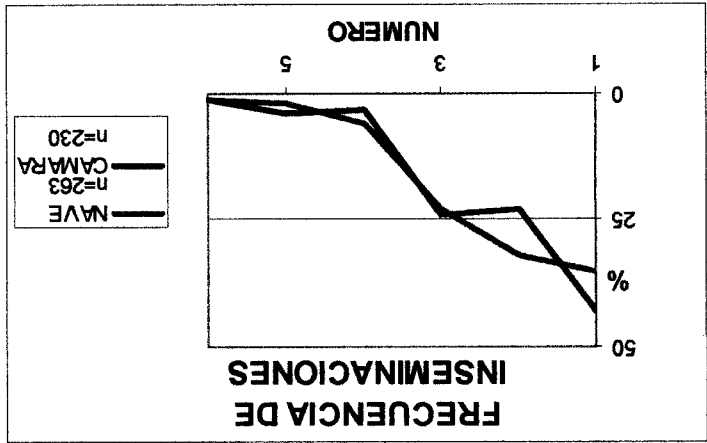
Figura 3

Figura 4